Comparison of Characteristics and Fatty Acid Profiles of Butters Produced from Cream Fermented by LAB Strains Isolated from Traditional Iranian Butter

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Abstract: Due to high nutritional value of butter, extensive studies have been undertaken to improve its functional properties focusing on the reduction of cholesterol. One of the health benefits of probiotics is their ability to reduce serum cholesterol. The main aim of this study was to investigate the characteristics, Fatty Acid Profiles, rheological behavior and sensory properties of butter samples produced by adding adjunct culture with the ability of cholesterol assimilation. *Lactobacillus brevis*, *Pediococcus pentosaceus*, *Neoscardovia arbecensis* and *Lactobacillus pentosus* were used as adjunct culture in the process of industrial butter. Results shown that using all examined strains as an adjunct starter reduced the amount of saturated fatty acids in butter and increased the amount of unsaturated fatty acids. In the butter sample prepared with *Lactobacillus brevis*, the highest amount of unsaturated fatty acids was observed (33.2%) and was the softest butter in rheological properties. Sensory analysis showed a significant differences between all treatments (P<0.05) than control and the sample with *Neoscardovia arbecensis* was more acceptable than the others.

It is concluded that due to the high health effect properties of the examined strains and their acceptable technological characteristics, we do encourage dairy industries involved in butter production to incorporate these strains in their product and introduce novel functional butter to the market.

Keywords: Butter, Chemical characteristics, fermentation, free fatty acid (FFA) composition, rheological properties, sensory properties.

1. INTRODUCTION

Due to high nutritional value of butter, extensive studies have been undertaken to improve the functional properties as well as the reduction of cholesterol in this product [1]. With increased knowledge of people to consume healthy foods (probiotic products), the trend to producing such products is increasing. At present, many attempts have been made to use probiotics in dairy products such as cheese, yogurt and ice cream [2-4]. One of the health effects of probiotics is their ability to reduce serum cholesterol [5].

The taste, flavor and aroma of cultured dairy products such as butter, highly depends on the type of microorganisms used as starter or adjunct starter. However, it is more aromatic and tangy, as well as having longer shelf life and better texture than normal nonfermented butter [6]. Probiotics are able to utilize fat in cream and convert it to phospholipids, fatty acids, mono and diglycerides [7]. Butter produced with probiotics provide better sensorial properties [8].

A study on the isolation of probiotic strains from traditional Iranian butter has recently been conducted. Based on the findings, *Lactobacillus brevis*, *Pediococcus pentosaceus*, *Neoscardovia arbecensis* and *Lactobacillus pentosus* were identified as suitable candidates for probiotic strains that cause a reduction on cholesterol content on butter [9]. The primary objective of this study was to evaluate the physicochemical and rheological properties of butter produced by these strains. To the best of our knowledge, no study has been conducted on the physical and chemical properties of butter produced with the examined strains.

2. MATERIALS AND METHODS

2.1. Preparation of Butter Sample

Preparation of butter was carried out in Pak dairy factory (Tehran, Iran). The cream was separated from milk by the separator and standardized as 35% fat cream. It was then pasteurized for 15 seconds at 85 °

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C and divided into four sections. The pasteurized cream was then cooled to 37°C and inoculated with *L. brevis*, *P. pentosaceus*, *N. arbecensis* and *L. pentosus* (5% v/v) at 37 ° C for 24 hr. It was then cooled to 14 ° C and churned. Butter samples (500 gr) were packed and kept in the refrigerator until the day of experiment [10].

2.2. Chemical Analysis

All butter samples were analyzed for moisture, non-fat solids, fat contents [11], acid value [12], saponification value [13], iodine value [14], peroxide value [15] and pH (measured by pH meter model Inolab 720, Germany).

