



## Effect of *Lactobacillus paracasei*, *L. helveticus* and *Bifidobacterium lactis* on fatty acid profile of sour cream

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ARTICLE INFO	ABSTRACT
<p><b>Article History:</b></p> <p>Received:2023/12/9</p> <p>Accepted:2024/4/9</p> <hr/> <p><b>Keywords:</b></p> <p>Fermentation, Functional, Probiotic, Sour Cream</p> <hr/> <p><b>DOI:</b> 10.22034/FSCT.21.157.50.</p> <hr/> <p>*Corresponding Author E- rezakarimi@guilan.ac.ir ; rzakarimi@gmail.com</p>	<p>Nowadays, probiotic products are among popular foods among the consumers due to their different health effects. One of the dairy products is sour cream, which has been less noticed, although it has high potential for inclusion of the probiotic microorganisms. The goal of the present research was considering the effects of probiotic cultures on fatty acid profile of sour cream. The cream samples were incorporated by <i>Lactobacillus casei</i>, <i>L. helveticus</i> and <i>Bifidobacterium lactis</i> as single cultures. The pH values, acidity and fatty acid profiles were evaluated at the time of 1, 15 and 30 days of storage period. The mentioned parameters were compared to the control cream. Concentrations of short-chain fatty acids in cultured cream samples differed depending on the used cultures. Moreover, probiotics caused the change in medium chain, saturated and polyunsaturated fatty acid content in fermented cream. Among the short-chain fatty acids and unsaturated fatty acids, <i>L. paracasei</i> caused the highest production of linoleic acid in 1 and 30 days of storage as well as the highest production of butyric acid in 15 and 30 days of storage. Generally, production of probiotic sour cream can be a functional value-added product in the dairy industry.</p>

## 1- Introduction

Consumer awareness about the effects of foods with high nutritional value and special health-promoting properties has increased the demand, size and success of the market for functional products (Tur and Bibiloni, 2016). Probiotic products are one of the most important categories of functional products (Karimi et al., 2011). Probiotic microorganisms have been used in various dairy products such as yogurt (Olson and Aryana, 2022), fermented milks (Sharma et al., 2023), cheese (Karimi et al., 2012), ice cream (Goktas et al., 2022), and even non-dairy products (Cosme et al., 2022). Among dairy products, those with a higher fat percentage have a greater protective effect for containing probiotics (Araújo et al., 2012). The selection of probiotics as added cultures depends on the health-promoting properties of those microorganisms (Fijan, 2014) as well as their effect on the sensory properties of the product (Karimi et al., 2012). Apart from the health-giving properties of probiotics, the health-giving properties of sour fermented dairy products have also been widely investigated due to their positive effect on human intestinal microflora (Paszczyk and Czarnowska-Kujawska, 2022). Sour cream has a special status among other types of cream, including breakfast cream, cooking cream, confectionary cream and other creams, and different types of sour cream can be different in different countries of the world based on the percentage of fat and the presence or absence of non-dairy components. In general, the fat percentage of sour creams ranges from 10 to 40 percent (Hoffmann, 2016). Some articles have reported fat percentages of sour cream as 18-20% (Niamsiri and Batt, 2009) and others as 18-25% more than milk fat percentage (Gibson and Newsham, 2018). According to Iran's standard definition, sour cream is a product obtained from the fermentation of cream by microorganisms or soured by acidifying the cream with the help of chemicals. The fat content of this product is at least 18% w/w and its acidity is at least 0.5% based on lactic acid. According to the standard definition, fermented cream is a product made from the fermentation of cream (with at least 10% fat) by a lactic starter consisting of bacteria such as *Streptococcus lactis*, *S. cremoris*, *S. lactis subsp. diacetylactis* and all types of *Leuconostoc* were prepared and its pH is

reduced with or without clot formation and its acidity is at least 0.6% in terms of lactic acid. Also, according to the standard definition, acidified cream is a product that is produced by acidifying cream, reconstituted cream or recombined cream, with the addition of permitted edible acids or acid regulators, and its pH is reduced with or without clot formation. The fat content of this product is at least 18% by weight and its acidity is at least 0.5% in terms of lactic acid (ISIRI-No.191, 2019). In general, consumption of fermented fatty dairy products such as sour cream can reduce the risk of cancers such as colon cancer and increase the function of the immune system. Sour cream can be one of the rich sources for bioactive fatty acids such as butyric acid and conjugated linoleic acid (CLA). This compound, CLA, has numerous properties such as anti-mutagenic, anti-cancer, anti-cholesterol, anti-osteoporosis (Rahman et al., 2011), anti-arteriosclerosis (Kritchevsky, 2000), anti-diabetes (Malinska et al., 2015), strengthening the immune system (Collomb et al., 2006) and skin papilloma inhibitor (Belury, 2002). The composition of fatty acids is important for two reasons, one for the sensory quality of dairy products and the other for its health effects, which were mentioned above.

