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ORIGINAL PAPER

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IMPROVEMENT OF VIABILITY OF PROBIOTIC BACTERIA, ORGANOLEPTIC QUALITIES AND PHYSICAL CHARACTERISTICS IN KEFIR USING TRANSGLUTAMINASE AND XANTHAN

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ABSTRACT

Background. Using kefir as a probiotic food carrier has many benefits. At the same time, it is considered an appropriate product for the dairy industry. The aim of this study was to evaluate the effect of xanthan gum and transglutaminase enzyme on the viability of probiotics and the organoleptic qualities and physicochemical characteristics of kefir.

Material and methods. Three levels of transglutaminase enzyme (50, 100 and 150 ppm), and xanthan gum (0.05%, 0.1% and 0.2%) were used. Sensory and physicochemical properties and viability of probiotic bacteria were measured over 2 weeks of storage at 4°C.

Results. By increasing the amounts of xanthan gum and transglutaminase, the viscosity of the samples was increased and syneresis was reduced significantly ($P < 0.05$). The kefir sample containing 150 ppm enzyme and 0.2% gum had the highest number of probiotic bacteria. Moreover, the highest organoleptic scores were found for this sample.

Conclusion. It can be concluded that adding 150 ppm transglutaminase and 0.2% xanthan improved the viability of probiotics and the physical and organoleptic characteristics of kefir.

Keywords: kefir, probiotic, transglutaminase, xanthan

INTRODUCTION

In recent years, the consumption of functional foods, including probiotic products, has become more popular. Probiotic bacteria have health-promoting features and the consumption of these bacteria is one way to reconstruct and modify intestinal microflora (Lourens-Hattingh and Viljoen, 2001). The health effects of probiotics include improvement of lactose intolerance, reduction of side effects associated with antibiotics, prevention of intestinal infections by the production of organic acids and antibacterial agents, prevention and treatment of cancer, strengthening the immune system, and the reduction of cholesterol (Aryana and McGrew, 2007).

Kefir is probiotic fermented milk, which is obtained by the inoculation of milk with kefir grains. During fermentation, lactic acid, CO_2, acetic acid, acetaldehyde, acetoin and diacetyl are produced, which produce the unique organoleptic properties of this product (Grønnevik et al., 2011; Yovanoudi et al., 2013). Kefir has been used for many years in Russia in order to control or treat several diseases. More recently, kefir consumption has become popular in other

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parts of the world, such as South East Asia, Eastern and Northern Europe, North America and Japan, due to its nutritional and therapeutic properties (Khurana and Kanawjia, 2007).

The consumption of low-fat or fat-free dairy products has increased in recent decades, due to the adverse effects of excess fat consumption on human health. Consumers demand low-fat dairy products which are nonetheless similar in taste and quality to full-fat products (Ünal et al., 2003). Increasing solids, not fat, of the milk, or adding natural or synthetic gums as stabilizers, are common and conventional methods used to improve the texture of low-fat dairy products. The quantities of these additives required to achieve similar proportions of solids to full-fat products can cause undesirable tastes and the overproduction of acids during storage, and can give the product a sandy texture (Özer et al., 2005). Given that the increase in dry matter can cause undesirable mouth feel, adding replacements such as transglutaminase enzyme and xanthan gum in small amounts may be important. It can also create better sensory properties than added dry matter. Meanwhile, the addition of transglutaminase, compared to the addition of dry matter, can lead to a reduction in the cost of production (Şanlı et al., 2011).