2.3. Determination of Free Fatty Acid Composition

Butter fat was extracted according to ISO 12966-2 [16]. The fatty acid methyl esters (FAMEs) were analyzed using a gas chromatograph (Young in, 6000, Korea) equipped with flame ionization detector and a capillary column (RTX column; 100 m, 0.2 mm, 0.3 mm). The oven temperature was programmed as follows: the initial temperature of 50°C was raised to 210°C at a rate of 5°C /min and was held at this temperature for 5 min, then was increased to 210°C and held at this temperature for 20 min. Helium was the carrier gas and sample injection volume was 1 µL. The identification of the peaks was achieved by retention times and by comparing them with authentic standards analyzed under the same conditions. Peak areas of triplicate injections were measured with an HP computing integrator. Results were expressed as (%) total fatty acids.

2.4. Rheological Properties

The rheological evaluation was performed by the rheometer (Anton Paar, MCR 301, Austria) and using parallel plate (25 mm plate diameter and 1 mm gap between them). The specimens were prepared by cylindrical coring device probe with a diameter of 12-15 mm and a height of 5 mm and were rested on a plate for 3 min after cutting. The device was equipped with a hot and cold water circulation system to supply the required temperature, which was set at 15°C for experiments with cold water. To determine the linear viscoelastic range, the strain amplitude sweep test was first performed on each sample at 1 Hz and the obtained strain range (γL) was determined at the boundary of the linear and nonlinear range for each sample. Then, the lowest γL value among the samples was used for angular sweep frequency test. Elastic modulus and viscose modulus were measured in the frequency range of 0.1 to 100 Hz [17].

2.5. Sensory Analysis

Some sensory properties of butter samples (appearance, flavor and consistency) were evaluated by 20 trained panelists (from 23 to 48 years old) using five point Hedonic scale test (by which 1 is very bad and 5 is very good) [18]. At a first meeting with all panelists, the project and the planned measures were explained and they were provided with the informed consent form. The mean scores obtained for each of the sensory properties was determined and the amount of differences between the samples and its significance were calculated using Duncan's multiple comparison method. All experimental protocols were carried out in accordance with the Iranian National Standard Number 162 [19]. The experimental protocols were approved by the Research Ethics Committee at Ferdowsi University of Mashhad on 15 Jun 2014 (code №: 31529).

2.6. Statistical Analysis

In order to evaluate the effect of the added isolated strain as adjunct culture on the cholesterol reduction of butter, all experiments were done in triplicate. The average rating of the three values was calculated for each sample (n = 3). One-way ANOVA was used to analyze the data following a general linear model in SPSS.14.0 (SPSS Inc., Chicago, IL, USA). Significance level was set at $p < 0.05$ to make comparisons between the means and obtain the standard deviation using Duncan test. All graphs were drawn by the software Microsoft EXCEL (version 2013).

3. RESULTS

3.1. Chemical Properties of Butter Samples

The chemical properties of butter samples prepared with selected strains are reported in the Table **1**. Statistically, there was significant difference ($P < 0.05$) between moisture, fat, non-fat dry matter, pH, acid value, iodine index and saponification value, among the samples. The fat content in butter samples prepared with *L. brevis*, *P. pentosaceus* and *L. pentosus* was significantly ($p < 0.05$) higher than the control, associated with a lower moisture content.

The analysis results of fatty acid profiles are presented in Table **2**. The iodine index and unsaturated fatty acids content of the butter sample prepared with