Various factors such as geographical conditions, physiological conditions of the milk producing animal from which the cream is taken and product processing conditions can affect the fatty acid composition of sour cream (Nunes and Torres, 2010). The fat must be homogenized before fermentation, and the degree of homogenization pressure depends on the fat content of the cream (Hoffmann, 2016). Bioactive fatty acids produced by probiotics can change the fatty acid composition of dairy products (Yilmaz-Ersan, 2013). Probiotic bacteria can also produce CLA (Yang et al., 2017). Some *Lactobacillus* strains (Palachum et al., 2018) including *L. plantarum* (Hosseini et al., 2015), *Propionibacterium* (Zárate, 2012), *Bifidobacterium* (Mei et al., 2022) and *Enterococcus* (Dapkevicius et al., 2021) can convert free linoleic acid to CLA (Sieber et al., 2004). Although there is limited information regarding the effect of probiotics on the composition and fatty acid profile of sour cream, in general, fermentation with probiotics can be an efficient approach to produce sour cream as a functional product and provide this opportunity to dairy industries to produce such

functional products. Based on the mentioned issues, the aim of this study is to investigate the effects of probiotic cultures on the fatty acid profile and sensory characteristics of sour cream.

## 2- Materials and Methods

### 1.1. Production of cream samples, cultures and preliminary analyses

Cream samples for this study were produced in Solico Kalleh dairy products company. In this study, the fat percentage of cream was standardized to 30%. Probiotic strains include *L. paracasei*, *L. helveticus* and *B. lactis* was supplied from Lallemand Health Solutions (LHS). One sample was considered as a control sample. It should be noted that probiotic strains were used and coded as single cultures according to Table 1. The cream taken from the

**Table 1** Probiotic bacteria used in each treatment

Treatments	Cultures
A	Control
B	<i>Lactobacillus paracasei</i> HA-196
C	<i>Bifidobacterium lactis</i> LAFTI® B94
D	<i>Lactobacillus helveticus</i> LAFTI® L10

### 1.2. Measurement of pH and acidity

The pH of the samples was measured using a pH meter according to the Iranian national standard No. 2852 during refrigerated storage. Before measuring pH, the pH meter was calibrated with standard buffers (pH 7 and pH 4).

To measure acidity, according to Iran's standard method, 18 grams of the sample was weighed in a beaker and distilled water was added as the same weight of the sample to it. Then, 0.5 ml of phenolphthalein reagent was added and titrated with 0.1 normal sodium hydroxide until pale pink color appeared. Acidity was reported in Dornic degree (ISIRI-No.2852, 2022).

### 2.3. Fatty acid profile analysis

separator was heated using a plate pasteurizer at a temperature of 80 °C for 25 seconds and cooling it to 42°C (as the optimum average inoculation temperature for all three bacteria) was done.

Then, DVS probiotic cultures were added directly at the concentration of 0.02 g per 100 g of cream sample. According to the initial cultures, it was found that the inoculum concentration was 10<sup>7</sup> CFU/g in the cream samples. After inoculation, the cream samples were placed in incubator at 42 °C for 12 hours until fermentation was done and the pH dropped. After fermentation, the samples were cooled to 4 °C and stored for 30 days. Tests of pH, acidity and fatty acid profiles were performed on the 1st, 15th and 30th days of storage.

Fatty acid content was determined using fatty acid methyl esters. First, 0.1 g of the extracted milk fat was poured into the test tube. Then, 1 ml of methanolic solution of 2N potassium hydroxide, 3 ml of N-hexane and 1 ml of vitamin C were added to the sample, and then it was kept in a hot water bath at a temperature of 50 °C for 15 minutes. Then the solution was vortexed and the supernatant was separated. Anhydrate sodium sulfate was added to it for drying. After filtering, the solution was directly used for gas chromatography. Chromatographic separation was performed using a Technochroma capillary column equipped with a flame ionization detector. The test took about 1 hour. The carrier gas was nitrogen, whose flow rate was 0.8 mL/min.

The heating program of the oven consisted of three steps (speed 15 °C/min to 160 °C and holding at this temperature for 25 minutes, then speed of 5 °C/min to 200 °C and holding at this temperature for 10 minutes and finally, the

speed of 5 °C/min to 220 °C and keeping at this temperature for 5 minutes). The injection volume was 1 microliter and the injection temperature was 240 °C. The split flow rate was 79.2 mL/min and the split ratio was 1:100. Peak identification was based on inhibition times and on desired standards. The results were reported as percentage concentration (grams per 100 grams of fat).

## 2.4. Statistical analysis

Experiments were conducted for different types of treatments and different retention times with full factorial designs. The results were analyzed by ANOVA method at a significance level of 5% with Duncan's multi-range test using SPSS 18.0 statistical program. The presence of significant differences is indicated by different lowercase letters.

