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Effects of Kefir Powder Fortification on Yogurt Quality

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ABSTRACT

The aim of this research was to improve functional properties and sensory of plain set type yogurt since yogurt bacteria have low probiotic properties. Total solid standardization (to 15% w v^{-1}) of milks was made with either using natural kefir powder as a treatment group (KTYO) or milk powder as a control group (YKON). Kefir powder was produced with freeze-dried kefir made from kefir grains. Microbial, chemical, sensory and physical properties of yogurts were determined during cold storage. The attributes determined on the yogurts were pH, total solids, titratable acidity, whey separation, sensory properties, color parameters and flavor. Numbers of *Lactobacillus* spp. in yogurt samples contained either kefir powder or milk powder were similar and did not change significantly during cold storage. *L. acidophilus* and *Bifidobacterium* spp. contents of KTYO ranged between 5.79-6.93 log cfu g^{-1} and 4.05-4.83 log cfu g^{-1} during the cold storage, respectively. There was no significant reduction in the number of *L. acidophilus* and *Bifidobacterium* spp. during the storage (P>0.05). In general, sensory properties of the YKON and KTYO were similar (P>0.05).

Keywords: Yogurt; Kefir powder; Probiotic; Fortification agent

Kefir Tozu Zenginleştirmesinin Yoğurt Kalitesi Üzerine Etkisi

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ÖZET

Bu araştırmanın amacı sade pıhtısı kırılmamış yoğurdun fonksiyonel ve duyusal özelliklerinin iyileştirilmesidir. Sütlerin toplam kuru madde standardizasyonu (% 15 w v^{-1}), uygulama grubunda (KTYO) doğal kefir tozuyla, kontrol grubunda (YKON) süt tozuyla yapılmıştır. Kefir tozu kefir danelerinden üretilen doğal kefirin liyofilize edilmesiyle üretilmiştir. Soğuk depolama süresince yoğurt örneklerinin mikrobiyolojik, kimyasal, duyusal ve fiziksel özellikleri belirlenmiştir. Örneklerin pH, titrasyon asitliği, serum ayrılması, duyusal değerlendirmesi, renk değerleri ve flavor bileşenleri çalışılmıştır. Kefir tozu veya süt tozu içeren yoğurt örneklerinin tümünde *Lactobacillus* spp. içeriği benzer olarak tespit edilmiş ve soğuk depolama sürecinde de önemli bir değişim gözlenmemiştir. KTYO örneğinin *L. acidophilus* ve *Bifidobacterium* spp. içeriği soğuk depolama süresince sırasıyla 5.79-6.93 log cfu g^{-1} ve 4.05-4.83 log cfu g^{-1} olarak tespit edilmiştir. Soğuk depolama süresince bu bakterilerin içeriğinde azalma tespit edilmemiştir (P>0.05). Kefir tozu ilaveli yoğurt örneklerinin duyusal değerlendirme bulguları süt tozu ilaveli yoğurt örneklerinin duyusal değerlendirmeleri ile benzer tespit edilmiştir (P>0.05).

Anahtar Kelimeler: Yoğurt; Kefir tozu; Probiyotik; Duyusal

1. Introduction

Yogurt has become a basic element of daily nutrition in many parts of the world because of the various health claims, therapeutic values and acceptance of its organoleptic properties (Routray & Mishra 2011). Gel structure, taste and flavor are the important attributes for consumer acceptance. Total solid content is significant for an appropriate gel texture formation of set type yogurt. Total solid adjustment to 15% could be carried out using addition of milk powder, evaporation or membrane filtration techniques. In recent years, it has been reported that yogurt does not have high potential for probiotic activity; therefore, yogurt has increasingly being used as a carrier of added probiotic bacteria for their potential health benefits (Ashraf & Shah 2011).