Table 1: Chemical Characteristics of Butters Prepared with Selected Strains

sample	Fat (%)	Non-fat solid (%)	Solid (%)	Moisture (%)	pH	Acid value	Iodine value	Saponification value	Peroxide value
control	69 ± 0.00^{a}	1.3 ± 0.06^{ab}	70.30 ± 0.05^{a}	29.70 ± 0.05^{b}	6.55 ± 0.05^{d}	0.28 ± 0.00^{a}	29.00 ± 0.10^{a}	224.8 ± 0.65^{a}	-
Butter sample prepared with *Lactobacillus brevis*	71 ± 0.00^{b}	1.48 ± 0.07^{bc}	72.48 ± 0.07^{b}	27.51 ± 0.07^{a}	4.27 ± 0.02^{b}	0.36 ± 0.00^{c}	31.72 ± 0.00^{d}	225.15 ± 0.65^{a}	-
Butter sample prepared with *Pediococcus pentosaceus*	71.16 ± 0.28^{b}	1.63 ± 0.15^{c}	72.8 ± 0.03^{bc}	27.20 ± 1.17^{a}	4.20 ± 0.02^{ab}	0.38 ± 0.01^{c}	30.54 ± 0.14^{c}	226.64 ± 0.00^{b}	-
Butter sample prepared with *Neoscardovia arbecensis*	69.67 ± 0.57^{a}	1.20 ± 0.00^{a}	70.87 ± 0.58^{a}	29.13 ± 0.58^{b}	4.37 ± 0.02^{c}	0.33 ± 0.01^{b}	29.44 ± 0.00^{b}	227.4 ± 0.64^{bc}	-
Butter sample prepared with *Lactobacillus pentosus*	72.17 ± 0.76^{c}	1.43 ± 0.06^{b}	73.6 ± 0.72^{c}	26.7 ± 0.72^{a}	4.15 ± 0.03^{a}	0.41 ± 0.01^{d}	31.55 ± 0.14^{d}	227.76 ± 0.00^{c}	-

Means within the treatments in the same column with the different letters differ significantly (*$p < 0.05$*).

Table 2: The Analysis Results of Fatty Acid Profiles

Fatty acids	Control (FA%)	Butter sample prepared with *L. brevis* (FA%)	Butter sample prepared with *P. pentosaceus*(FA%)	Butter sample prepared with *N. arbecensis*(FA%)	Butter sample prepared with *L. pentosus*(FA%)
C4:0	1.6	1.0	2.5	1.8	1.7
C6:0	3.3	1.1	2.0	3.7	1.7
C8:0	1.9	0.7	1.4	2.8	1.0
C10:0	5.5	2.1	2.8	6.9	2.9
C11:0	-	-	0.5	0.2	-
C12:0	6.0	2.6	3.6	6.9	3.5
C13:0	0.3	0.1	0.1	0.2	-
C14:0	16.7	9.8	11.7	16.4	11.4
C15:0	2.2	1.1	1.2	0.8	1.2
C16:0	33.1	37.2	32.0	28.0	32.5
C17:0	0.9	0.4	0.7	0.8	1
C18:0	4.3	10.1	8.3	6.0	9.3
C20:0	-	0.3	0.3	0.3	0.3
C22:0	0.3	0.3	0.1	0.1	0.1
Total Saturated	76.1	66.8	67.2	74.9	66.6
C10:1c	0.7	0.1	0.4	0.6	0.5
C12:1t	0.2	-	-	-	-
C12:1c	0.2	0.1	0.1	0.3	0.2
C14:1t	-	-	-	0.1	-
C14:1c	1.5	1.5	1.9	1.7	1.7
C15:1	0.3	0.1	0.1	1.4	0.2
C15:2	-	0.2	0.2	0.4	-
C16:1t	0.3	-	-	0.5	-

(Table 2). Continued.

Fatty acids	Control (FA%)	Butter sample prepared with *L. brevis* (FA%)	Butter sample prepared with *P. pentosaceus*(FA%)	Butter sample prepared with *N. arbecensis*(FA%)	Butter sample prepared with *L. pentosus*(FA%)
C16:1c	2.0	0.7	2.1	1.6	1.7
C17:1	0.5	-	0.7	0.5	0.7
C17:2	0.9	-	0.4	0.1	0.2
C18:1t	-	-	-	-	-
C18:1c	12.1	25.8	21.7	14.6	23.9
C18:2t	0.6	-	0.2	0.1	-
C18:2c	2	3.4	2.8	1.8	3
C20:1	0.3	0.6	0.1	0.2	-
C18:3t	-	-	0.5	-	-
C18:3c	-	0.6	0.5	0.4	0.8
C18:3y	-	-	-	-	-
C18:2,9c,11t	-	-	0.1	0.2	-
C18:2,10t,12c	-	-	-	0.1	-
C22:1	0.7	0.1	0.1	-	-
Total Unsaturated	22.3	33.2	31.9	24.6	32.9

L. brevis were higher than other samples (Tables **1**, **2**). The total saturated fatty acids percentage in all samples was less than the control and total unsaturated fatty acids percentage was higher than the control. In other words, stains used as an adjunct culture were able to reduce the amount of saturated fatty acids in butter and increase the amount of unsaturated fatty acids.