## 3-Results and discussion

### 3.1. pH and acidity values

The results related to pH values and acidity of sour cream samples during the storage period are shown in Table 2. As expected, the pH value decreases with increasing storage time. Meanwhile, the amount of pH reduction during the 30-day storage period from the first day (and not the beginning of inoculation) in samples inoculated with *B. lactis* is more than other samples. This issue could be due to the higher initial pH of this sample compared to samples inoculated with other probiotic species. In other words, on the first day of storage, the pH of the sample containing *B. lactis* is higher than others, which can be due to weaker activity *B. lactis* compared to *L. paracasei* and *L. helveticus* on the first day of storage. On the other hand, on the first day of storage, the pH drop in the sample containing *L. helveticus* is more, which indicates a stronger

activity of this probiotic species. Also, on the last day of storage, the pH level in the samples containing *L. helveticus* and *B. lactis* showed the lowest and highest pH levels, respectively. The pH level on other days of storage also indicates the fact that *B. lactis* and *L. helveticus* are respectively the weakest and strongest species in terms of lowering pH. It should be noted that the control sample suffered a slight decrease in pH, which could be due to possible contaminating microorganisms during storage. In general, at the end of the 30-day storage period, the pH value from the lowest to the highest belongs to the samples containing *L. helveticus*, *L. paracasei* and *B. lactis*.

The results of acidity measurement also show a similar trend of pH changes in terms of comparing the activity of probiotic species. Expectedly, the acidity increased in all samples with the passing days of storage. On the first day of storage, the highest amount of acidity was related to the sample containing *L. helveticus* and the lowest acidity (regardless of the control sample) related to the sample containing *B. lactis*. This trend was also observed on the last day of storage, which indicates the more acidifying power of *L. helveticus* compared to *L. paracasei* and more acidifying power of *L. paracasei* compared to *B. lactis*. It should be noted that a very slight increase in the acidity of the control sample was also observed, which, as mentioned earlier, could be due to possible slight contamination during the 30-day storage of the product. In general, at the end of 30 days of storage of the samples, the amount of acidity from the highest to the lowest respectively belonged to the samples containing *L. helveticus*, *L. paracasei* and *B. lactis*.

**Table 2** pH values and acidities of the cream samples during the storage

Treatments	pH values*			Acidity*		
	Storage days			Storage days		
	1 d	15 d	30 d	1 d	15 d	30 d
Control	6.75±0.05 <sup>a</sup>	6.59±0.04 <sup>a</sup>	6.45±0.03 <sup>a</sup>	9.2±0.2 <sup>d</sup>	10.4±0.2 <sup>d</sup>	11.1±0.1 <sup>d</sup>
<i>L. paracasei</i>	4.43±0.04 <sup>c</sup>	4.28±0.03 <sup>c</sup>	4.18±0.01 <sup>c</sup>	37.7±0.2 <sup>b</sup>	43.4±0.2 <sup>b</sup>	46.1±0.1 <sup>c</sup>
<i>B. lactis</i>	5.18±0.01 <sup>b</sup>	4.99±0.01 <sup>b</sup>	4.52±0.02 <sup>b</sup>	28.7±0.1 <sup>c</sup>	34.4±0.1 <sup>c</sup>	50.1±0.1 <sup>b</sup>

<i>L. helveticus</i>	4.2±0.04 <sup>d</sup>	4.09±0.03 <sup>d</sup>	3.95±0.03 <sup>d</sup>	48.7±0.2 <sup>a</sup>	61.4±0.2 <sup>a</sup>	65.1±0.1 <sup>a</sup>
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\*Different lowercase superscript in a same column indicate significant differences between treatments

### 3.2. Fatty acids profile

The results related to the profile of fatty acids of sour cream samples during the 1st, 15th and 30th days of the storage period as a percentage (grams per 100 grams of fat) in the cream samples are shown in Tables 3, 4 and 5, respectively. The presence of 13 fatty acids was found in the gas chromatography analysis of fatty acids from cream samples. Among saturated fatty acids, myristic acid, palmitic acid and stearic acid were the main fatty acids in all samples. But among unsaturated fatty acids, oleic acid was the predominant fatty acid in all samples. This finding was consistent with the results of another study on fermented cream with probiotic bacteria (Yilmaz-Ersan, 2013). Polyunsaturated fatty acids such as linolenic acid were not measured in this research. On the first day of storage, the amount of butyric acid, myristoleic acid and stearic acid in the cream sample containing *L. helveticus* was more than the others. Also, caproic acid and oleic acid in the sample of cream containing *B. lactis* was at the highest level compared to the rest of the cream samples. In the case of caprylic acid, the control sample and the sample containing *B. lactis* had the highest amount. For capric acid, myristic acid and arachidic acid, the highest amount was seen in the control sample, but for lauric acid and linoleic acid, the highest amount was in the sample containing *L. paracasei* was observed. The highest amount of palmitic acid was seen in samples containing *L. paracasei* and *L. helveticus*. This was while the lowest amount of palmitoleic acid in the sample containing *B. lactis* was found. The lowest quantitative difference among the samples was seen for the fatty acids of arachidic acid, linoleic acid and palmitoleic acid. In general, among important short-chain fatty acids and important unsaturated acids, the highest amount of butyric acid and linoleic acid was seen in the samples containing *L. helveticus* and *L. paracasei*, respectively. Generally, in milk fat and related products, the amount of short chain fatty acids such as butyric acid is nutritionally important. It should be noted that the fatty acid content of various fermented dairy products,