Kefir is produced by activity of natural starter culture, kefir grains, which contain a diverse range of inherent lactic acid bacteria and yeast in a unique polysaccharide structure (Guzel-Seydim et al 2011); therefore it is a significant source of probiotics and prebiotics. Kefir contains *Lactobacillus kefiranofaciens, Lactobacillus kefirgranum, Lactobacillus helveticus, Lactobacillus casei, Lactobacillus brevis, Lactobacillus acidophilus, Lactococcus lactis, Streptococcus thermophilus and Kluyveromyces marxianus* (Kök Taş et al 2012). Several studies have showed that the health benefits associated with kefir are gastrointestinal proliferation, antibacterial spectrum, anticarcinogenic effect, hypocholesterolemic effect, antidiabetic properties, antimutagenic activity, β-galactosidase activity, scavenging activity, lactic acid content, effect on lipid and blood pressure level, protection against apoptosis, antiallergic properties, anti-inflammatory action, bacterial colonization, and immune system booster (Guzel-Seydim et al 2011; Ahmed et al 2013). The aim of this research was to use freeze-dried natural kefir powder for total solid increment in set type yogurt production to improve its functional and sensory properties.

2. Material and Methods

2.1. Yogurt production

Cow milk was provided by the Unsut Dairy Plant, Suleyman Demirel University, Isparta, Turkey. Kefir powder was kindly provided by the Danem Co., Technopark at Suleyman Demirel University, Isparta, Turkey.

In this study, yogurts were made with using addition of natural kefir powder (treatment group was abbreviated as KTYO) and nonfat milk powder (control group was abbreviated as YKON) into milk for 15% total solid standardization.

Control yogurt (YKON) was produced as followed; raw milk was standardized to 15% (w w^{-1}) total solids with skimmed milk powder at 30 °C, homogenized with warring blender for 2 min, heated to 85 °C for 15 min and cooled to 46 °C and inoculated with 2% (w w^{-1}) commercial yogurt starter culture (w w^{-1}) (YC-350, Yo-Flex; thermophilic yogurt culture, DVS Chr. Hansen). After transferring into 200 mL plastic cups yogurts incubated at 42 °C until pH 4.6 and stored at 4 °C.

Yogurt enriched with kefir powder (KTYO) was made as followed: 12% solid content of raw milk heated to 85 °C for 15 min and cooled to 46 °C and standardized to 15% (w w^{-1}) total solids with kefir powder than immediately inoculated with 2% (w w^{-1}) commercial yogurt starter culture (YC-350, Yo-Flex; thermophilic yogurt culture, DVS Chr. Hansen). After transferring into 200 mL plastic cups yogurts incubated at 42 °C until pH 4.6 and stored at 4 °C. Yogurt samples were analyzed during cold storage (4 °C) at days 1^{st}, 7^{th}, 14^{th} and, 21^{st}.

2.2. Microbiological analyses

Yogurt samples during storage were analyzed to determine changes in their microbial content. The lactobacilli counts were determined by using de Man, Rogosa and Sharpe (MRS) medium (Accumedia 7543, East Lansing, MI, USA) after incubation at 37 °C under 6% CO_2 at 37 °C for 48 h. The *Lactococcus* spp. and *Streptococcus thermophilus* were plated on M17 medium (Oxoid, Basingstoke, UK) and incubated under 6% CO_2 at 37 °C for 48 h. Yeasts were grown on Potato Dextrose Agar (Merck, Darmstadt, Germany) with 0.14% added lactic acid at 25 °C for 5 days (Mossel et al 1995). *L. acidophilus* and *Bifidobacterium* spp. were cultured on MRS with sorbitol (10%) and on MRS with NNLP (20%) containing neomycin sulfate (100 mg L^{-1}), nalidixic acid (50 100 mg L^{-1}), lithium chloride (3000 100 mg L^{-1}) and paramycin sulfate (200 100 mg L^{-1}), respectively (Özer et al 2008).

2.3. Chemical analyses

The pH of yogurt was measured using digital pH meter (WTW, Measurement System, FL, USA). Titratable acidity and total solids were determined according to AOAC International (1992) methods. The extent of syneresis was measured according to Atamer & Sezgin (1986).

2.4. Color measurement

L* (whiteness to blackness), a* (redness to greenness) and b* (yellowness to blueness) values of the yogurts were determined by Minolta (Minolta Corp, Ramsey, NJ, USA).