3.2. Rheological Properties

The curves for changes in the elastic modulus (G') and the viscous modulus (G'') against the strain are shown in Figure **1**. In all samples, G' is larger than G'' and, as other researchers have pointed out, it can be concluded that the butter is a viscoelastic solid [17, 28]. In the structure of butter, liquid fat has the role of continuous phase, which crystalline fat, fatty globule and water particles dispersed therein. The structure of the lipid membrane is resistant to stretching and has an elastic behavior, while the liquid fat around the fat globules has a viscose role and runs against high tensions. The high ratio of G' to G'' indicates that the elastic modulus has a higher contribution to dynamic viscosity than the viscosity modulus [29].

In order to increase the confidence coefficient for positioning in the linear viscoelastic range, the frequency scan test was performed at 0.05% strain and frequency 0.1-100 Hz. G' is shown to be directly correlated to a product’s hardness [7]. Despite the low dependence of G'' on the frequency compared to G', this can indicate a change from viscous to elastic behavior at high frequencies (Figure **2**). Such behavior is also expressed for butter by other researchers [29].

In order to better interpret the variation trend of the elastic and viscose modulus with the frequency,

Bohlin equation was used, the width from the origin and calculated slope for the lines are given in Table **3**.

The width from origin is related to the elastic modulus at 1 rad/s frequency and is as firmness of butter [17]. The reason for the significant difference between the widths from origin of the samples is probably due to the difference in the nature of the fat in samples.

3.3. Sensory Evaluation

As it is illustrated in Table **4**, comparing the average points about appearance, flavor and consistency show a significant difference among samples and control ($p<0.05$). Butter sample prepared with *N. arbecensis* gained the highest points about appearance, flavor and consistency. Oxidation is the main cause of reduction in the quality and shelf life of high-fat dairy products. Peroxide is produced by the reaction of oxygen with unsaturated fatty acids, which leads to decomposition

