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Research article

EFFECTS ON SOME QUALİTY PROPERTİES OF YOGURT ICE CREAM ADDİNG ENCAPSULATED PROBİOTİC BACTERİA TO YOGURT ICE CREAM PRODUCED FROM COW AND BUFFALO MİLK

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ABSTRACT

In this study, the effects of adding encapsulated and free-form probiotic bacteria (*Lactobacillus acidophilus* ATCC 4356) to yogurt ice cream produced from cow and buffalo milk on physical and chemical properties were investigated. Changes in the storage process of yogurt ice creams were determined and the effect of probiotics was analyzed. The prebiotic effects of inulin on probiotic bacteria were also investigated in the study. When applied together with yogurt bacteria, inulin increased the activity and effect rate of probiotic cultures in the same way. *L. acidophilus*, one of these probiotic bacteria, has an ideal growth temperature in the pH range of 5.5-6.0. This bacteria can also multiply in the pH range of 3.7-4.3. The reason we use inulin in yogurt ice cream is that in addition to the beneficial effects of inulin on health, inulin has a prebiotic effect on probiotic bacteria without affecting the sensory properties of the product to which it is added. The study also aimed to determine whether inulin can increase the quality of the yogurt ice cream we will produce due to its effect of preventing the formation of ice crystals in ice cream. Buffalo yogurt ice cream with probiotic bacteria and inulin contained the highest level(18.09%) of acetic acid. The long-chain saturated fatty acids (palmitic and stearic acids), which are undesirable in terms of health, were lower(35.79% and13.52%,respectively) in control buffalo yogurt ice cream samples than that of control cow milk(37.82% and 17.17%). When probiotic bacteria were encapsulated, the hardness of the ice cream samples increased. When probiotic bacteria encapsulated in cow's milk were added, the hardness was found to be at the highest level of 716.34 g, while the hardness of the ice cream sample made by adding probiotic bacteria encapsulated in buffalo milk and inulin was found to be at the highest level (912.48 g). A more homogeneous structure was detected in samples added with inulin and encapsulated probiotic.

1. Introduction

The demand for functional foods has increased significantly, especially in probiotic and prebiotic products. This study aimed to increase the quality of yogurt ice cream and to preserve the viability of probiotic bacteria. The quality of yogurt ice cream made from buffalo and cow milk was investigated and the effects of their mixtures on quality were also examined. The need for foodstuffs is also increasing in parallel with the increase in the world population. Milk and dairy products have an important share among these foodstuffs. Milk is the only foodstuff that contains the majority of the nutrients required for nutrition as well as the enzymes and metabolites involved in cell reactions. In addition to being so important in human nutrition, reasons such as its bulkiness, difficulty in transportation and rapid deterioration make it necessary to process milk into more durable products (Milci, 2008). Among these durable dairy products, ice cream, which has shown significant developments in the world and Türkiye in recent years (Dervişoğlu, 1995). Since the ice cream industry is a rapidly developing , many products based on ice cream are being developed and offered to the consumer. The fact that it appeals to consumers from all walks of life and all ages increases the importance of ice cream. For example, dietetic or diabetic ice creams are produced specifically for those who avoid consuming excess calories and have heart, circulatory or diabetic patients. In addition, ice creams with different flavors and structures are produced in fruit, chocolate, nuts and plain varieties; with only water, sugar, fruit or fruit flavor; and with a fat ratio of 16% and total dry matter of 40% for those who want to gain weight. Various ice creams are also made from fermented milk products mixed with fruit syrups, stabilizers-emulsifiers and sugar. The most well-known and consumed ice cream made from fermented milk products is yogurt ice cream (Demir, 2001). Today, consumers prefer healthy, reliable and balanced foods in order to be able to eat a balanced diet. Therefore, food manufacturers are trying to respond to these demands of consumers by developing new food types such as suitable diet foods (low-fat foods), modified foods (additive-free/organic foods) and functional foods (probiotic and prebiotic foods) (Tonguç, 2006). However, unfortunately, the variety of probiotic foods produced in our country is not at the desired level. The importance of the subject was emphasized by taking decisions on "Increasing the variety of processed products by developing food processing methods and processes: Development of functional foods with special functions