including sour cream, can vary depending on the strain used, the fat percentage of the product, temperature and fermentation time (Nieuwenhove et al., 2012). In general, the probiotic strains used in this research increased butyric, stearic and oleic acids compared to the control sample (without inoculation). Among the different samples, the sample containing *B. lactis* had the highest amount of oleic acid, which was consistent with the results of the Yilmaz-Ersan study in 2013. With the exception of the control sample, the amount of arachidic acid of all samples containing probiotics was not significantly different ( $p>0.05$ ). It has been reported that the amount of saturated fatty acids in sour cream ranges from 64 to 65 grams per 100 grams of fat (Yilmaz-Ersan, 2013). In the recent research, on the first day, this value for samples containing *L. paracasei*, *B. lactis* and *L. helveticus* was 66.21, 65.73 and 67.36 grams per 100 grams of fat, respectively. In another study on a product called Dahi or fermented milk produced in India, it was observed that the addition of probiotic bacteria to the product increased saturated fatty acids compared to the control sample (Yadav et al., 2007). Regarding unsaturated fatty acids, as mentioned before, butyric acid and oleic acid were higher in probiotic-containing samples than in the control sample. Although the high level of unsaturated fatty acids can increase fat oxidation and subsequently overshadow the quality of dairy products due to the spread of bad taste, these fatty acids have health benefits for humans, one of which is reducing the risk of cardiovascular diseases (Perdomo et al., 2015, Shramko et al., 2020, Lu et al., 2024).

**Table 3** Profile of Fatty acids (FAs) of cultured creams after 1 days of storage (%)

FAs	Treatments*			
	Control	<i>L. paracasei</i>	<i>B. lactis</i>	<i>L. helveticus</i>
C4:0	1.45±0.02 <sup>d</sup>	1.89±0.01 <sup>c</sup>	1.95±0.02 <sup>b</sup>	2.43±0.03 <sup>a</sup>
C6:0	1.24±0.03 <sup>c</sup>	1.36±0.03 <sup>b</sup>	1.62±0.01 <sup>a</sup>	1.34±0.01 <sup>b</sup>
C8:0	0.77±0.04 <sup>a</sup>	0.50±0.03 <sup>b</sup>	0.59±0.02 <sup>a</sup>	0.52±0.01 <sup>b</sup>
C10:0	2.83±0.02 <sup>a</sup>	2.55±0.02 <sup>c</sup>	2.66±0.04 <sup>b</sup>	2.57±0.03 <sup>c</sup>
C12:0	3.45±0.03 <sup>b</sup>	3.52±0.01 <sup>a</sup>	3.24±0.03 <sup>c</sup>	3.23±0.02 <sup>c</sup>
C14:0	11.07±0.01 <sup>a</sup>	11.01±0.02 <sup>b</sup>	10.60±0.04 <sup>d</sup>	10.74±0.01 <sup>c</sup>
C14:1	1.22±0.02 <sup>c</sup>	1.31±0.01 <sup>b</sup>	1.32±0.02 <sup>b</sup>	1.39±0.04 <sup>a</sup>
C16:0	35.05±0.03 <sup>c</sup>	35.64±0.03 <sup>a</sup>	35.25±0.02 <sup>b</sup>	36.61±0.04 <sup>a</sup>
C16:1	1.71±0.05 <sup>a</sup>	1.76±0.02 <sup>a</sup>	1.62±0.02 <sup>b</sup>	1.79±0.04 <sup>a</sup>
C18:0	9.31±0.01 <sup>d</sup>	9.71±0.02 <sup>c</sup>	9.78±0.01 <sup>b</sup>	9.88±0.05 <sup>a</sup>
C18:1	21.08±0.04 <sup>d</sup>	23.40±0.02 <sup>b</sup>	23.90±0.02 <sup>a</sup>	22.28±0.02 <sup>c</sup>
C18:2	3.04±0.02 <sup>b</sup>	3.14±0.04 <sup>a</sup>	3.05±0.04 <sup>b</sup>	3.04±0.02 <sup>b</sup>
C20:0	0.17±0.01 <sup>a</sup>	0.04±0.01 <sup>b</sup>	0.04±0.01 <sup>b</sup>	0.04±0.02 <sup>b</sup>

\*Different lowercase superscript in a same row indicates significant differences between treatments

On the 15th day of the storage period, the samples containing *L. paracasei* had the highest amount of butyric acid and caproic acid. Samples containing *B. lactis* had the highest levels of caprylic acid, lauric acid and stearic acid. Also, samples inoculated with *L. helveticus*, produced the highest amount of oleic acid ( $p < 0.05$ ). However, these samples (*L. helveticus*) had insignificantly the highest amounts of myristoleic acid and linoleic acid. It should be noted that the samples containing

probiotics had lower amounts of capric acid, myristic acid, palmitic acid and palmitoleic acid than the control sample (without inoculation). None of the samples were significantly different in terms of arachidic acid content ( $p > 0.05$ ). In general, in terms of the most important short-chain fatty acid, *L. paracasei* causes the highest production of butyric acid and in terms of the most important unsaturated fatty acid, *L. helveticus* caused the highest production of oleic acid and linoleic acid.