The microbial transglutaminase enzyme is one of the transferase enzymes which catalyzes the acyl transfer reaction between the gamma carboxyl glutamine and first type amines, such as the amine epsilon group of lysine, which causes the formation of new intramolecular and intermolecular cross links (Motokia and Segurob, 1998). These links can change the structure and function of proteins (Özer et al., 2005). Cross linking of milk proteins by microbial transglutaminase improves functional properties, including hydration ability, rheological properties, and emulsification (Şanlı et al., 2011). This network of different proteins also may affect the growth and viability of probiotics (Farnsworth et al., 2006). Microbial transglutaminase is an enzyme that causes not only decreasing pH during the fermentation, but also protein enrichment of milk for yogurt production (Bönisch et al., 2007a). Often, some compounds, such as non-fat dry milk, whey protein concentrate, and sodium caseinate are used to enrich milk protein for yogurt production, with the aim of obtaining yogurt with a desirable structure and light viscosity (Bönisch et al., 2007b). Casein, especially sodium caseinate, is the best substrate for microbial transglutaminase among the milk proteins. The ability for cross linking between the proteins in milk depends on their molecular structures (Şanlı et al., 2011). Transglutaminase makes the production of dairy products such as ice cream, cheese and yogurt with reduced fat levels, or solids instead of fat, possible (Motokia and Segurob, 1998).

Gums and hydrocolloids are polymeric materials which are dissolved or spared in water. They are added to food formulations in order to increase the viscosity, and many of them form gels at relatively low concentrations (Lee and Inglett, 2006). Some compounds, such as gelatin, pectin, inulin, and gums are used in order to improve the properties of yogurt (Amaya-Llano et al., 2008; Sahana et al., 2008). Xanthan gum is an anionic and hygroscopic polysaccharide which is applied as a setting agent for yogurt in low concentrations (Sikora et al., 2008). Kefir is not popular among Iranian people, as the texture is not considered acceptable. In this study, we tried to improve the consistency and organoleptic properties of kefir using xanthan gum and the transglutaminase enzyme and in order to raise its acceptability to people in Iran, by creating an appropriate texture similar to the yogurt. Hence, consumers can consume it with their food and they can benefit from its health-promoting properties.

MATERIALS AND METHODS

Materials

A kefir starter culture was purchased from Chr. Hansen Company (Denmark). The three DVS starters made by Christian Hansen were CHN-22, ABT-2 and LAF-4.

CHN-22 included *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris* and *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis.*

ABT-2 included *Lactobacillus acidophilus, Bifidobacterium bifidum* and *Streptococcus thermophilus.*

LAF-4 included *Kluyveromyces marxianus* subsp. *marxianus.*

Xanthan (Merck, Germany) and microbial transglutaminase (Activa YG, Ajinomoto, Japan) were used.

Starter preparation

DVS kefir starters were added into 1 L reconstituted skimmed milk according to the manufacturer's instructions and vortexed for 30–45 min (150 rpm). 2 ml of this mixture was added into 1 L pasteurized milk with 0.5% fat.

Kefir production

Milk with 10.5% solids not fat and 0.5% fat was pasteurized (90°C for 10 min). After cooling to 55°C, three levels of transglutaminase (50, 100 and 150 ppm) and xanthan (0.05, 0.1 and 0.2%) were added to milk. Additionally, a control sample (without the enzyme and gum) was produced. The kefir starter, prepared as described above, was added to milk at 35°C. The microbial population of the starter was 10^7 CFU/ml at the time of inoculation. The samples were incubated at 35°C. Incubation was stopped when pH reached 4.7. The kefir samples were stored at 4°C for two weeks. The treatments used in this study are shown in Table 1.

Table 1. The treatments of this study

Kefir sample	Transglutaminase, ppm	Xanthan, %
Control	–	–
1	50	0.05
2	50	0.1
3	50	0.2
4	100	0.05
5	100	0.1
6	100	0.2
7	150	0.05
8	150	0.1
9	150	0.2

Physicochemical analysis

Acidity was determined after mixing 10 mL of samples with 10 mL distilled water and titrating with 0.1 N NaOH using 0.5% phenolphthaelin, according to the AOAC method (AOAC, 2005). Syneresis was determined by centrifuging the kefir at 222 g for 10 min at 10°C, and it was expressed as volume of separated whey per 100 ml of kefir (Ünal et al., 2003). Determination of viscosity was conducted by Brookfield viscometer (DV-II+pro, USA) that was used at shear rate 58. The temperature of the kefir samples was 5°C (Ünal et al., 2003).