such as increasing resistance to diseases, maintaining form, regulating metabolic activities, assisting treatment, boosting immunity. Considering the interest of the Türk people in yogurt, it is highly probable that yogurt ice cream will gain a good place in the market. Since probiotics are live microorganism strains and need to be stored in the cold in order not to lose their effects, it is reported that ice cream and similar foods are foods that can prevent the loss of probiotic microorganisms even during long-term storage. On the other hand, the temperature change that occurs during the freezing and melting of ice cream and similar foods prevents the development of probiotic microorganisms, reduces their metabolic activity and can even completely destroy them. Therefore, it is obvious that the probiotic properties of foods in which probiotic microorganisms are used in their production should be monitored after production and during storage. When the subject is evaluated in the light of all this information, it is an important factor to determine whether the probiotic microorganisms in the production of yogurt ice cream maintain their probiotic properties during the production and storage of yogurt ice cream. It is also obvious that it is necessary to determine a standard production method that will ensure the highest level of preservation of probiotic properties in yogurt ice cream production. Although various studies have been conducted in recent years to preserve the viability of probiotic bacteria using the microencapsulation technique in probiotic dairy products, no study has been identified on the use of the microencapsulation technique in the production of yogurt ice cream. In this study, the probiotic properties of probiotic bacteria in the production of yogurt ice cream were tried to be preserved at the highest possible level using the microencapsulation technique. Simply revealing the probiotic properties of yogurt ice cream is not enough for the product to find a place in the market and be preferred. Therefore, the quality characteristics of yogurt ice cream should also be revealed and these characteristics should be monitored during storage. Advances in science and technology, consumers' more conscious choices and increasing interest in healthy living make the production of healthier products a necessity (Tonguç, 2006). However, in our country, the development of new probiotic foods has not been emphasized as much as necessary in research on health and nutrition. In this study, the physical and chemical properties of the products obtained by adding different forms of inulin and probiotic bacteria to yogurt ice cream produced from cow and buffalo milk were examined. In addition, the effect of inulin in preventing the formation of ice crystals in yogurt ice cream and the protection of probiotic bacteria by microencapsulation were aimed to increase product quality. In this context, the main purpose of the study is to improve the production processes of probiotic yogurt ice cream and to maximize the health benefits of these products. Studies show that yogurt ice cream is a product that can meet consumer demand with both its nutritional and health-beneficial properties. Especially in countries where yogurt consumption is widespread, such as Türkiye yogurt ice cream has a high potential in the market. However, the production processes of this product need to be improved and standardized. This study aims to eliminate the lack of information in this area by investigating the effects of different milk types and probiotic additives used in the production of yogurt ice cream on product quality.

2. Materials and methods

2.1. Materials

Yogurt ice cream production was carried out in the milk processing facility under the supervision of the Food Engineering Department of Atatürk University, Faculty of Agriculture. MRS broth (Merck) medium was used in the activation of bacterial strains and microbiological counts in the study. Buffered Peptone Water (Merck) was used in the preparation of dilutions and microbiological analyses. Sodium alginate (Sigma) was used in the coating of microorganisms and $Na_2HPO_4.7H_2O$ (Merck) was used in the preparation of buffer solutions.

2.2. Methods

2.2.1.Preparation of culture for use in yogurt ice cream production

L. acidophilus ATCC 4356 strain was first inoculated into 9 ml of MRS Broth and left for 18 hours of anaerobic incubation at 37°C. At the end of incubation, it was re-inoculated into 100 ml of MRS Broth and left for 18 hours of anaerobic incubation at 37°C. After incubation, it was centrifuged at 6000 rpm at 4°C for 10 minutes to collect bacterial cells and washed twice with sterile peptone water. The collected bacterial cells were suspended in sterile peptone water containing 0.1% peptone and their absorbance was measured at 600 nm in a spectrophotometer and a growth sphere was obtained by inoculating on MRS agar. Thus, the approximate number of microorganisms was determined to determine the numbers at the end of subsequent incubation (Adhikari et al. 2003). Accordingly, L. acidophilus ATCC 4356 was multiplied to ~1010 cfu/g at the end of 18 hours of anaerobic incubation at 37°C.

2.2.2.Probiotic yoghurt production

First of all, the fat and dry matter ratio of buffalo milk was determined. The fat ratio of cow milk was taken as the basis and the fat ratio of buffalo milk was standardized with fresh cream and the non-fat dry matter ratio was standardized with non-fat milk powder. Milk standardized to be 12% with nonfat dry matter adding was heat treated at 90 °C for 10 minutes and then cooled to 43 °C. Milk cooled to fermentation temperature was inoculated with 0.5% (w/v) inulin, 2% (w/v) yoghurt starter culture and probiotic bacteria as specified in and fermented at 43 °C until its pH reached 4.6. As a result of incubation, the temperature of the yoghurt was reduced to 4 °C and its curd was broken. Yoghurt samples, whose curd was broken and cooled to 4 °C, were stored at this temperature for 24 hours. The milk cooled to fermentation temperature was inoculated with 0.5% (w/v) inulin and 2% (w/v) yogurt starter culture as specified with the encapsulated form of probiotic bacteria and fermented at 37 °C until its pH reached 4.6. As a result of incubation, the temperature of the yogurt was reduced to 4 °C and its curd was broken down. The yogurt samples, whose curd was broken down and cooled to 4 °C, were stored at this temperature for 24 hours.

2.2.3. Encapsulation of probiotic bacteria into alginate

Alginate microcapsules were obtained using a modified encapsulation method (Krasaekoopt et al. 2003; Ortakcı 2010). According to this method, 20 mL of *Lb. acidophilus* culture concentrate, which was multiplied to ~10^7 cfu/ml, was slowly added to 80 mL of sterile alginate solution containing 2.5% sodium alginate using a magnetic stirrer for approximately 10 minutes and immobilized. The alginate-bacteria suspension we obtained was extruded dropwise into 500 mL of 0.2 M $CaCl_2$ solution, which allows alginate to form spherical beads in the gel, using a sterile 21 G syringe. When the droplets were dropped into the $CaCl_2$ solution, alginate encapsulated the cells by trapping them in a 3D lattice structure as a result of cross-ionic bonding and gel spheres (microcapsules) were formed (Krasaekoopt et al. 2003). In the formation mechanism of calcium-alginate microspheres, the functional properties of alginate, which is a linear heteropolysaccharide composed of D-mannuronic acid and L-gluronic acid, are closely related to the composition and frequency of L-gluronic and D-mannuronic acid. Divalent cations such as Ca^{2+} preferentially bind to the L-gluronic acid polymer and alginate encapsulates the cells in a 3D lattice structure as a result of cross-ionic bonding (Krasaekoopt et al. 2003). Finally, the filtration process was carried out using Whatmann 4 filter paper to obtain the microcapsules in the $CaCl_2$ solution. The filtered microcapsules were stored in a sterile petri dish at 4°C to be added to the freezing on the same day.

