



Chemical Changes in Strained Dairy Product Produced with Organic Milk by Using Kefir Grains and Yogurt Culture during Refrigerated Storage

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ABSTRACT

The objective of this study was to produce a strained dairy product made from organic milk using yogurt culture and kefir grains as a new product development. Strained dairy product was analyzed for carbohydrates, organic acids, volatile organic compounds (VOCs), basic chemical composition as well as overall acceptability at 1, 7, 14, 21 and 28 days of refrigerated storage. While galactose content in dairy products increased, lactose and glucose contents decreased on 14th day of storage. The main VOC was ethanol, accounted for about 58% of total volatiles. An increase in succinic acid (from 312 mg/kg to 638 mg/kg), acetic acid (632 mg/kg-843 mg/kg), ethyl octanoate (1.07%-4.15%) and a decrease in viscosity (5754 mPas-1050 mPas) and total solids (24.33%-20.64%) towards the end of storage were observed. Refrigerated storage for more than 21 days could not be recommended since the product was found unacceptable by the panelists but the product consumption at the first 21 days of storage may be an advantage for lactose intolerance humans due to its low lactose level.

Keywords: Strained dairy product, Yogurt, Kefir, Organic acids, Volatile organic compounds

Kefir Tanesi ve Yoğurt Kültürü ile Organik Sütten Üretilen Süzme Süt Ürününün Soğuk Depolanması Sırasında Kimyasal Değişimler

ÖZ

Yoğurt kültürü ve kefir tanesi, organik süt kullanılarak üretilen; süzme yeni bir ürün üretimi amaçlanmıştır. Süzülmuş üründe depolama boyunca (1., 7., 14., 21., 28. günlerde) karbonhidrat ve organik asit içeriği, uçucu bileşenler, temel kimyasal kompozisyon analizleri yapılmış ve genel kabul edilebilirlik değerlendirilmiştir. Depolamanın 14. gününde galaktoz miktarı artarken, laktoz ve glukoz miktarları azalmıştır. Üründe en fazla oranda belirlenen uçucu bileşen etanol olup; toplam uçucu bileşenlerin yaklaşık %58'ini oluşturmuştur. Depolamanın sonuna doğru süksinik asit (312 mg/kg'dan 638 mg/kg'a), asetik asit (632 mg/kg'dan 843 mg/kg'a) ve etil oktanoatta (%1.07'den %4.15'e) önemli düzeyde bir artma; viskozite (5754-1050 mPas) ve toplam kurumaddede (%24.33-%20.64) bir azalma gözlemlenmiştir. Yirmi bir günden daha uzun süre depolanan ürün panelistler tarafından kabul edilemez olarak değerlendirilmiştir. Ancak depolamanın 21. gününe kadar ürünün tüketimi, düşük laktoz içeriği nedeniyle laktoz intoleransı insanlar için bir avantaj olarak değerlendirilebilir.

Anahtar Kelimeler: Süzme süt ürünü, Yoğurt, Kefir, Organik asitler, Uçucu bileşenler

INTRODUCTION

Dairy products such as yogurt, kefir are produced by fermentation of milk with lactic culture and kefir grains, respectively. The roles of both yogurt culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) and kefir grains (*L. brevis*, *L. cellobiosus*, *L. acidophilus*, *L. casei*, lactococci and *L. lactis*, *Str. thermophilus*, *L. mesenteroides*, *L. cremoris*, *Kluyveromyces*, *Candida*, *Torulopsis*, and *Saccharomyces* sp.) can be summarized as milk acidification and synthesis of aromatic compounds. However, the shelf-life of these products is limited with almost 3-4 weeks with respect to keeping quality traits [1-3]. Removing whey of acidified product can extend the shelf-life and also water soluble nutrients such as lactose, vitamin and minerals are lost from acidified product along with whey [4]. Therefore, the nutritional value of product may be different from kefir and yogurt. Concentration process allows further fermentation by lactic acid bacteria and yeast resulting in a modified flavor of the final product.

Culture-containing dairy foods such as yogurt and kefir are an excellent source of many of milk nutrients and also they have beneficial effects for health due to their inherent lactic acid bacteria and yeast since the organic acids in yogurt and the other probiotic bacteria such as *L. bulgaricus*, *L. acidophilus* tend to exert preservative effect by controlling the growth of contaminating spoilage and pathogenic organisms [5]. Lactic acid fermentation also contributes to improved storage qualities, physical properties and flavor. Kefir has a distinctive flavor due to the CO₂, ethanol, lactic acid and the other volatile organic compounds [6-8]. These flavor compounds in yogurt and kefir are influenced by the chemical composition of milk base, type of milk, processing conditions (e.g. heat treatment, draining of whey), the inoculation rate, activity and strains of starter culture used and incubation period [1].