Probiotic bacterial count

MRS bile agar (Merck, Germany) was used for enumeration of *Bifidobacterium bifidum* and *Lactobacillus acidophilus*. The pour plate method was applied. Incubation was conducted anaerobically at 37°C for 72 h (Ghaleh Mosiyani et al., 2017).

Sensory evaluation

Sensory evaluation was carried out by 10 trained panelists. Organoleptic parameters included flavor, texture and overall acceptance. A ranking method was used. The parameters were rated: 5 (very good), 4 (good), 3 (moderate), 2 (bad) or 1 (very bad).

Statistical analysis

All experiments were carried out in triplicate. The data obtained was subjected to one way analysis of variance (ANOVA), followed by the Duncan's multiple range test to determine significant differences ($p < 0.05$) between samples, using the SPSS 18 software.

RESULTS AND DISCUSSION

Physicochemical characteristics

The acidity levels of the kefir samples are shown in Table 2. The acidity of the samples increased significantly ($P < 0.05$) during cold storage. This increase could be due to the production of lactic acid and other organic acids by lactic cultures (Joung et al., 2016). Sample 2 (containing 100 ppm enzyme and 0.05% gum) had the highest acidity on the 14th day and the lowest acidity was related to sample 5 (containing 100 ppm enzyme and 0.01% gum) on the first day. The increase in acidity in kefir when gum was applied was reported by Fuller (1999). In a similar study, commercially available starters were used and the acidity of yogurt samples was investigated during storage at 6°C. According to the results, titratable acidity of the samples increased (Kneifel et al., 1993).

Table 2. Acidity levels (Dornic Degree) of kefir samples during cold storage (mean ±SD)

Sample	First day	7th day	14th day
Control	53 ±2.11^{Cd}	59 ±3.27^{Bd}	66 ±2.13^{Ad}
1	56 ±1.26^{Cab}	62 ±2.37^{Bab}	68 ±2.37^{Aab}
2	57 ±2.01^{Ca}	62 ±4.03^{Ba}	67 ±3.31^{Aa}
3	56 ±1.33^{Cc}	61 ±2.23^{Bc}	67 ±1.13^{Ac}
4	53 ±2.16^{Cd}	55 ±2.12^{Bf}	65 ±2.18^{Af}
5	49 ±1.13^{Ce}	53 ±3.22^{Bh}	61 ±3.23^{Ah}
6	52 ±2.13^{Ce}	57 ±3.27^{Be}	68 ±1.16^{Ae}
7	52 ±2.26^{Ce}	59 ±1.15^{Be}	66 ±2.18^{Ae}
8	51 ±2.21^{Cg}	56 ±1.31^{Bg}	63 ±2.41^{Ag}
9	57 ±1.03^{Cbc}	61 ±2.02^{Bbc}	69 ±3.03^{Abc}

Values in the same column shown with similar lowercase letters are not significantly different.
Values in the same rows shown with similar capital letters are not significantly different.

Syneresis

The syneresis of the kefir samples is shown in Table 3. Syneresis of the samples increased significantly ($P < 0.05$) during cold storage. The control sample had the highest syneresis on the 14th day and the lowest syneresis was related to sample 9 (containing 150 ppm enzyme and 0.2% gum) on the first day. Other samples showed significant differences ($P < 0.05$). In a similar study, transglutaminase was added to milk. The results showed that adding transglutaminase after pasteurization of milk can decrease the syneresis of yogurt (Şanlı et al., 2011). Aryana and McGrew (2007) reported that the syneresis of yogurt increased with increasing acid production. Our findings were similar to these results.

Viscosity

The viscosity of the kefir samples is shown in Table 4. Sample 9 (containing 150 ppm enzyme and 0.2% gum) had the highest viscosity on the first day and the lowest viscosity was related to the control sample on the 14th day. In a similar study, Kuraishi et al. (2001) reported that the viscosity of set and stirred yogurt samples improved when transglutaminase was added.