2.2.4. Determination of microcapsule dimensions:

A 250 mL graduated cylinder was used to determine the dimensions of the microcapsules. 100 capsules were added to a cylinder containing 20 mL of pure water, the volume increase was noted, and then the diameter was calculated from the sphere volume formula based on the volume of 1 capsule. Thus, the average size of a capsule was determined. In order to determine the dimensions of the capsules obtained by coating the *Lb. acidophilus* ATCC 4356 strain with 2.5% sodium alginate, 50 capsules were added to a 25 mL graduated cylinder containing 10 mL of pure water, resulting in a volume increase of 0.75 mL. Accordingly, the approximate volume of 1 capsule was determined as 0.015 mL. Reid et al. (2005) obtained beads of approximately 3 mm in size in their studies in which they performed microencapsulation of probiotics by extrusion method using whey proteins as coating material.

Volume of a sphere $V = \frac{4}{3}\pi r3$

$$0{,}015 = \frac{4}{3} \times 3{,}14 \times r3 \quad (1)$$

$$r3 = 0{,}00358 \rightarrow r = 0{,}153 \text{ cm} \quad R = 2r \rightarrow 2 \times 0{,}153 = 0{,}306 \text{ cm} \quad (2)$$

2.2.5. Production of Yogurt Ice Cream Samples

After adding stabilizer (0.5%), emulsifier (0.25%) and sugar (18%) to the yogurt in the ice cream mix tank, the mix was heated to 70 ° C and kept at the same temperature for 10 minutes. The mixed samples were then cooled to 42°C and encapsulated probiotic bacteria (10^9 cfu/ml milk) were added and kept for 30 minutes, and the mixture temperature was then cooled to 5°C and kept in the tank for 6 hours before being turned into ice cream in an ice cream machine and packaged. The packaged yogurt ice creams were taken to the hardening rooms. In the study, ice cream samples containing *Lb. acidophilus* were treated with probiotic bacteria in 5 different treatments (free probiotic bacteria+inulin, encapsulated probiotic bacteria+inulin, without probiotics, free probiotic bacteria, encapsulated probiotic bacteria) and a 2× 5 factorial design with two replications was set up and conducted according to a completely random trial plan. *Lactobacillus acidophilus* was provided in lyophilized form in its original package.

2.2.6.Determination of fatty acids

Fat samples will be obtained by ether extraction in the ice cream sample. Then, methyl ester of the sample was prepared. Identification of fatty acids was made by gas chromatography according to retention times. Fatty acid standards will be used to determine the retention times of fatty acids. Injection and detector temperatures were made at 250 and 260 °C, respectively. Temperature program; the oven was kept at 140 °C for 1 minute and then increased by 4 °C per minute and kept at 240 °C for 5 minutes. Arithmetic means and standard deviations of the percentage (%) areas of the peaks in the chromatograms obtained in two parallels were calculated and given in tables (da Silveira et al.,2017).

2.2.7.Determination of Volatile Compounds

Volatile compounds of yogurt ice cream samples were performed as described by Condurso et al. (2008). Extractions were performed by solid phase microextraction (SPME) using 50/30µm thick Sulpeco® divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS) fibers. For this, 11 g of each sample was taken and transferred to a clear bottle and 10 mL of saturated NaCl aqueous solution was added to the bottle at 40 ° C. During the extraction, the sample was continuously stirred with a magnetic stir bar (750 rpm speed). Identification of volatile compounds was performed by GC-ME (Gas Chromatography - Mass Spectrometry). (Condurso et al. 2008).

2.2.9.Texture analysis in ice cream

Texture profile analysis (TPA) in yogurt ice creams was determined by modifying the method used by El-Nagar et al. (2002) using the TA.XT Plus texture analyzer (Stable Microsystems, Godalming, Surrey, UK). Texture analysis of yogurt ice creams was performed after samples taken from the deep freezer were kept at 15 °C for 15 minutes. The analysis was performed using a 5 mm cylinder probe and the test speed was 1 mm/s, the waiting time was 5 s, the trigger force was 50 kg, and the distance was 25 mm. The hardness values of the yogurt ice cream samples were expressed in N (Newton).

2.2.8. Determination of microscopic appearances of ice cream samples

A microscopic image analysis method was developed based on the studies conducted by Küçükçetin et al. (2009) and Körzendörfer et al. (2018). In this study, real-scale images were used (74 and 171 pixels/cm, respectively). However, an image scale of 100 µm was used in this study (15,198 pixels/cm). Approximately 0.5 mL of mixed yogurt was transferred to a microscope slide (2.5 cm × 7.5 cm) equipped with 2 microscope slides (2.5 cm × 2.5 cm × 0.175 ± 0.015 mm) fixed with nail polish at each end. This created an area of 2.5 cm × 2.5 cm where the yogurt spread with an average thickness of 0.175 ± 0.015 mm. Two slides were prepared for each yogurt. As soon as the yogurt microscope slides were prepared, they were observed using an optical microscope with a 10x objective (Olympus BX51; Olympus, Center Valley, PA). Five random gray images (resolution 300 dpi) were taken using an adapted camera (Photometrics CoolSNAPfx; Roper Scientific, Ottobrunn, Germany). This technique allowed us to see differences in gel density on the microscopic slides. Light passes through the slides from areas filled with loose (or no) gel, but is blocked by the dense compact gel. When the area is darker, the gel is denser. The darkest shapes in the images were considered as intact clusters (gel pieces) from the fixed yogurt (microgels), and their surface areas were measured by image analysis using ImageJ2 software (Küçükçetin et al., 2009).