2.5. Flavor compounds

Agilent 7697A Headspace and Agilent 7890A Gas Chromatography with 5975C MS were used for determination of acetaldehyde, ethanol, acetone and diacetyl (Yılmazer & Seçilmiş 2006).

2.6. Sensory analyses

Sensory evaluation of plain yogurt samples was conducted by 12 taste panelists. Panelists were selected from volunteer undergraduate/graduate students and academic staff of the Department of Food Engineering. The panelists (n= 12: 8 women, 4 men, aged 19-43 years old) received a 30-h training session including basic tastes and flavor identification, and using a 5-point product specific scale with references (Meilgaard et al 1999). The samples were presented to the panelists for every week (1st, 7th, 14th and, 21st). Yogurts were presented in 30 g sample cups with plastic lids with three digit codes. The panelists were asked to evaluate the color, appearance, odor, taste, texture (hand), texture (mouth) and overall acceptability, based on a 5 point scale; between like extremely = 5 point and dislike extremely = 1 point.

2.7. Statistical analyses

Data analyses were performed using SPSS statistical software Version 22 (SPSS Inc., Chicago, IL). Microbial and physico-chemical data were analyzed using repeated measurement ANOVA. A factorial arrangement was set up to study the influence of two treatment and four storage time using 3 replicates. Tukey A test was performed for group means comparison. P value < 0.05 was considered statistically significant for all analysis.

3. Results and Discussion

The changes in the viable counts of *Lactococcus* spp., *Streptococcus thermophilus* and *Lactobacillus* spp. during storage of yogurt were presented in Table 1. *Lactobacillus* spp. contents of yogurt samples contained either kefir powder or milk powder were similar and did not have any significant changes during cold storage (P>0.05).

YKON sample had higher numbers of *Lactococcus* spp., *Streptococcus thermophilus* (9.12 log cfu g^{-1}) than the KTYO sample (8.83 log cfu g^{-1}) at first day, respectively (P<0.05). Cocci numbers were higher than *Lactobacillus* spp. in both of the samples. It is important that *Lactobacillus acidophilus*, *Bifidobacterium* spp. and yeasts were detected only in the KTYO product due to natural kefir powder inoculation (Table 2). The cell counts of *L. acidophilus*, *Bifidobacterium* spp. and yeasts ranged between 5.79-6.93, 4.05-4.83 and 3.64-4.95 log cfu g^{-1} during cold storage of KTYO product,

Table 1- *Lactococcus* spp., *S. thermophilus* and *Lactobacillus* spp. content of yogurt samples during cold storage at 4 °C

Samples	*Storage time (day)*			
	1st	*7th*	*14th*	*21st*
	Lactococcus spp. and *Streptococcus thermophilus* (log cfu g^{-1})			
YKON*	9.12±0.05[a]	9.13±0.05[a]	9.11±0.05[a]	9.16±0.03[a]
KTYO	8.83±0.02[a]	8.74±0.08[b]	8.69±0.07[b]	8.80±0.08[b]
	Lactobacillus spp. (log cfu g^{-1})			
YKON	8.01±0.10[a]	8.19±0.13[a]	8.15±0.14[a]	7.88±0.29[a]
KTYO	7.92±0.04[a]	8.22±0.18[a]	8.18±0.22[a]	8.10±0.29[a]

[a,b], statistical differences (P<0.05) for treatments are indicated by different superscripts in the same column; treatment group has been abbreviated as KTYO; control group has been abbreviated as YKON

Table 2- *L. acidophilus, Bifidobacterium* spp. and yeast content (viability) of KTYO sample during cold storage at 4 °C

Microorganism	*Storage time (day)*			
	1st	*7th*	*14th*	*21st*
L. acidophilus (log cfu g^{-1})	6.93±0.54[a]	6.40±0.54[b]	6.13±0.80[c]	5.79±0.80[d]
*Bifidobacterium s*pp. (log cfu g^{-1})	4.83±1.77[a]	4.39±1.47[b]	4.25±1.42[b]	4.05±1.58[c]
Yeast (log cfu g^{-1})	3.64±0.77[c]	4.19±1.17[b]	4.36±1.56[b]	4.95±1.04[a]