Table 3. Syneresis of kefir samples during cold storage (mean ±SD), %

Sample	First day	7th day	14th day
Control	71 ±0.08^{Ca}	78 ±0.07^{Ba}	83 ±0.09^{Aa}
1	60 ±0.05^{Cb}	68 ±0.07^{Bb}	73 ±0.08^{Ab}
2	57 ±0.06^{Cc}	62 ±0.18^{Bc}	66 ±0.18^{Ac}
3	55 ±0.18^{Cd}	57 ±0.18^{Bd}	61 ±0.09^{Ad}
4	53 ±0.14^{Ce}	56 ±0.08^{Be}	61 ±0.09^{Ae}
5	50 ±0.08^{Cf}	54 ±0.08^{Bf}	56 ±0.08^{Af}
6	48 ±0.08^{Cg}	51 ±0.08^{Bg}	55 ±0.08^{Ag}
7	42 ±0.18^{Ch}	48 ±0.18^{Bh}	51 ±0.18^{Ah}
8	38 ±0.17^{Ci}	45 ±0.15^{Bi}	49 ±0.13^{Ai}
9	36 ±0.08^{Cj}	41 ±0.09^{Bj}	45 ±0.06^{Aj}

Values in the same column shown with similar lowercase letters are not significantly different.
Values in the same rows shown with similar capital letters are not significantly different.

Table 4. Viscosity (centipoise) of kefir samples during cold storage (mean ±SD)

Sample	First day	7th day	14th day
Control	4.08 ±1.04^{Ak}	3.92 ±0.14^{Bk}	3.72 ±0.24^{Ck}
1	166.8 ±0.17^{Aj}	121.9 ±0.82^{Bj}	110.7 ±0.26^{Cj}
2	174.28 ±0.11^{Ah}	136.11 ±0.48^{Bh}	125.35 ±0.78^{Ch}
3	186.8 ±0.67^{Ag}	151.9 ±0.62^{Bg}	140.7 ±0.70^{Cg}
4	269.49 ±0.75^{Af}	255.3 ±0.99^{Bf}	238.56 ±0.74^{Cf}
5	286.88 ±0.36^{Ae}	261.45 ±1.48^{Be}	246.033 ±0.97^{Ce}
6	299.6 ±0.12^{Ad}	278.7 ±0.45^{Bd}	258.3 ±0.14^{Cd}
7	419.02 ±0.27^{Ac}	393.71 ±0.45^{Bc}	362.32 ±1.05^{Cc}
8	435.12 ±0.50^{Ab}	412.74 ±0.27^{Bb}	386.86 ±0.05^{Cb}
9	462.47 ±0.46^{Aa}	442.65 ±0.17^{Ba}	412.78 ±0.28^{Ca}

Values in the same column shown with similar lowercase letters are not significantly different.
Values in the same rows shown with similar capital letters are not significantly different.

Transglutaminase increased viscosity by improving water holding capacity. The viscosity of the samples decreased significantly ($P < 0.05$) during storage. This decrease may be explained by microbial enzyme action on the casein micelle matrix during the storage period (Kosikowski, 1982).

Probiotic bacterial count

The population of probiotic bacteria in the kefir samples is shown in Table 5. Sample 9 (containing 150 ppm enzyme and 0.2% gum) had the highest number of probiotic bacteria on the first day. The lowest number was found in the control sample on the 14th day. Transglutaminase can protect probiotics from damage due to its low pH (Heidebach et al., 2009a), and this can be a suitable approach for the more effective application of probiotic bacteria in food products (Heidebach et al., 2009b). In a similar study, Farnsworth at al. (2006) reported that adding transglutaminase increased the viability of probiotic bacteria in goat's milk yogurt. The population of probiotics decreased significantly ($P < 0.05$) during cold storage, which could be due to the production of lactic acid and other organic acids by lactic cultures leading to decreasing pH and increasing acidity (Joung et al., 2016). The increasing redox potential and concentration of hydrogen peroxide was due to the metabolic activity of lactic acid bacteria, which can lead to a decrease in the number of bacteria during storage (Dave and Shah, 1997). In a similar study, Vasiljevic and Shah (2008) reported that the amount of probiotics in yogurt samples decreased during storage. Moreover, Pourahmad et al. (2011) found that the population of probiotic lactobacilli in soy milk kefir samples decreased significantly ($P < 0.05$) during cold storage.