Table 1. Volatile Analysis Results of Yogurt Ice Cream Samples(%)

Treatment	Ethanol	2-3-bütan dion	Heksanal	Cyclopentacyclohexane	1-bütanol 3-methyl	2-bütanon 3-hydroxyl	1-heksanol	Maltol	Cyclohexane	Asetic acid	2-3-bütan diol	Butanoic acid	Silenadiol	Hexanoic acid
CC	0.67 ±0.03	2.77 ±0.10	2.15 ±0.38	4.31 ±0.85	2.91 ±0.77	3.28 ±0.77	4.06 ±0.85	nd	4.72 ±0.87	11.62 ±1.13	9.14 ±1.15	2.82 ±0.85	3.21 ±0.88	6.02 ±0.87
C+P	0.32	1.61 ±0.05	5.04 ±0.85	4.77 ±0.95	4.52 ±0.98	3.19 ±0.68	5.04 ±1.35	10.14 ±1.83	4.52 ±0.78	4.68 ±0.77	1.61 ±0.35	3.89 ±0.89	0.61 ±0.22	7.24 ±0.98
C+P+I	0.44 ±0.003	3.79 ±0.35	2.74 ±0.45	4.37 ±0.77	1.03 ±0.35	7.22 ±1.82	2.74 ±0.56	1.41 ±0.35	3.72 ±0.58	6.78 ±0.85	0.66 ±0.18	2.63 ±0.38	nd	4.64 ±0.77
C+EP	nd	10.52 ±1.35	2.73 ±0.35	nd	nd	nd	nd	nd	nd	12.46 ±1.35	1.90 ±0.77	1.85 ±0.88	nd	2.72 ±0.55
C+EP+I	11.56 ±0.35	4.71 ±1.70	nd	3.01 ±0.56	4.41 ±1.05	20.85 ±2.95	4.37 ±0.97	nd	nd	7.39 ±1.15	nd	nd	nd	5.54 ±0.72
BC	3.44 ±0,27	nd	3.86 ±0,.55	4.85 ±0.87	nd	nd	nd	nd	4.85 ±0.73	nd	nd	4,11 ±0.91	nd	6.61 ±0.76
B+P	0.56 ±0.02	6.77 ±1.35	4.41 ±0.77	nd	1.13 ±0.35	20.34 ±1.35	3.31 ±0.78	nd	nd	13.20 ±1.35	6.77 ±0.98	6.12 ±0.88	nd	nd
B+P+I	0.76 ±0.03	0.39 ±0,05	0.35 ±0.03	nd	nd	18.43 ±1.53	nd	nd	nd	18.09 ±1.75	nd	6.20 ±0.66	nd	7.74 ±0.66
B+EP	0.48 ±0.02	nd	2.01 ±0.75	nd	nd	17.50 ±1.35	4.64 ±0.95	nd	nd	13.04 ±1,05	nd	5.81 ±0.95	nd	nd
B+EP+I	1.60 ±0.04	nd	2.21 ±0.35	nd	4.11 ±0.88	10.02 ±0.95	22.78 ±2.35	nd	nd	nd	nd	nd	nd	nd

CC: Plain Cow Yogurt Ice Cream (Control), C+P: Cow milk+Probiotic Bacteria C+P+I:Cow milk+ Probiotic Bacteria C+EP: Cow Milk+ Encapsulated Probiotic+, C+EC+I: Cow milk+Encapsulated Bacteria + Inulin, BC: Plain Buffalo (Control), B+P: Buffalo milk+Probiotic Bacteria, B+P+I: Buffalo Milk +Probiotic + Inulin, B+EC: Buffalo milk+Encapsulated Bacteria, B+EI+I: Buffalo milk +Encapsulated Probiotic + Inulin nd:not dedected

2.2.10. Statistical analysis in ice cream

The study was established and conducted according to the Completely Randomized Trial Plan with 2 different milks (cow and buffalo)x5treatment(plain,probiotic,enkapsulated probiotic,probiotic+inulin and encapsulated probiotic+inulin) with 2 replications. The obtained data were subjected to variance analysis using SPSS 20 package program (SPSS 20 for Windows, SPSS Inc., USA) and the mean values of the main variation sources that were significant were analyzed with Duncan multiple comparison test.