[a,b], statistical differences (P<0.05) during the storage are indicated by different superscripts in the same row

respectively. There were significant differences in the cell counts of *L. acidophilus*, *Bifidobacterium* spp. and yeasts during storage (P<0.05). It is difficult to keep Bifidobacteria active in food during storage due to oxygen sensitivity and low acid tolerance. However, high oxygen utilization ability of *S. thermophilus* prevents reduction of Bifidobacteria (Lourens-Hattingh & Viljoen 2001). Inclusion of freeze dried kefir with probiotics affected the microbiota of yogurt and the functional properties of yogurt were improved.

Total solid contents of YKON and KTYO were 15.90% and 15.51%, respectively (P>0.05). Chemical properties of yogurt samples were shown in Table 3. The pH level of YKON and KTYO sample ranged between 4.10-4.38 and 4.03-4.41

Table 3- Physical and chemical properties of yogurts during cold storage at 4 °C

Samples	*Storage time (day)*			
	1st	*7th*	*14th*	*21st*
	pH			
YKON	4.38±0.04[a]	4.34±0.07[a]	4.17±0.10[ab]	4.10±0.01[b]
KTYO	4.41±0.04[a]	4.22±0.06[ab]	4.16±0.13[bc]	4.03±0.03[c]
	Titratable acidity (%)			
YKON	1.12±0.03[a]	1.28±0.02[a]	1.32±0.08[b]	1.35±0.04[b]
KTYO	1.06±0.02[a]	1.22±0.08[a]	1.25±0.15[a]	1.35±0.08[b]
	Whey separation (mL)			
YKON	3.44±1.11[a]	2.07±0.20[ab]	1.64±0.14[b]	2.03±0.05[ab]
KTYO	3.47±0.18[a]	2.31±0.44[ab]	2.52±0.22[ab]	1.68±0.24[b]
	Total solids (%)			
YKON	15.85±0.21[a]	15.92±0.03[a]	15.82±0.35[a]	16.02±0.24[a]
KTYO	15.74±0.43[a]	15.59±0.45[a]	15.28±0.61[a]	15.43±0.46[a]

[a,b], statistical differences (P<0.05) during the storage are indicated by different superscripts in the same row; treatment group has been abbreviated as KTYO; control group has been abbreviated as YKON

during the cold storage, respectively. A gradual decrease in pH through the storage was noted both of the samples at the 14th day ($P<0.05$). There were no significant differences among the pH values of all yogurt samples during the storage period ($P>0.05$). Acidity is one of the most critical parameters affecting the viability of the probiotics in yogurt (Dave & Shah 1997; Ranadheera et al 2012). The titratable acidities of YKON and KTYO samples were ranged between 1.12-1.35% and 1.06-1.35% during the storage. The titratable acidities of yogurt samples increased at during storage ($P<0.05$).

The volumes of serum separation from the YKON and KTYO samples were 1.64-3.44 mL and 1.68-3.47 mL during the cold storage, respectively. There was no significant difference in the amount of syneresis between control yogurt and the yogurt enriched with kefir powder ($P>0.05$). However, syneresis was significantly affected by the storage time (Table 3). There was significant decrease in syneresis amount of yogurt samples between 1st day and 21st day ($P<0.05$).

The L*, a* and b* values were presented in Table 4. L*, a* and b* values are important factors for the appearance of the food products. The L* and a* values of KTYO sample was significantly higher than those of YKON sample ($P<0.05$). The b* value of YKON sample was significantly higher than that of KTYO sample ($P<0.05$). Cais-Sokolinska & Pikul (2006) determined high correlation coefficient between the L*, a* and b* color parameters and syneresis and titratable acidities. It was observed that the L*, a* and b* color parameters decreased during the storage time (Cais-Sokolinska & Pikul 2006).