**Table 4** Profile of Fatty acids (FAs) of cultured creams after 15 days of storage (%)

Fatty acids	Treatments*			
	Control	<i>L. paracasei</i>	<i>B. lactis</i>	<i>L. helveticus</i>
C4:0	1.91±0.02 <sup>b</sup>	2.3±0.03 <sup>a</sup>	1.92±0.04 <sup>b</sup>	1.83±0.02 <sup>c</sup>
C6:0	1.58±0.04 <sup>c</sup>	1.71±0.03 <sup>a</sup>	1.64±0.01 <sup>b</sup>	1.47±0.03 <sup>d</sup>
C8:0	0.50±0.02 <sup>b</sup>	0.57±0.02 <sup>a</sup>	0.58±0.03 <sup>a</sup>	0.46±0.01 <sup>c</sup>
C10:0	2.93±0.01 <sup>a</sup>	2.59±0.04 <sup>c</sup>	2.82±0.02 <sup>b</sup>	2.56±0.04 <sup>c</sup>
C12:0	3.30±0.02 <sup>c</sup>	3.36±0.02 <sup>b</sup>	3.41±0.02 <sup>a</sup>	3.20±0.03 <sup>d</sup>
C14:0	11.20±0.05 <sup>a</sup>	10.64±0.04 <sup>c</sup>	10.96±0.05 <sup>b</sup>	10.68±0.06 <sup>c</sup>
C14:1	1.33±0.01 <sup>c</sup>	1.36±0.01 <sup>b</sup>	1.40±0.02 <sup>a</sup>	1.40±0.03 <sup>a</sup>

C16:0	36.32±0.07 <sup>a</sup>	35.78±0.06 <sup>c</sup>	35.89±0.03 <sup>b</sup>	35.73±0.07 <sup>c</sup>
C16:1	1.80±0.01 <sup>a</sup>	1.76±0.01 <sup>b</sup>	1.77±0.02 <sup>b</sup>	1.76±0.02 <sup>b</sup>
C18:0	9.68±0.04 <sup>b</sup>	9.29±0.05 <sup>c</sup>	9.88±0.04 <sup>a</sup>	9.70±0.03 <sup>b</sup>
C18:1	21.64±0.06 <sup>d</sup>	23.18±0.05 <sup>b</sup>	21.84±0.04 <sup>c</sup>	23.79±0.07 <sup>a</sup>
C18:2	3.07±0.01 <sup>b</sup>	3±0.02 <sup>c</sup>	3.12±0.02 <sup>a</sup>	3.13±0.03 <sup>a</sup>
C20:0	0.05±0.01 <sup>a</sup>	0.05±0.0 <sup>a</sup>	0.05±0.01 <sup>a</sup>	0.05±0.01 <sup>a</sup>

\*Different lowercase superscript in a same row indicate significant differences between treatments

On the 30th day of storage, samples containing *L. paracasei* produced the highest amount of butyric acid and linoleic acid compared to other samples. While in comparison with other samples, the samples containing *B. lactis* had the highest amount of oleic acid. Also, samples containing *L. helveticus* had the highest amount of caprylic acid compared to other samples ( $p < 0.05$ ). The samples containing probiotics were lower in terms of caproic acid, capric acid,

lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid and stearic acid than the control sample (not inoculated). It is worth noting that samples containing probiotics did not differ significantly in terms of palmitoleic acid content ( $p > 0.05$ ). In general, in terms of the most important short-chain fatty acid and unsaturated fatty acid, *L. paracasei* caused the highest production of butyric acid and linoleic acid compared to other samples.