3. Results and discussions

3.1. Volatile Compound Analysis Results of Ice Cream Samples

The volatile compound composition of yogurt ice cream samples made by adding probiotic encapsulated bacteria was shown in Table 1. As can be seen from the table, the ethanol rate of yogurt ice cream samples added with encapsulated probiotic bacteria and inulin was found to be at the highest level (11.56%). However, only the ethanol rate of cow yogurt ice cream samples added with encapsulated probiotic bacteria was found to be below the detectable level. The amount of ethanol found in yogurt samples added with probiotic bacteria by Kara (2023) was considerably higher than the findings of this research. This may be due to the fact that the processes applied during the production of ice cream from yogurt (heating and freezing) removed ethanol. The 2-3 butanediol rate of yogurt ice cream samples varied between below the detectable level and 10.52%. While the hexanol level was below the detectable level in cow yogurt ice cream samples added with encapsulated probiotic bacteria and inulin, it was found to be 5.04% in yogurt ice cream samples added with probiotic bacteria. Kara (2023) found the 1 hexanol ratio in probiotic yogurt samples to be between 0.32% in his study. Kara's findings are quite high in these research results. The cyclopentane ratio of the samples varied between nd and 4.85%. The 1 butanol 3 methyl ratio was found between nd and 4.52%. Kara (2023) found the 1 butanol 3 methyl ratio in probiotic yogurt samples to be between nd and 11.15%. This finding was higher in these research results. Generally, when probiotic bacteria are encapsulated, some volatile components decrease and some increase. In particular, the 2 butanol 3 hydroxyl aromatic component was found to be significantly high (20.85%) in encapsulated probiotic bacteria and inulin-added cow yogurt ice cream samples. The maltol ratio in probiotic yogurt ice cream samples was determined to be as high as 10.14% in probiotic bacteria-added yogurt ice cream samples. In plain buffalo yogurt ice cream samples, the cyclohexane ratio was found to be at the highest level as4.85%. The acetic acid ratio in yogurt ice cream samples varied between nd and 18.09%. Buffalo yogurt ice cream with probiotic bacteria and inulin added contained the highest level of acetic acid. While 2-3 butanediol could found not be detected in some samples, 2-3 butanediol ratio was detected at a high level of 9.14% in plain cow yogurt ice cream samples. The butanoic acid ratio of yogurt ice cream samples varied between nd and 6.20%. The cilenodiol ratio of yogurt ice cream samples varied between nd and 3.21%. Cilenodiol could not be detected in many samples (8 samples). Generally, hexanoic acid was detected in all of the cow probiotic yogurt ice cream samples, while hexanoic acid was detected only in the probiotic bacteria and inulin added samples of buffalo yogurt ice cream samples. Kara (2023)'s research on probiotic yogurt and the probiotic yogurt ice cream volatile compound results obtained in this study were found to be significantly different. It was concluded that many volatile components (benzyl alcohol, cyclohexane2-Methyl-5,1-butanol 3-methyl, ethanol) could not pass into yogurt ice cream during the production of ice cream from yogurt and therefore these volatile components could not be detected in yogurt ice cream. In this case, it was concluded that the main flavor and volatile components of ice cream originated from sugar and milk. In the study conducted by Chamari et al. (2024), they found the acetone ratio as

23.55%, the ethanol ratio as 15.47%, the 3-methyl butanal ratio as 4.95%, the 2-3 butanedione ratio as 5.69%, the 3-methyl 1 butanol ratio as 5.24% and the 1 hexane ratio as 4.82% in samples made with Lactobacillus casei probiotic ice cream. On the other hand, the researchers found the acetone ratio as 17.97%, the ethanol ratio as 9.76%, the acetaldehyde ratio as 7.48.3% and the methyl 1-butanol ratio as 4.81% in the symbiotic ice cream samples they made with a mixture of Lactobacillus casei and pyycoerythrin. The ethanol ratio findings of Chamari et al (2024) were found to be parallel to the highest ethanol ratio (11.56%) found in this study and quite higher than other samples. The 2-3 butanedione value found in probiotic ice cream by Chamari et al (2024) iwas parallel to the findings of this research. 3-methyl butanal, 2-3 butanedione, 1 hexene, acetaldehyde and isobutyraldehyde found in probiotic and symbiotic ice cream samples by Chamari et al (2024) were not among the volatile compounds detected in this study.

3.2. Fatty Acid Results Of Ice Cream Samples

Fatty Acid Analysis Results of Yogurt Ice Cream Samples were given in Table 2.

Low-chain fatty acids such as butyric, caproic, caprylic and capric fatty acids are fatty acids that create flavor and aroma and were higher in yogurt ice cream samples made from buffalo milk than in samples made from cow milk (Table 2). Reducing saturated fat in human diet reduces the risk of cardiovascular disease and other diet-related disorders (Anonymous, 2011). In contrast, long-chain saturated fatty acids (palmitic and stearic acids), which are undesirable in terms of health, were lower in buffalo yogurt ice cream samples than in cow milk. The ratio of linoleic acid, which is an essential fatty acid, was lower in yogurt ice cream samples made from cow milk than in buffalo yogurt ice cream samples. The ratio of palmitic acid in ice cream samples added to cow and buffalo milk with probiotic bacteria and inulin was higher than in control samples. The ratio of palmitooleic acid and myristooleic acid in yogurt ice cream samples made from cow milk was found to be lower than in yogurt ice cream samples made from buffalo milk. When probiotic bacteria were encapsulated, the oleic acid ratio was higher than the control samples and the non-encapsulated probiotic bacteria addition. The stearic acid ratio of plain yogurt ice cream samples made from cow's milk was higher than the yogurt ice cream samples made with probiotic bacteria and inulin added to the milk. In buffalo yogurt ice cream samples, the addition of probiotic bacteria and inulin to the milk did not affect the stearic acid ratio (Table 2). Corradini et al. (2014) found in a study that adding palm oil and coconut oil to the ice cream mixture did not affect the fatty acid profile. The butyric, caproic and oleic acid ratios found in ice cream samples made from milk by Özdemir (2023) are parallel to the findings of this study. Özdemir (2023) found that the myristic and linoleic acid ratios in plain ice cream samples were higher than these research findings, and the palmitic and stearic acid ratios were lower.