Yogurt flavor consists of more than 90 different flavor compounds. The significant flavor compounds are acetaldehyde, acetone, acetoin, and diacetyl in yogurt (Guzel-Seydim et al 2005). *L. bulgaricus* and *S. thermophilus* both are required for typical flavor of yogurt (Yalçın 1985); flavor production of starter culture is very important in plain yogurts. In this study, it was found that YKON had significantly higher contents of acetaldehyde and acetone than KTYO samples ($P<0.05$) (Table 5). The typical aroma of yogurt resulted when especially acetaldehyde was greater than 8 mg kg^{-1} (Routray & Mishra 2011). Both KTYO and YKON samples had contained acetaldehyde more than 8 mg kg^{-1}. Certain amount of acetaldehyde in KTYO was possibly metabolized to ethanol not only during fermentation but also during storage. Ethanol content of KTYO sample was significantly higher than YKON since yeast content (Table 2) of kefir powder affected the microbial metabolism ($P<0.05$). It was noted that natural kefir microflora results in lactic acid and ethanol fermentation together (Guzel-Seydim et al 2011). Diacetyl contents of yogurt samples contained either kefir powder or milk powder were similar at the first storage day ($P>0.05$).

Table 4- CIE L*, a* and b* values of yogurt samples during the storage (n= 3)

Samples	*Storage time (day)*			
	1st	*7th*	*14th*	*21st*
	CIE L*			
YKON	84.01 ± 0.04^a	83.96 ± 0.08^a	83.86 ± 0.07^a	83.92 ± 0.06^a
KTYO	84.32 ± 0.05^a	84.44 ± 0.04^b	84.37 ± 0.03^b	84.45 ± 0.11^b
	CIE a*			
YKON	-2.93 ± 0.01^b	-2.99 ± 0.04^b	-3.02 ± 0.01^b	-3.03 ± 0.06^b
KTYO	-2.62 ± 0.07^a	-2.68 ± 0.02^a	-2.67 ± 0.04^a	-2.60 ± 0.06^a
	CIE b*			
YKON	7.29 ± 0.02^a	7.35 ± 0.11	7.42 ± 0.15	7.45 ± 0.19
KTYO	6.58 ± 0.16^a	6.96 ± 0.10	6.96 ± 0.16	6.90 ± 0.16

a,b, statistical differences ($P<0.05$) for treatments are indicated by different superscripts in the same column; treatment group has been abbreviated as KTYO; control group has been abbreviated as YKON

Table 5- Changes in flavor compounds contents of yogurt samples during cold storage at 4 °C

Samples	*Storage time (day)*	
	1st	*21st*
	Acetaldehyde (mg kg^{-1})	
YKON	27.38±2.41[a]	22.36±0.37[a]
KTYO	12.23±7.86[b]	12.14±1.25[b]
	Acetone (mg kg^{-1})	
YKON	0.95±0.02[a]	1.09±0.02[a]
KTYO	0.54±0.02[b]	0.66±0.09[b]
	Ethanol (mg kg^{-1})	
YKON	5.55±0.77[a]	9.13±2.71[a]
KTYO	36.56±15.66[b]	138.16±61.77[b]
	Diacetyl (mg kg^{-1})	
YKON	1.58±0.21[a]	1.98±0.23[a]
KTYO	1.49±0.69[a]	1.08±0.25[b]

[a,b], statistical differences (P<0.05) for treatments are indicated by different superscripts in the same column; treatment group has been abbreviated as KTYO; control group has been abbreviated as YKON

The sensory scores of the yogurt samples for taste, color, odor, appearance, texture with spoon and texture with mouth were detailed in Figure1.

Color, odor, appearance, texture with spoon, texture with mouth and taste scores of YKON samples ranged between 4.33-4.46, 3.96-4.17, 4.08-4.17, 4.38-4.50, 4.08-4.25 and 3.83-4.58 during the storage, respectively. Color, odor, appearance, texture with spoon, texture with mouth and taste scores of KTYO samples ranged between 4.00-4.29, 3.58-3.83, 3.38-4.21, 3.75-4.13 and 3.38-4.00 during the storage, respectively.