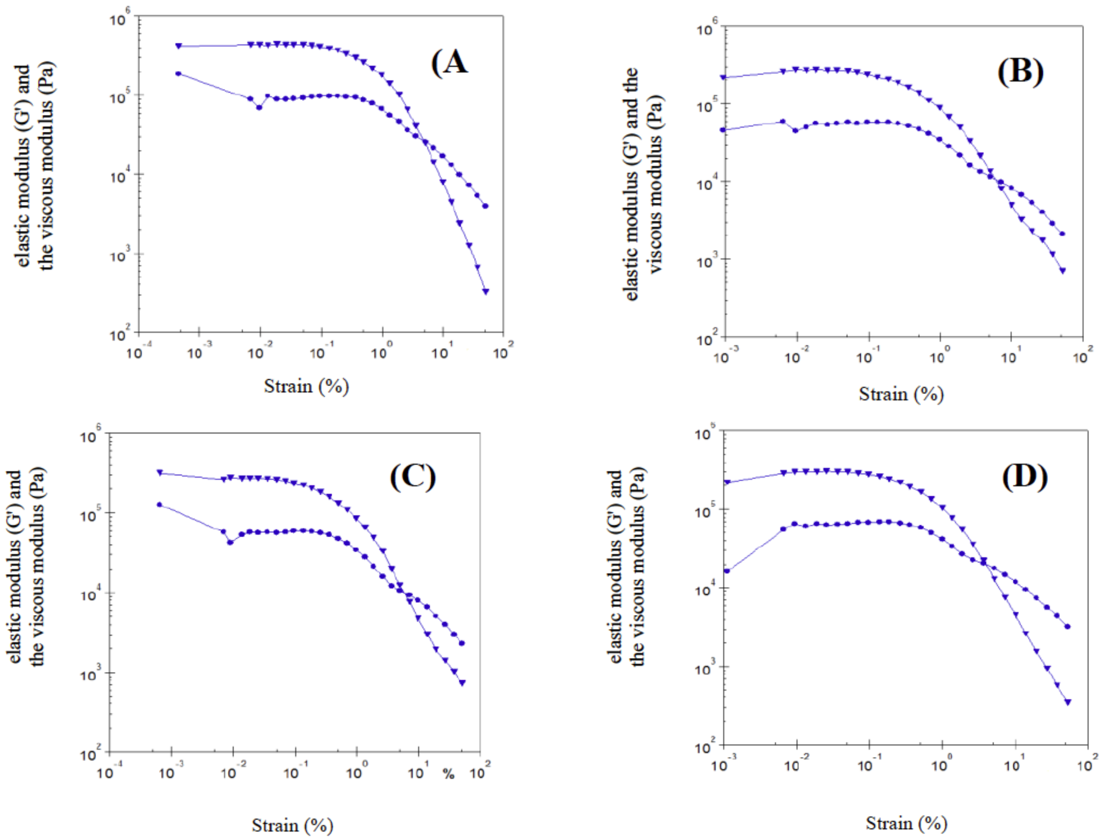

Figure 1: The curves for changes in the elastic modulus (G') and the viscous modulus (G'') against the strain in frequency 1 Hz (▾elastic modulus (G') and • viscous modulus (G'')) **A**) butter sample prepared with *Lactobacillus brevis.* **B**) Butter sample prepared with *Pediococcus pentosaceus.* **C**) Butter sample prepared with *Neoscardovia arbecensis.* **D**) Butter sample prepared with *Lactobacillus pentosus.*