3.3.Texture Analysis Results of Ice Cream Samples

Texture analysis results of yogurt ıce cream samples were given in Table 3.

The structure of ice cream depends on various factors such as the size of the ice crystals, the type and levels of fat, the protein structure and the processing methods. While the free water in ice cream acts as a plasticizer that increases properties such as flexibility and cohesion, higher fat content generally softens the texture by weakening the protein matrix. In contrast, low moisture and low fat levels increase hardness and gumminess. Protein content also plays an important role in cheese texture. A higher protein content has a positive effect on chewiness and hardness and a negative effect on gumminess. However, there are direct cause-effect relationships between certain biochemical changes and textural properties.

Table 2. Fatty Acid Analysis Results of Yogurt Ice Cream Samples

Treatment	C:4/0	C:6/0	C:8/0	C:10/0	C12/0	C14/0	C14/1	C16/0	C16/1	C18/0	C18/1	C18/2	C20/0
CC	1.55 ±0.32	1.18 ±0.18	0.76 ±0.03	1.63±0.55	2.03±0.32	8.82±0.32	0.71±0.32	37.82±0.32	1.02±0.32	17.17±0.32	22.05±0.32	1.9±0.327	0.39±0.32
C+P	1.85 ±0.33	1.46 ±0.35	0.96 ±0.04	2.15±0.72	2.94±0.82	9.94±1.32	0.78±0.03	35.96±3.38	1.09±0.35	1.05±0.32	2.89±0.52	2.06±0.42	0.31±0.03
C+P+I	1,41±0.32	1.11 ±0.17	0.77 ±0.03	1.67±0.43	2.46±0.72	8.46±1.32	0.70±0.32	40.29±4.32	1.02±0.32	16.42±2.34	20.98±4.37	1.84±0.33	0.35±0.03
C+EP	1,26 ±035	0.98 ±0.09	0.78 ±0.03	1.43±0.34	2.99±0.52	8.99±1.32	0.71±0.02	40.69±4.72	1.13±0.33	16.28±1.32	26.19±3.32	1.85±0.32	0.28±0.03
C+EP+I	0.58 ±0,03	0,48 ±0.09	0.39 ±0.03	0.82±0.32	1.03±0.32	3.76±0.75	0.27±0.02	40.04±5.38	0.47±0.03	13.11±2.33	26.35±4.32	1.66±0.32	0.25±0.03
BC	2.00 ±0.35	1.48 ±0.38	0.91 ±0.03	1.99±0.34	2.41±0.72	9.83±1.34	0.85±0.47	35.79±4.31	1.26±0.55	13.52±2.55	24.30±3.32	1.70±0.42	0.33±0.03
B+P	1.79±0.44	1.38 ±0.09	0.90 ±0.05	2.00±08	2.46±0.66	9.91±1.32	0.83±0.03	36.37±4.32	1.29±0.66	13.76±2.32	23.66±3.55	1.73±0.35	0.34±0.03
B+P+I	1,88 ±0,35	1.43 ±0.85	0.90 ±0.05	2.01±0.72	2.45±0.74	9.85±1.33	0.84±0.05	35.93±5.32	1.27±0.82	13.55±3.33	24.23±4.39	1.71±0.72	0.35±0.03
B+EP	1.75 ±0,38	1.38 ±0.71	0.89 ±0.03	2.05±0.32	2.50±0.82	9.54±1.74	0.81±0.06	38.34±5.24	1.32±0.36	14.29±2.35	21.92±3.32	1.87±0.78	0.30±0.03
B+EP+I	2.00 ±0.36	1.48 ±0.35	0.91 ±0.05	1.99±0.52	2.41±0.37	9.83±0.32	0.85±0.03	35.79±6.32	1.26±0.22	13.52±2.32	24.30±3.32	1.70±0.77	0.33±0.02

CC: Plain Cow Yogurt Ice Cream (Control), C+P: Cow milk+Probiotic Bacteria C+P+I:Cow milk+ Probiotic Bacteria C+EP: Cow Milk+ Encapsulated Probiotic+, C+EC+I: Cow milk+Encapsulated Bacteria + Inulin, BC: Plain Buffalo (Control), B+P: Buffalo milk+Probiotic Bacteria, B+P+I: Buffalo Milk +Probiotic + Inulin, B+EC: Buffalo milk+Encapsulated Bacteria, B+EI+I: Buffalo milk +Encapsulated Probiotic + Inulin **)**