Several references are available, however, on the volatile compounds and organic acids in kefir and yogurt [2, 6-12], no scientific literature on biochemical properties of strained product made from organic milk using kefir grains and yogurt culture are existent. Various dairy products such as yogurt and cheeses have previously been produced using kefir as culture [13, 14]. To the best of our knowledge, such a product was produced for the first time. Therefore, we aimed to determine some quality attributes of strained product and their changes during refrigerated storage. For this purpose, the volatile organic compounds (VOCs), organic acids, physical and sensory properties as well as basic chemical composition of strained dairy product were analyzed because those are recognized as important traits in fermented dairy products for consumer acceptability.

MATERIALS AND METHODS

Materials

Kefir grains (approximately 0.32 to 3.4 mm in diameter, small cauliflower florets in appearance) and commercial yogurt culture CH-1 (DVS) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were provided from Ankara University, Department of Dairy Science (Ankara, Turkey) and Chr.Hansen-Peyma (Istanbul, Turkey), respectively. Organic UHT (Ultra High Temperature) cows' milk was obtained from a retail market. Organic acid and lactose standards were purchased from Sigma-Aldrich GmbH (Steinheim, Germany) and Supelco (Bellefonte, PA, USA), respectively.

Manufacture of Strained Dairy Product using Kefir Grains and Yogurt Culture

Strained dairy product was produced according to the procedure presented in Fig. 1. Organic cow milk is used for strained product manufacturing since kefir grains display a good activity in organic milk compared with conventional milk also organic milk kefir is more aromatic [15]. Kefir grains were inoculated at the rate of 0.2 g/100 g milk to organic cow milk at 25°C. After incubation at 25°C for 28 h to pH 4.6, the grains were separated from the fermented milk by filtration through a sieve and fermented milk was heated to 43°C and inoculated at rate of 1 g/100 g with CH-1 type yogurt culture and incubated at 43°C up to 4.4 pH. Decrease from pH 4.6 to 4.4 took place approximately 2.5 h. This may be due to the fact that the high ethanol content of the product suppresses the activity of yogurt bacteria. After cooling one night at 4°C, the fermented product was gently mixed and transferred into a cotton cloth bag. To prevent recontamination, the cloth bag was hung to drain the whey (4°C) until about 25% total solids. Dry salting (1 g/100 g) was applied to the product in order to the development of taste and the preservation of product. Production was repeated three times, and all analyses were performed in triplicate. Samples were analyzed at 7-day intervals during refrigerated storage.

Chemical Analyses

The total solids, fat, protein, ash and acidity as lactic acid of the strained dairy product were analyzed according to methodology recommended by the Association of Official Analytical Chemist Methods [16]. The pH was measured using a pH meter (Orion, Thermo, Beverly, MA, USA).

According to the procedure described by Fernandez-Garcia and McGregor [17] organic acids and carbohydrates were analyzed in an automated HPLC system (HPLC-20 AD Prominence, Shimadzu, Kyoto, Japan) using an ion exchange column (Aminex HPX-87 H, 300x7.8 mm, BIO-RAD, Hercules, CA, USA). Organic acids and carbohydrates were detected at 210 nm with a UV/VIS detector (SPD-20 AV, Shimadzu, Kyoto, Japan) and refractive index detector (RID-10A,

Shimadzu, Kyoto, Japan), respectively. Linear regression curves based peak areas were calculated for

the individual organic acid and carbohydrate covering a broad range of concentrations.