**Table 5** Profile of Fatty acids (FAs) of cultured creams after 30 days of storage (%)

FAs	Treatments*			
	Control	<i>L. paracasei</i>	<i>B. lactis</i>	<i>L. helveticus</i>
C4:0	1.99±0.02 <sup>d</sup>	2.48±0.05 <sup>a</sup>	2.37±0.02 <sup>b</sup>	2.06±0.03 <sup>c</sup>
C6:0	2.07±0.04 <sup>a</sup>	1.60±0.02 <sup>d</sup>	1.78±0.03 <sup>c</sup>	2.02±0.05 <sup>b</sup>
C8:0	0.56±0.02 <sup>c</sup>	0.63±0.04 <sup>b</sup>	0.69±0.06 <sup>b</sup>	0.94±0.03 <sup>a</sup>
C10:0	3.31±0.03 <sup>a</sup>	2.83±0.01 <sup>d</sup>	2.95±0.02 <sup>c</sup>	3.13±0.02 <sup>b</sup>
C12:0	4.03±0.05 <sup>a</sup>	3.36±0.04 <sup>c</sup>	3.42±0.04 <sup>c</sup>	3.55±0.06 <sup>b</sup>
C14:0	12.31±0.06 <sup>a</sup>	10.87±0.04 <sup>c</sup>	10.68±0.05 <sup>d</sup>	11.16±0.02 <sup>b</sup>
C14:1	1.69±0.02 <sup>a</sup>	1.51±0.04 <sup>b</sup>	1.38±0.05 <sup>c</sup>	1.53±0.03 <sup>b</sup>
C16:0	39.70±0.09 <sup>a</sup>	35.90±0.07 <sup>b</sup>	35±0.04 <sup>d</sup>	35.33±0.05 <sup>c</sup>
C16:1	2.03±0.03 <sup>a</sup>	1.83±0.04 <sup>b</sup>	1.81±0.06 <sup>b</sup>	1.8±0.07 <sup>b</sup>
C18:0	10.81±0.06 <sup>a</sup>	9.9±0.06 <sup>b</sup>	9.64±0.04 <sup>c</sup>	9.82±0.07 <sup>b</sup>
C18:1	12.13±0.04 <sup>d</sup>	22.87±0.06 <sup>b</sup>	23.26±0.06 <sup>a</sup>	20.23±0.04 <sup>c</sup>
C18:2	3.04±0.02 <sup>d</sup>	3.53±0.03 <sup>a</sup>	3.18±0.07 <sup>b</sup>	3.09±0.02 <sup>c</sup>
C20:0	0.13±0.01 <sup>a</sup>	0.08±0.02 <sup>b</sup>	0.08±0.01 <sup>b</sup>	0.13±0.01 <sup>a</sup>

\*Different lowercase superscript in a same row indicate significant differences between treatments

In different studies, different strains of probiotics have been used. Among the fatty

acids, butyric acid and conjugated linoleic acid should be considered from the nutritional point of view. Butyric acid is one of the short chain fatty acids that are natural components of milk



fat. This fatty acid plays an important role against cancer by preventing cell proliferation and causing cancer cell death (apoptosis) (Siregar et al., 2016, Pattayil and Balakrishnan-saraswathi, 2019). Linoleic acid is an important precursor of conjugated linoleic acid or CLA, which is converted through biohydrogenation during lactic fermentation (Kuhl and Lindner, 2016). The amount of CLA in dairy products varies due to the animal's diet and can be in the range of 0.1% to 2% of milk fat (Khanal and Olson, 2004). Changes in the amount of CLA in fermented products according to the type and strain of starters and probiotics used in the process, fermentation time and temperature, substrate composition (Nieuwenhove et al., 2012), source and concentration of linoleic acid (Kuhl and Lindner, 2016) and reaching pH is acidic (Kim and Liu, 2002). The production of other conjugated fatty acids is also regulated by various factors such as pH and temperature (Gorissen et al., 2011). It has been shown that strains of lactobacilli (Alonso et al., 2003), bifidobacteria (Coakley et al., 2003, Raimondi et al., 2016) and propionibacteria (Wang et al., 2007) can efficiently convert linoleic to CLA. Different probiotic strains have been used to increase CLA in fermented dairy products such as fermented milk (Florence et al., 2009), yogurt (Rouhi et al., 2008), drinking yogurt (Colakoglu and Gursoy, 2011), cheese (Gursoy et al., 2012) and so on. Ekinici et al. (2008) studied the effect of probiotics on the fatty acid profile of cultured cream with a fat content of 52.80%. They used different probiotic bacteria such as *L. acidophilus*, *B. bifidum*, *S. thermophilus* and *L. bulgaricus*, *Propionibacterium thoenii* and *P. jensenii* and also a mixed culture (combination of *L. acidophilus*, *B. bifidum*, *S. thermophilus* and *L. bulgaricus*) were used to ferment cream enriched with sunflower oil, soybean oil and hazelnut oil. The highest amount of CLA (0.73 mg of CLA per gram of fat) was observed in samples inoculated with *B. bifidum*. Fermentation with *P. jensenii* significantly resulted in higher concentrations of butyric acid compared to fermentation with other bacteria. None of the *Propionibacterium* caused a significant improvement in CLA concentration. It was also seen that in most of the samples, the count of probiotics was higher than  $10^6$  cfu.g<sup>-1</sup> (Ekinici et al., 2008). Domagala et al. (2009) studied the content of CLA, linoleic acid and vaccenic acid in fermented cream. They used

mesophilic culture, thermophilic culture, yogurt culture and cheese culture by adding *Propionibacterium* strains and probiotics. It was found that one of the yogurt starters (ABY-2) increased the amount of CLA in sour cream compared to fresh cream. At the same time, the amount of CLA in creams fermented with other starters was lower than the amount before fermentation. The amount of linoleic acid and vaccenic acid generally decreased after fermentation in all samples except in the sample inoculated with ABY-2 starter. Additionally, *Propionibacterium* did not induce CLA production from linoleic acid (Domagala et al., 2009).