Sensory characteristics

The flavor scores of kefir samples are shown in Table 6. The highest scores were related to sample 9 (containing 150 ppm enzyme and 0.2% gum) and sample 8 (containing 100 ppm enzyme and 0.05% gum) on the 7th day. Sample 1 and the control sample on the 14th day had the lowest flavor scores.

The scores for texture of the kefir samples are shown in Table 7. Sample 9 (containing 150 ppm enzyme and 0.2% gum) and sample 8 (containing 100 ppm

Table 5. Probiotic bacterial count in kefir samples during cold storage (mean ±SD), log CFU/mL

Sample	First day	7th day	14th day
Control	7.36 ±0.13^{Aj}	7.13 ±0.21^{Bj}	6.44 ±0.01^{Cj}
1	7.15 ±0.31^{Ah}	6.75 ±0.03^{Bh}	6.45 ±0.04^{Ch}
2	7.32 ±0.11^{Ad}	7.16 ±0.01^{Bd}	6.74 ±0.21^{Cd}
3	7.15 ±0.14^{Af}	6.91 ±0.09^{Bf}	6.44 ±0.08^{Cf}
4	7.12 ±0.21^{Ai}	6.96 ±0.11^{Bi}	6.14 ±0.31^{Ci}
5	7.24 ±0.21^{Ac}	6.88 ±0.01^{Bc}	6.78 ±0.08^{Cc}
6	7.16 ±0.01^{Ae}	7.06 ±0.07^{Be}	6.31 ±0.03^{Ce}
7	7.23 ±0.06^{Ag}	6.78 ±0.07^{Bg}	6.36 ±0.03^{Cg}
8	7.14 ±0.00^{Ab}	6.47 ±0.01^{Bb}	6.14 ±0.02^{Cb}
9	7.35 ±0.04^{Aa}	6.86 ±0.09^{Ba}	6.48 ±0.21^{Ca}

Values in the same column shown with similar lowercase letters are not significantly different.
Values in the same rows shown with similar capital letters are not significantly different.

Table 6. The flavor scores of kefir samples during cold storage (mean ±SD)

Sample	First day	7th day	14th day
Control	4.25 ±0.14^{Bgf}	4.75 ±0.23^{Agf}	3.12 ±0.15^{Cgf}
1	4.36 ±0.10^{Bg}	4.57 ±0.23^{Ag}	3.01 ±0.27^{Cg}
2	4.41 ±0.16^{Bef}	4.62 ±0.27^{Aef}	3.25 ±0.38^{Cef}
3	4.44 ±0.13^{Be}	4.69 ±0.12^{Ae}	3.36 ±0.24^{Ce}
4	4.52 ±0.39^{Bd}	4.82 ±0.16^{Ad}	4.14 ±0.26^{Cd}
5	4.55 ±0.12^{Bc}	4.81 ±0.14^{Ac}	4.23 ±0.37^{Cc}
6	4.63 ±0.09^{Bbc}	4.79 ±0.23^{Abc}	4.36 ±0.11^{Cbc}
7	4.65 ±0.05^{Bbc}	4.88 ±0.04^{Abc}	4.32 ±0.11^{Cbc}
8	4.76 ±0.36^{Bb}	4.91 ±0.37^{Ab}	4.36 ±0.06^{Cb}
9	4.89 ±0.03^{Ba}	4.95 ±0.29^{Aa}	4.53 ±0.13^{Ca}

Values in the same column shown with similar lowercase letters are not significantly different.
Values in the same rows shown with similar capital letters are not significantly different.