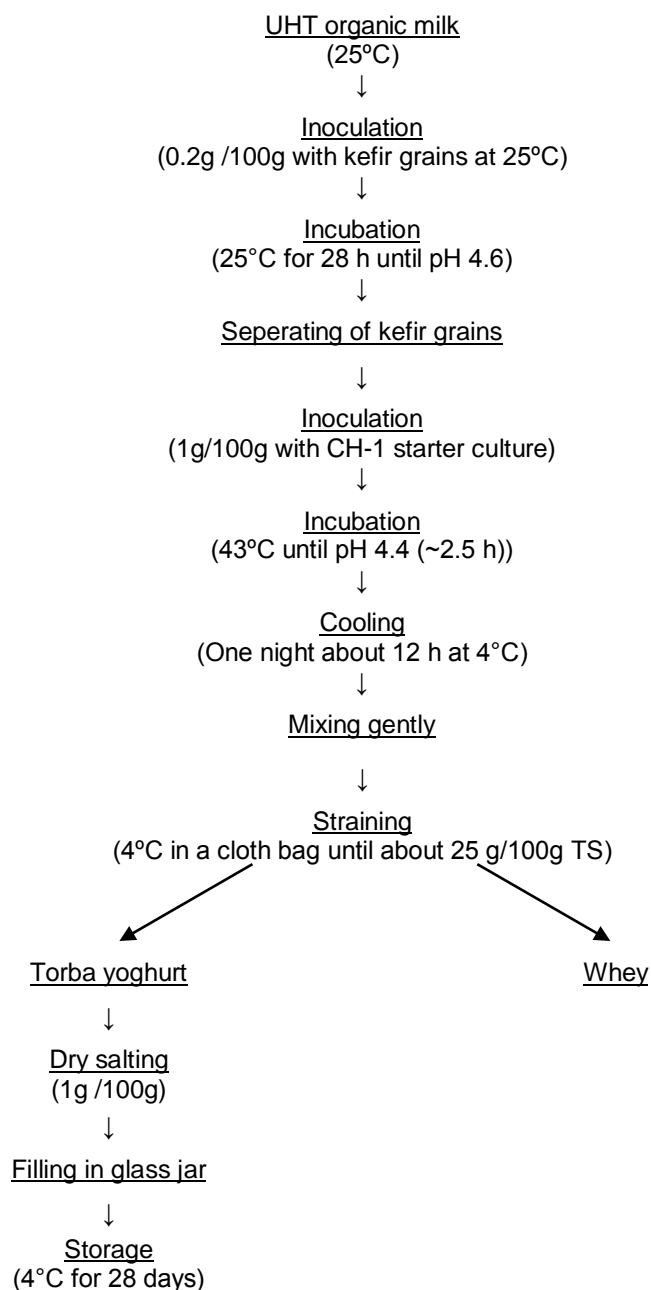


Figure 1. Flow diagram of strained dairy production

The extraction and characterization of the volatile compounds were carried out by headspace (HS)/solid phase microextraction (SPME)/Gas Chromatography (GC)/Mass Spectrometry (MS) analysis, which were able to detect the most volatile compounds. Ten g of the strained dairy sample was immediately transferred in 20 mL head space vial containing 1 g NaCl (Agilent, USA). The vials were sealed using crimp-top caps with PTFE/silicone headspace septa (Agilent, USA) and immediately frozen at -20°C until use. Prior to analysis, frozen samples were thawed at 4°C overnight. At the time of solid phase microextraction analysis, the vials were placed in a water bath with temperature control

and stirring. The sample vials were equilibrated for 30 min at 60°C in water bath then a 50/30 µm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fibre (Supelco, Bellefonte PA.,USA) was exposed to the sample headspace for 40 min at 60°C. Several preliminary tests were carried out to optimize solid phase microextraction (SPME) system. Identification and estimation of volatile compounds was carried out according to procedure described by Guler et al. [18].

Apparent Viscosity

Apparent viscosity (millipascal-seconds, mPas) was measured according to Felfoul et al. [19] with minor modification using a Selecta rotational viscometer with Spindle No 7 at 200 rpm (J. P. Selecta, Barcelona, Spain). Viscosity measurements were carried out at 5°C, with the sample in a 250 mL beaker. Strained product was gently stirred for 20 s before analysis.

Sensory Analysis

Sensory evaluation was performed by 10 experienced panelists. The panel consisted of academic staff and students from the Department of Food Engineering in Mustafa Kemal University, Hatay, Turkey. Strained product was removed from refrigerator (4°C) 1 h prior to sensory evaluation, kept at room temperature (22±2°C). According to procedure stipulated by the Bodyfelt et al. [20], the product is graded on a 20-point scale as follows: 5 points maximum for whey separation, 5 points maximum for spreadable, 5 points maximum for odour and 5 points maximum for taste. By using a 9-point hedonic scale (1=dislike extremely, 5=neither like nor dislike, 9=like extremely), panelists rated overall acceptability.