Yilmaz-Ersan (2013) examined fatty acid composition of fermented cream with *B. lactis*, *L. acidophilus* and *L. rhamnosus*. It was shown that the amount of oleic acid and  $\alpha$ -linolenic acid in the cream fermented with *B. lactis* was higher than its value in the control sample. Also, fermented cream with *B. lactis* had the highest amount of monounsaturated fatty acids, polyunsaturated fatty acids and long chain fatty acids compared to other samples. A sample of fermented cream with *L. acidophilus* had the highest amount of saturated fatty acids and medium chain fatty acids compared to the rest of the samples. In general, the main difference between cream samples inoculated with different probiotics was the degree of saturation of fatty acids. It was also concluded that adding probiotics does not significantly change the amount of butyric acid, caproic acid and CLA (Yilmaz-Ersan, 2013). Lin et al. (1995) reported that in sour cream with 18.4% fat, the values of 4.13 and 0.76 mg were per gram of fat and per gram of product, respectively. In cream with 33.2% fat, these concentrations were 4.30 and 1.43 mg/g, respectively (Lin et al., 1995). In another study on cream products, CLA concentrations ranged from 6.1 to 6.2 mg per gram of fat (Jiang et al., 1997). Xu et al. (2005) studied the effects of *L. rhamnosus*, *P. freudenreichii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii* individually or co-cultured with *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* in fermented milk. They found that *L. rhamnosus* in co-culture with yogurt starters produced the highest amount of CLA. They also reported that the presence of yogurt starters increased CLA formation by propionibacteria (Xu et al., 2005). In a similar



study on fermented milk, it was observed that the inoculation rate of *L. rhamnosus* and yogurt starters have no significant effect on the amount of CLA (Xu et al., 2006). Dave et al. (2002) studied the composition of fatty acids during yogurt processing. They observed that fermentation with yogurt starters (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) and probiotics (*L. acidophilus* and bifidobacteria) did not change the amount of CLA, trans-vaccinic acid and omega-3 fatty acids (Dave et al., 2002). Yadav et al. (2007) showed that the addition of probiotics increased the amount of saturated fatty acids in Dahi product (traditional Indian yogurt) compared to the control sample (Yadav et al., 2007). In another study on synbiotic fermented milk containing *S. thermophilus* and *L. acidophilus*, the use of maltodextrin increased CLA by at least 38% compared to the control sample (Oliveira et al., 2009).

Khosravi-Darani et al. (2014) investigated the effect of probiotic bacteria including *L. acidophilus* La-5, *B. bifidum* and *P. freudenreichii* on the biological production of CLA in yogurt containing whey powder and grape seed oil. The best conditions for CLA production were 4% (w/v) whey powder, 4% (v/v) grape seed oil, pH 6, fermentation temperature 35°C and incubation time of 27 hours. It was shown that the amount of CLA in probiotic yogurt increased from 8.01 mg per gram of fat in the control sample to 11.03 mg per gram of fat in probiotic yogurt containing grape seed oil, which was an increase of 40% (Khosravi-Darani et al., 2014). The change in CLA concentration of yogurt produced from cow's milk and sheep's milk was investigated during 14 days of storage. Storage at refrigerator temperature led to a significant decrease and a significant increase in cow's milk yogurt and sheep's milk yogurt, respectively. In other words, it can be concluded that the origin of milk can affect CLA content (Serafeimidou et al., 2013). Złoch et al. (2022) studied the effect of adding *L. paracasei* on the fatty acid profile and vitamin D3 content of the cream. They found that *L. paracasei* increases the ratio of unsaturated to saturated fatty acids and also increases the amount of CLA. Also, *L. paracasei* increased vitamin D precursor levels after 6 hours and increased vitamin D3 levels after 24 and 48 hours (Złoch et al., 2022). The use of

bifidobacteria caused a slight increase in CLA (1.4 times) in fermented milk after 7 days of storage at 4°C (Florence et al., 2012). *B. animalis* Bb-12 increased CLA in colostrum after incubation (Rodríguez-Alcalá et al., 2011). In reconstituted milk containing 0.2% hydrolyzed sesame oil, some mesophilic starters such as *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc mesenteroides* subsp. *mesenteroides* have a higher production amount of CLA compared to probiotic genera such as *Lactobacillus*, *Propionibacterium*, *Enterococcus* and *Pediococcus* (El-salam et al., 2010). In a study, the amount of CLA produced by 126 strains of 31 *Bifidobacterium* species was investigated in MRS broth enriched with 0.5 g/L linoleic acid. Most of the *Bifidobacterium* species producing CLA belong to *B. breve* and *B. pseudocatenulatum* which among them the strain B. WC0421 was the best producer of CLA (Raimondi et al., 2016). Also, in another study, CLA production was evaluated in 36 different *Bifidobacterium* strains in MRS broth culture medium enriched with 0.5 mg/ml linoleic acid. After 7 hours of microbial growth and incubation at 37°C for 72 hours, it was found that among bifidobacteria, strains of *B. breve*, *B. bifidum* and *B. pseudolongum* can produce different isomers of CLA ranging from 19.5% to 53.5% (Gorissen et al., 2010). In another study, the ability to produce CLA in *B. breve* LMC520 was investigated with different amounts of linoleic acid in different cultivated conditions. The highest amount of CLA was obtained after 24 hours of incubation in MRS medium with 1 mM linoleic acid at pH 5.5 under anaerobic conditions (Park et al., 2009). Despite the positive effect of probiotics on CLA production in many studies, in a series of researches, probiotics have no effect on CLA amount. Manzo et al. (2015) reported that *L. acidophilus* La-5 and *B. animalis* Bb-12 not only did not increase the amount of CLA in pasteurized cow's milk, but also did not increase it in baby food with high linoleic acid content and prebiotic galactooligosaccharides. Their findings strengthened the hypothesis that the amount of CLA measured in fermented products was originally present in cow's milk. According to this research, since the added probiotics did not lead to significant changes in CLA values, the amount of CLA was not related to probiotic fermentation. Therefore, it can be concluded in such cases that CLA was