There were no significant differences in the appearance scores of the YKON sample during the storage (P>0.05). The appearance scores of the KTYO sample decreased significantly at the 21st day (P<0.05). Foamy surface (1-2 mm thickness) of the KYTO was noticed due to CO_2 production of yeasts in kefir. Texture with spoon scores of YKON sample

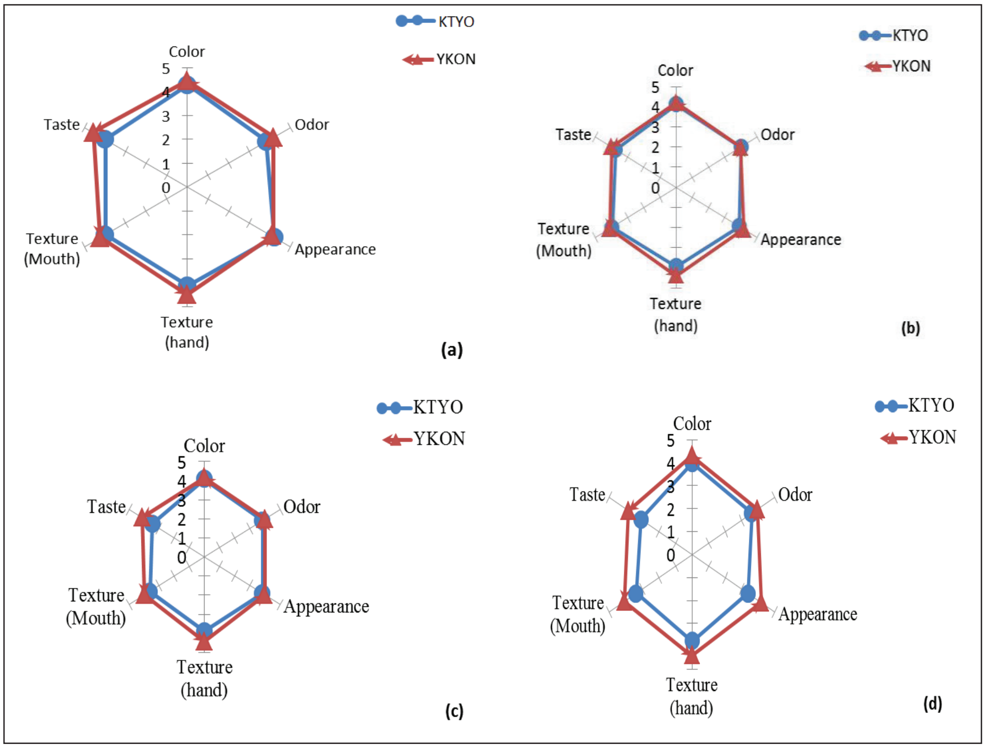

Figure 1- Sensory evaluation of yogurt samples during cold storage (n= 12) at 4 °C ((a)1, (b)7, (c)14, (d)21)

were significantly higher than that of KTYO sample (P<0.05). There were no significant differences between texture with mouth scores of both samples (P>0.05). The texture (mouth) scores of the samples decreased significantly at the 21st day (P<0.05). Taste scores of the YKON sample were significantly higher than KTYO sample (P<0.05) possibly due to less amount of acetaldehyde and higher amount of ethanol contents in KTYO. There were no significant differences in the color and odor scores of control yogurt and the yogurt enriched with kefir powder (P>0.05). The general sensory evaluation scores of the YKON and KTYO were similar (P>0.05).

4. Conclusions

This study showed that a combination of kefir powder and yogurt starter cultures would be used to renovate the traditional yogurt production. Kefir powder was included for mainly total solid standardization. Furthermore, it was concluded that kefir powder also had a significant role during fermentation. Probiotic properties of traditional yogurt improved with the kefir powder. The refreshing taste and health benefits of kefir could be transferred to commercial yogurt by this application. This study formed a new product for consumers in terms of acceptable sensory properties. In order for consumers to gain health benefits from yogurt, kefir powder could be a significant fortification agent for yogurt. It is not only increased total solids but also enhanced probiotic content.

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