Table 3. Texture Analysis Results of Yogurt Ice Cream Samples

Treatments	Adhesiveness	Springiness	Hardness	Cheviness	Gumminess	Chosiveness	Resilience
CC	-35.12±5.45	0.55±0.03	408.79±8.34	83.25 ±5.75	15.05 ±10.32	0.37±0.02	0.13±0.01
C+P	-24.62±3.32	0.78±0.02	101.77±5.32	36.95 ±3.20	47.34 ±.3.25	0.46±0.02	0.16±0.01
C+P+I	-19.07±4.35	0.78±0.02	140.85±7.32	47.05 ±3.32	60.28 ±3.52	0.42±0.02	0.14±0.01
C+EP	-57.23±7.32	0.66±0.03	716.34 ±25..72	239.83 ±28.45	364.29 ±35.32	0.51±0.02	0.23±0.02
C+EP+I	-12.91±3.32	0.63±0.22	210.66±6.32	50.77 ±4.32	80.80 ±7.70	0.38±0.03	0.13±0.02
BC	-17.32±4.32	0.53±0.35	233.30±7.77	42.74 ±4.45	80.47 ±7.32	0.35±0.02	0.13±0.01
B+P	-32.62±5.32	0.87±0.44	94.36±4.32	46.54 ±4.32	53.77 ±6.32	0.57±0.03	0.18±0.01
B+P+I	-25.69±3.32	0.68±0.08	306.55±14.82	91.68 ±7.78	135.67 ±15.32	0.44±0.02	0.15±0.01
B+EP	-35.24±4.44	0.77±0.12	285.61 ±15.32	106.05 ±8.32	137.63 ±14.74	0.48±0.02	0.17±0.01
B+EP+I	-66.08±6.32	0.67±0.03	912.47±78.56	253.52 14.89	377.92 ±27.32	0.41±0.01	0.20±0.01

CC: Plain Cow Yogurt Ice Cream (Control), C+P: Cow milk+Probiotic Bacteria C+P+I:Cow milk+ Probiotic Bacteria C+EP: Cow Milk+ Encapsulated Probiotic+, C+EC+I: Cow milk+Encapsulated Bacteria + Inulin, BC: Plain Buffalo (Control), B+P: Buffalo milk+Probiotic Bacteria, B+P+I: Buffalo Milk +Probiotic + Inulin, B+EC: Buffalo milk+Encapsulated Bacteria, B+EI+I: Buffalo milk +Encapsulated Probiotic + Inulin **)**

However, when probiotic bacteria were encapsulated, the hardness of the ice cream increased. However, when probiotic bacteria encapsulated in cow's milk were added, the hardness was found to be at the highest level of 716.34 g, while the hardness of the ice cream sample made by adding probiotic bacteria encapsulated in buffalo milk and inulin was found to be at the highest level (912.48 g). Inulin also was increased the hardness of low-fat or nonfat ice cream, probably due to its high degree of polymerization and long chain length. When inulin was added to water or milk, it has the ability to form inulin microcrystals due to its long chain length. These microcrystals interact to create a creamy texture (Niness, 1999) and probably increase the hardness of ice cream.Addition of 5% inulin to low-fat ice cream (Aime et al. 2001) or 5% modified starch to a low-fat yogurt-ice base (El-Nagar et al. 2002) increased the hardness compared to the high-fat control sample. In contrast, the addition of probiotic bacteria to the ice cream composition decreased the hardness of ice cream. Rheological properties such as mix viscosity affect the hardness of ice cream (Balthazar et al., 2017) because the increase in the viscosity of the mix and the decrease in molecular mobility in the mix are associated with ice crystal growth (Bahramparvar & Mazaheri Tehrani, 2011). The growth of ice crystal size and ice phase volume is associated with the increase in the hardness of ice cream (Javidi et al., 2016). Hardness is the resistance of food against any applied force. In other words, it is the resistance of solid foods to the pressure between the grinding teeth and semi-solid foods to the pressure between the tongue and the palate (Ertaş and Doğruer, 2010; Kesenkaş et al., 2012).. As can be seen from Table 3, the hardness values of ice cream samples varied between 34.75 g and 113.25 g. The effect of stabilizer type and addition ratio on the hardness values of ice creams is statistically significant (Çetin Abay, 2017), and in his study on the possibilities of using konjac plant gum as a stabilizer instead of salep in ice cream production, he found the effect of stabilizer addition ratio on the hardness value to be statistically significant. In the literature, the effect of volume increase on the hardness values of ice creams has been investigated and it has been determined that ice creams with high volume increase are softer (Muse and Hartel, 2004). It is reported that this situation is due to the compressible phase showing less resistance to the applied force in ice creams with high volume increase (Muse and Hartel, 2004).). The cohesiveness values had a similar trend with hardness, in agreement with Aime et al. (2001). It was determined that the ice cream with added whey protein and low fat was significantly more cohesive than the other samples. The lowest cohesiveness value was found in the normal ice cream sample, probably due to the milk fat content (Akalın et al.,2008). When probiotic bacteria encapsulated in cow milk were added, the gumminess was found at the highest level of 364.30, while the ice cream sample made by adding probiotic bacteria and inulin encapsulated in buffalo milk had the highest gumminess (377.92). Ice cream samples showed significant differences in terms of chewiness. When probiotic bacteria were encapsulated, it was increased the chewiness of the ice cream samples. The stickiness value of the ice cream samples varied between -66.08 g.min and -12.91 g.min. The stickiness value of the ice cream samples made by encapsulating probiotic bacteria was found to be lower than the other samples.