actually produced in the rumen of the animal and preserved during fermentation (Manzo et al., 2015). It is not easy to compare different products according to the unique physicochemical characteristics of each product and specific production conditions, as well as comparing different strains in terms of the metabolic characteristics of each strain. Therefore, it is necessary to identify the role of pure and single cultures of different probiotic species in order to understand the mechanisms that change the composition of fatty acids in sour cream. Fatty acid content of fermented dairy products depends on the composition of primary milk and bacterial metabolic activity during fermentation. The profile of fatty acids in sour cream is continuously changing during fermentation due to the growth of bacteria. Hence, it is necessary to investigate the effects of bacteria on the distribution of fatty acids, which can determine the characteristics of the final product.

#### 4-Conclusion

The findings of the present research showed that the effect of probiotics on the chemical characteristics (pH and acidity) and fatty acid profile of sour cream depends on the strain used. The amount of pH lowering power and increasing acidity from the highest to the lowest respectively belong to the samples containing *L. helveticus*, *L. paracasei* and *B. lactis*. It was observed that the fermentation of cream with probiotic strains changes the amount of short-chain fatty acids and unsaturated fatty acids. In general, among short-chain fatty acids and unsaturated fatty acids, *L. paracasei* produced the highest amount of linoleic acid on the 1st and 30th days of storage and also the highest production of butyric acid on the 15th and 30th days of storage. Totally, the inoculation of probiotic bacteria in dairy products such as sour cream is important for the production of functional dairy products. More research in this field is needed in terms of examining the effect of the type of probiotic strains on changing the physicochemical characteristics and changing the profile of fatty acids. It is suggested to evaluate different species and strains with high potential in the production of bioactive fatty acids during processing and storage of sour cream.

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## تاثیر لاکتوباسیلوس کازئی، *L. هلوتیکوس* و *بیفیدوباکتریوم لاکتیس* بر رخ نمای اسیدهای چرب خامه ترش

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اطلاعات مقاله	چکیده
<p>تاریخ های مقاله :</p> <p>تاریخ دریافت: ۱۴۰۲/۹/۱۸</p> <p>تاریخ پذیرش: ۱۴۰۳/۱/۲۱</p>	<p>امروزه، محصولات پروبیوتیک بدلیل خواص سلامتیبخش مختلف جزو فرآورده های غذایی محبوب در بین مصرف کنندگان هستند. یکی از محصولات لبنی، خامه ترش بوده که در عین حالیکه پتانسیل بالایی برای دربرگیری میکروارگانیسم های پروبیوتیک دارد، توجه چندانی به آن نشده است. هدف تحقیق حاضر بررسی اثرات کشت های پروبیوتیک بر رخ نمای اسیدهای چرب و ویژگی های حسی خامه ترش بود. نمونه های خامه بالاکتوباسیلوس کازئی، <i>L. هلوتیکوس</i> و <i>بیفیدوباکتریوم لاکتیس</i> به صورت کشت منفرد تلقیح شدند. مقادیر pH، اسیدیته و رخ نمای اسیدهای چرب، در روزهای ۱، ۱۵ و ۳۰ دوره نگهداری مورد ارزیابی قرار گرفتند. پارامترهای گفته شده با نمونه خامه شاهد مقایسه شدند. غلظت اسیدهای چرب کوتاه زنجیر در نمونه های خامه بسته به نوع کشت مورد استفاده تغییر کردند. همچنین، پروبیوتیک ها باعث تغییر در اسیدهای چرب متوسط زنجیر و اسیدهای چرب اشباع و غیراشباع در خامه تخمیر شده شدند. از میان اسیدهای چرب کوتاه زنجیر و اسیدهای چرب غیراشباع، <i>L. پاراکازئی</i> باعث تولید بیشترین میزان اسید لینولئیک در روزهای ۱ و ۳۰ نگهداری و همچنین بیشترین تولید اسید بوتیریک در روزهای ۱۵ و ۳۰ نگهداری شد. به طور کلی، تولید خامه ترش پروبیوتیک می تواند یک محصول فراسودمند با ارزش افزوده بالا در صنعت لبنیات باشد.</p>
<p>کلمات کلیدی:</p> <p>پروبیوتیک، تخمیر، خامه ترش، فراسودمند</p>	
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