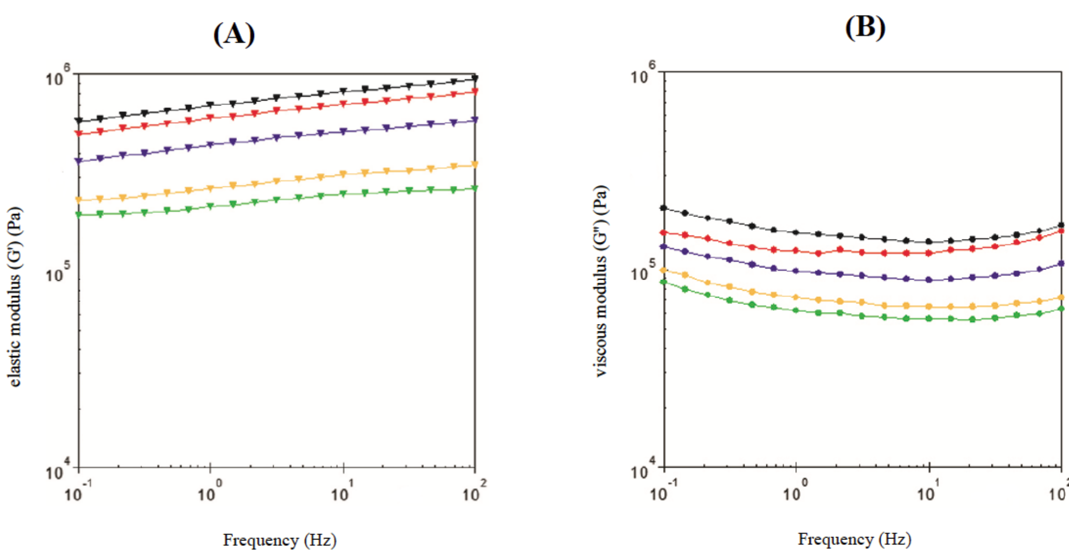

Figure 2: A) The curves for changes in the elastic modulus (G') against the frequency in strain 0.5% (▾elastic modulus for control, ▾ elastic modulus for butter sample prepared with *Neoscardovia arbecensis,* ▾elastic modulus for butter sample prepared with *Pediococcus pentosaceus,* ▾elastic modulus for butter sample prepared with *Lactobacillus pentosus,* ▾elastic modulus for butter sample prepared with *Lactobacillus brevis.*) **B**) The curves for changes in the the viscous modulus (G'') against the frequency in strain 0.5% (• viscous modulus for control, • viscous modulus for butter sample prepared with *Neoscardovia arbecensis,* • viscous modulus for butter sample prepared with *Pediococcus pentosaceus,* • viscous modulus for butter sample prepared with *Lactobacillus pentosus,* • viscous modulus for butter sample prepared with *Lactobacillus brevis.*)

Table 3: Equation of Lines Obtained, Width of Origin and Slope for Butter Samples

butter sample prepared with	Curve Line Equation G'	slope	width from origin
control	Log G´= 0.02 logω+ 5.95	0.02[a]	5.95[e]
Lactobacillus brevis	Log G´= 0.03 logω+ 5.29	0.03[c]	5.29[a]
Pediococcus pentosaceus	Log G´= 0.02 logω+ 5.58	0.02[b]	5.58[d]
Neoscardovia arbecensis	Log G´= 0.02 logω+ 5.71	0.02[b]	5.71[b]
Lactobacillus pentosus	Log G´= 0.03 logω+ 5.37	0.03[c]	5.37[c]

Means within the treatments in the same column with the different letters differ significantly ($p < 0.05$).

Table 4: Sensory Evaluation of Butters

Sample	Appearance	Flavor	consistency
Control	3.34±1.03[a]	2.43±0.87[a]	3.40±0.97[a]
Butter sample prepared with *Lactobacillus brevis*	3.37±0.9[a]	3.25±0.91[b]	3.28±0.95[a]
Butter sample prepared with *Pediococcus pentosaceus*	3.65±0.9[ab]	3.62±1.09[b]	3.65±0.78[ab]
Butter sample prepared with *Neoscardovia arbecensis*	4.12±1.03[b]	3.65±1.15[b]	4.06±0.94[b]
Butter sample prepared with *Lactobacillus pentosus*	3.65±1.15[ab]	3.46±1.10[b]	3.68±0.93[b]

Means within the treatments in the same column with the different letters differ significantly ($p < 0.05$).

of fatty acids and carbonyls production, with unpleasant flavor and aroma in butter [30].

4. DISCUSSION

As the present study showed, butter samples prepared by adding adjunct culture with the ability of cholesterol assimilation exhibit high-end healthy product. The results of chemical properties obtained from this study are consistent with the results of previous studies [7, 20, and 21]. Ewe and loo, evaluated the physicochemical and rheological properties of butter produced by *L. helveticus* fermented cream. They suggested that the *L. helveticus* due to having a hydrophobic S-layer that had better binding to fat globules, retained a higher composition of fats in the resulting butter upon churning [7]. Fermentation significantly ($p < 0.05$) affected the acid value where a 46% higher acid value than the control was observed in butter sample prepared with *L.pentosus*. The higher acid value suggested a higher number of free fatty acids were generated upon triacylglycerol hydrolysis by these strains. Fermentation activity of the lactic acid bacteria in cream could have produced organic acids which in turn lead to increase the acid value and decrease pH in the butter [7].

Regarding the peroxide index, because this experiment was carried out over a short period of storage time, this indicator was within the standard range or even lower than that for all samples.

The analysis results of fatty acid profiles of this study were similar to the results of Bezerra *et al*., which investigated the effect of adding probiotic bacteria on the fatty acid profile of cheese, as well as Florence *et al*. who studied the effect of adding probiotic bacteria on the fatty acid profile of the local milk and sterilized milk [22, 23].