3.4. Microscopic Views of Ice Creams

In general, encapsulated bacteria are clearly seen in encapsulated yogurt ice cream samples. In control yogurt ice cream samples, only large fat globules and protein particles are seen together with bacteria. Large air bubbles are seen in samples added with cow probiotic bacteria. It was determined that when inulin was added together with probiotic bacteria, the size of fat particles and protein particles was reduced and a more homogeneous structure was formed. A more homogeneous structure was detected in samples added with inulin and encapsulated probiotic. When unencapsulated or encapsulated probiotics were added to buffalo milk, the microscopic appearance obtained showed a more homogeneous distribution compared to cow milk. This may be due to the addition of external cream to cow milk. When encapsulated probiotics were added to buffalo milk, a more homogeneous structure was obtained compared to cow milk yogurt ice cream**.**

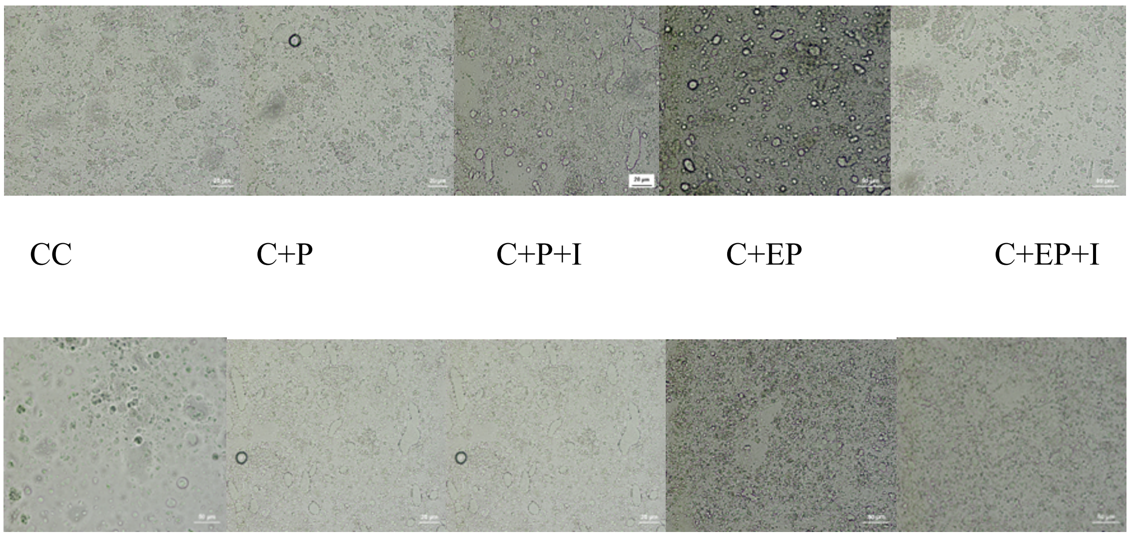

Figure 1. Microscopic appearances of probiotic and symbiotic ice cream samples

CC: Plain Cow Yogurt Ice Cream (Control), C+P: Cow milk+Probiotic Bacteria C+P+I:Cow milk+ Probiotic Bacteria C+EP: Cow Milk+ Encapsulated Probiotic+, C+EC+I: Cow milk+Encapsulated Bacteria + Inulin, BC: Plain Buffalo (Control), B+P: Buffalo milk+Probiotic Bacteria, B+P+I: Buffalo Milk +Probiotic + Inulin, B+EC: Buffalo milk+Encapsulated Bacteria, B+EI+I: Buffalo milk +Encapsulated Probiotic + Inulin

When the microscopic structures of the ice cream samples were examined, it was determined that the capsules in encapsulated bacteria could be easily seen (C+EP and B+EP). In addition, cow milk ice cream was more homogeneous than buffalo milk ice cream. It was determined that the addition of inulin to the ice cream formulation was increased the homogeneity of the ice cream.

4.Conclusions

In the study, the effects of adding encapsulated and free-form probiotic bacteria (*Lactobacillus acidophilus* ATCC 4356) to yogurt ice cream produced from cow and buffalo milk on some physical and chemical properties were investigated. Buffalo yogurt ice cream with probiotic bacteria and inulin had the highest level of acetic acid. It was concluded that many volatile components (benzyl alcohol, cyclohexane2-Methyl-5,1-butanol 3-methyl, ethanol) could not found into yogurt ice cream during the production of ice cream from yogurt. In this case, it was said that the main flavor and volatile components of ice cream originated from sugar and milk. Low-chain fatty acids such as butyric, caproic, caprylic and capric fatty acids are fatty acids that have flavor and aroma and were higher in yogurt ice cream made from buffalo milk than that of from cow milk. In contrast, long-chain saturated fatty acids (palmitic and stearic acids), which are undesirable in terms of health, were lower in buffalo yogurt ice cream samples than that of cow milk. When probiotic bacteria were encapsulated, the oleic acid ratio was higher than the control samples and the non-encapsulated probiotic bacteria addition. The stearic acid ratio of plain yogurt ice cream samples made from cow's milk was higher than the yogurt ice cream samples made with probiotic bacteria and inulin added to the milk.When probiotic bacteria were encapsulated, the hardness of the ice cream increased. When probiotic bacteria encapsulated in cow milk were added, the gumminess was found at the highest level. The stickiness value of the ice cream samples made by encapsulating probiotic bacteria was found to be lower than that of other samples. When probiotic bacteria were encapsulated, it was increased the chewiness of the ice cream samples. It was determined that the addition of inulin to the ice cream formulation was increased the homogeneity of the ice cream. The addition of encapsulated probiotic bacteria and inulin to ice cream made with buffalo milk had a positive effect on the fatty acid and aroma profile and texture properties of the ice cream.

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