Statistical Analysis

A factorial arrangement (5x3) was set up to study the influence of the following factor: storage time (5) using

three replicates. A total of 15 samples were investigated on 1, 7, 14, 21 and 28 days. All analyses were conducted at least twice. One-way (ANOVA) variance analyses of all data obtained from strained dairy product for effects of storage period was performed using SPSS (Version 17.00) statistic program [21]. The mean differences were analyzed using Duncan's multiple-range test at least $P<0.05$ significance.

RESULTS AND DISCUSSION

Physicochemical Aspects of Strained Dairy Product

As presented in Table 1, there were no significant differences in total solids contents of strained product during the first 14 days of storage. However, it decreased significantly ($P<0.001$) at the latter days of storage. A sharp decrease in total solids, and also fat, protein and titratable acidity (Fig. 2) could be related to substances such as CO_2 , H_2O_2 and NH_3 formed from degradation of protein and fat by microbial enzymes in strained product since they lead to the alkalization and decrease in total solids of fermented products [1, 22]. The pH value increased significantly ($P<0.05$) on day 7 (Fig. 2). The increase in acidity up to 14 days is not accompanied by a strong decrease in pH, probably due to HCO_3^- formation as a result of reaction between H^+ and CO_2 and/or the presence of substances such as citrate capable of neutralizing H^+ present, and also the increases in esters (Table 2).

Table 1. Basic chemical composition of strained product during refrigerated storage

Chemical Composition	Storage days					P
	1	7	14	21	28	
Total solids (g/100g)	24.33±0.52 ^a	24.14±0.80 ^a	24.77±0.26 ^a	21.98±0.88 ^b	20.64±0.52 ^b	***
Fat (g/100g)	9.33±0.52 ^b	9.83±0.51 ^b	11.00±0.27 ^a	9.45±0.27 ^b	8.75±0.22 ^b	**
Protein (g/100g)	8.83±0.21 ^b	9.91±0.15 ^b	11.50±0.16 ^a	10.88±0.13 ^{ab}	9.66±0.09 ^b	**
Carbohydrate (g/100g)	3.59±0.32 ^a	1.82±0.08 ^b	0.40±0.05 ^b	0.15±0.01 ^c	0.14±0.01 ^c	***
Salt (g/100g)	1.05±0.09 ^b	1.28±0.03 ^b	1.67±0.04 ^a	1.52±0.06 ^{ab}	1.76±0.03 ^a	**
Ash (g/100g)	0.95±0.08 ^b	1.25±0.05 ^b	1.78±0.07 ^a	1.71±0.02 ^a	1.75±0.04 ^a	*

^{a-c} Different letters in the same row are significantly different (* $P<0.05$, ** $P<0.01$, *** $P<0.001$) between values for storage days.

The mean values of total solids, fat, protein and ash of strained dairy product were similar to those reported by Guler & Sanal [4] for Torba yogurt and by Musaiger et al. [23] for Labneh. The pHs were slightly higher and titratable acidity values were lower than those previously reported for Torba yogurt [4]. This could be attributed to the low inoculation ratio of yogurt culture also the use of kefir grains as culture.

Carbohydrates

As shown in Fig. 3., lactose and glucose decreased significantly ($P<0.01$) up to 14 and 21 days of storage, respectively. Afterwards, they did not change. However, galactose increased significantly ($P<0.01$) during the first 14 days of storage. This is probably a consequence of hydrolysis of lactose by lactic acid bacteria [24] and by yeasts [2]. Galactose was not detected between 21 and 28 days of storage. In this time, it may be utilized by yeasts and/or heterofermentative bacteria as carbon source [2, 22]. After 14 days of storage, microbial flora

in strained product could have changed in favor of heterofermentative bacteria and yeasts due to the increases in ethanol and acetic acid production.

Organic Acids

Organic acids are shown in Figure 4. As expected, the primary organic acid was lactic acid. Its concentration ranged from 11154 mg/kg to 12422 mg/kg. Lactic acid decreased significantly ($P<0.05$) on day 14, after that it remained stable. This result was consistent with increases in galactose and ethanol concentrations on day 14. After this period, possible biochemical changes could be carried out via glucose and galactose by heterofermentative lactic acid bacteria or yeast due to the high ethyl acetate (Fig. 5), succinic acid (Fig. 4b) and acetic acid (Fig. 4c) concentrations. The lactic acid concentration was higher than that reported by Guler [10] for yogurt and by Keskenas et al. [25] for kefir. This may be due to the high total solid content of strained product.