Table 7. The scores of texture of kefir samples during cold storage (mean ±SD)

Sample	First day	7th day	14th day
Control	4.65 ±0.12^{Af}	4.33 ±0.02^{Bf}	3.28 ±0.17^{Cf}
1	4.69 ±0.11^{Ai}	4.41 ±0.14^{Bi}	3.33 ±0.32^{Ci}
2	4.74 ±0.22^{Ah}	4.44 ±0.38^{Bh}	3.37 ±0.11
3	4.77 ±0.01^{Ag}	4.51 ±0.18^{Bg}	3.48 ±0.23^{Cg}
4	4.79 ±0.23^{Ae}	4.52 ±0.25^{Be}	4.14 ±0.14^{Ce}
5	4.81 ±0.18^{Ad}	4.55 ± 0.31^{Bd}	4.23 ±0.17^{Cd}
6	4.83 ±0.15^{Ac}	4.63 ±0.07^{Bc}	4.36 ±0.02^{Cc}
7	4.88 ±0.08^{Ac}	4.65 ±0.19^{Bc}	4.32 ±0.06^{Cc}
8	4.91 ±0.14^{Ab}	4.76 ±0.38^{Bb}	4.36 ±0.13^{Cb}
9	4.95 ±0.23^{Aa}	4.89 ±0.24^{Ba}	4.53 ±0.11^{Ca}

Values in the same column shown with similar lowercase letters are not significantly different.
Values in the same rows shown with similar capital letters are not significantly different.

Table 8. The overall acceptance scores of kefir samples during cold storage (mean ±SD)

Sample	First day	7th day	14th day
Control	4.25 ±0.15^{Bi}	4.75 ±0.07^{Ai}	3.12 ±0.18^{Ci}
1	4.36 ±0.35^{Bj}	4.57 ±0.11^{Aj}	3.14 ±0.28^{Cj}
2	4.41 ±0.14^{Bh}	4.62 ±0.46^{Ah}	3.25 ±0.41^{Ch}
3	4.44 ±0.26^{Bg}	4.69 ±0.13^{Ag}	3.36 ±0.44^{Cg}
4	4.52 ±0.18^{Bf}	4.81 ±0.22^{Af}	4.14 ±0.14^{Cf}
5	4.58 ±0.31^{Be}	4.84 ±0.39^{Ae}	4.23 ±0.24^{Ce}
6	4.61 ±0.11^{Bd}	4.90 ±0.14^{Ad}	4.36 ±0.06^{Cd}
7	4.64 ±0.43^{Bc}	4.87 ±0.48^{Ac}	4.41 ±0.14^{Cc}
8	4.76 ±0.11^{Bb}	5.00 ±0.06^{Ab}	4.47 ±0.14^{Cb}
9	4.89 ±0.05^{Ba}	5.00 ±0.13^{Aa}	4.59 ±0.11^{Ca}

Values in the same column shown with similar lowercase letters are not significantly different.
Values in the same rows shown with similar capital letters are not significantly different.

enzyme and 0.2% gum) on the 7th day had the highest texture scores. The lowest texture score was related to sample 1 (containing 50 ppm enzyme and 0.05% gum) on the 14th day.

The overall acceptance scores of the kefir samples are shown in Table 8. The highest overall acceptance score was related to sample 9 (containing 150 ppm enzyme and 0.2% gum) on the 7th day. Sample 1 (containing 50 ppm enzyme and 0.05% gum) on the 14th day had the lowest overall acceptance score.

In a similar study, Saint-Eve et al. (2004) found that adding hydrocolloids such as xanthan gum could improve the texture of low-fat yogurt. Moreover, Wróblewska et al. (2009) reported that kefir with microbial transglutaminase enzyme obtained higher scores for sensory quality than a control.

CONCLUSIONS

Kefir samples could maintain adequate levels of probiotic bacteria during two weeks of cold storage. The kefir sample containing 150 ppm transglutaminase and 0.2% xanthan had the highest number of probiotic bacteria. By increasing the amounts of the gum and the enzyme, the viscosity of the samples increased and syneresis decreased significantly ($P < 0.05$). The trend in acidity changes in the test samples was similar to the control sample. The kefir sample containing 150 ppm transglutaminase and 0.2% xanthan had the highest organoleptic scores. Therefore, this sample was selected as the best sample.

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