Percentage of short fatty acids (Butyric acid, Caproic acid, Caprylic acid and Capric acid) in control sample and examined butter samples prepared with *L. brevis, P. pentosaceus*, *N. arbecensis, L. pentosus* were 12.3, 4.9, 8.7, 15.2 and 6.6 respectively. The results of similar published works on the impact of fermentation on changing fatty acid profile are inconsistent. Santos Junior *et al*. have noted that adding starter to dairy products releases fatty acids due to lipolysis, and due to the function of lipases derived from the starter on the positions 1 and 3 of triglycerides and on the other hand, considering the fact that the majority of short and intermediate chain fatty acids are at the position 3 of triglycerides in milk fat, release and reduction of short chain fatty acids are further increased [24]. Short chain fatty acids have low molecular weight and high solubility, so they can easily enter buttermilk and thus be lower in butter. On the other hand, as the butyric acid and caproic acid are soluble in water, the solubility increase with decreasing pH and the tendency to transfer to buttermilk increases [25]. While Ekinci *et al*., investigated the effect of cream fermentation with different probiotic bacteria as well as the addition of sunflower, soybean and hazelnut oils on fatty acids of cream and concluded that short-chain fatty acids such as butyric, caproic and capric acids were significantly affected by used culture media in sour cream and they increased compared to the control group where no fermentation took place. They also reported that the rate of change varied depending on the type of culture used and it was higher for sour cream with *L. acidophilus* than the others. However, unsaturated long-chain fatty acids were significantly affected by the type of oil added to them [26].

Loric acid, myristic acid, and palmitic acid are known as atherogenic factors, and their high level in food have a high risk of cardiovascular diseases [27]. Total of these three fatty acids for control sample, and examined butter samples prepared with *L. brevis, P. pentosaceus*, *N. arbecensis, L. pentosus* were 55.8, 49.6, 47.3, 51.3 and 47.4 respectively. In other words, fermentation of butter with examined strains lead to reduction of these fatty acids.

In terms of rheological properties, results from the present study showed that the G' and G'' values of butter sample prepared with the examined strains were lower than the control, suggesting that butter samples prepared with these strains were softer than the control at room temperature. The G' was greater than G'' in the range of the desired frequencies. For all samples, the viscous module decreases with increasing frequency while the elastic modulus increases.

Butter is a plastic material and its properties depend on crystallization degree of milk fat. The milk fat, which is the main ingredient in butter, contains a mixture of triglycerides with different melting points (-40 - +40 °C) and depending on the type of fatty acids, this parameter can change, as liquid fat content increases by increasing the unsaturated fatty acids with low melting point, fat will be more fluid [31].

The unsaturated fatty acid content of butter sample prepared with *L. brevis* was the highest (33.2%) (Table **2**) and was the softest butter (lower width from origin) (Table **3**). In other words, increasing the liquid fat content of the butter led to an increase the firmness. The other reason for the low width from origin and the increased softness in butter samples can be related to high amount of moisture and the increase in the diameter of water particles.

There was also a significant difference ($p < 0.05$) between the specimens in relation to the calculated slope (Table **3**). The small amount of slope showed a lower dependency of modulus to frequency and in fact showed a more stable structure of the butter. Fat viscosity depends on the amount of unsaturated fatty acids and chain length of fatty acids, the slope decreases by decreasing unsaturated fatty acids and increasing short chain fatty acids. Among the samples of butter prepared with the examined strains, butter sample prepared with *N. arbecensis* has the lowest amount of unsaturated fatty acids and the highest amount of short chain fatty acids (Table **2**). In other words, butter prepared with *N. arbecensis* has more strength than other samples after the control sample. Undesirable flavor and aroma such as bitterness and rancidity have not been detected by the panelists in this study.

5. CONCLUSION

Butter produced using fermented cream can scarcely be found in the market. Butter produced from fermented cream with the examined strains contained a higher amount of fat, acid value, unsaturated fatty acids and a softer texture than the control. Due to the high health effect properties of the examined strains and their acceptable technological characteristics, we do encourage dairy industries involved in butter production to incorporate these strains in their product and introduce novel functional butter to the market.

ABBREVIATIONS

FFA = free fatty acid

FAME = fatty acid methyl ester

ANOVA = analysis of variance

G' = elastic modulus

G'' = viscous modulus

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