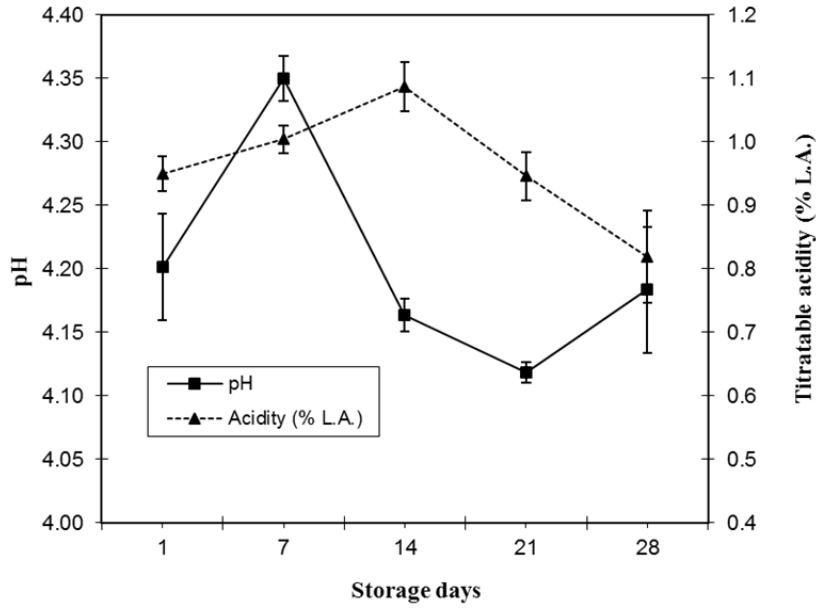


Figure 2. Variations in pH values and titratable acidity of strained product manufactured using kefir grains and yogurt culture during refrigerated storage. The error bars are indicated standard deviation (n=3)

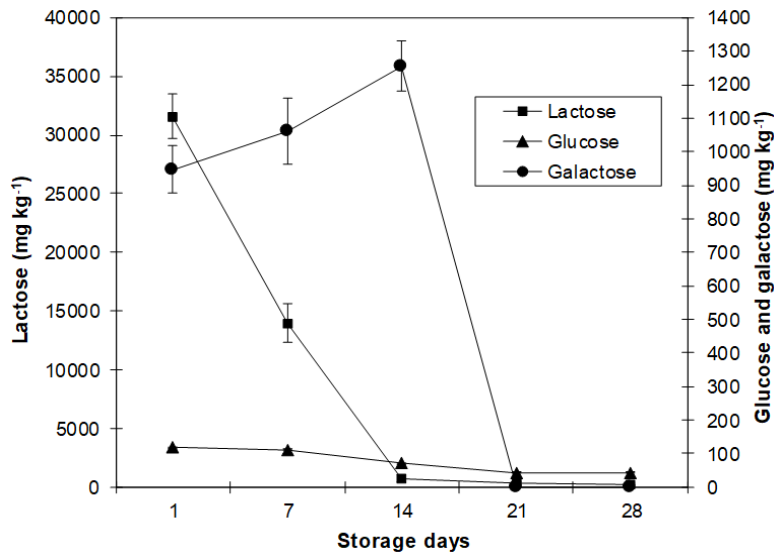


Figure 3. Variations in carbohydrates of strained product manufactured using kefir grains and yogurt culture during refrigerated storage. The error bars are indicated standard deviation (n=3)

Insignificant change in pyruvic acid was observed up to 14 days whereas it decreased significantly ($P < 0.05$) on day 21 and slightly increased at the end of storage. This result was complied with decrease in glucose and increases in acetic acid and ethyl acetate since pyruvic acid is an intermediate product in glucose metabolism. It is probably converted to ethanol and acetic acid by acetic acid bacteria and yeast. Citric acid concentration decreased significantly ($P < 0.01$) from 1806 mg/kg on day 1 to 1237 mg/kg on day 14 (Fig.4b.), after that it showed a slight decrease trend towards to the end of storage. This result was inconsistent with that reported by Guzel-Seydim et al. [8] for kefir whereas it was similar to the findings of Gronnevik et al. [2] for kefir and of Guler [10] for yogurt. Succinic acid was found for the

first time in yogurt and kefir. It may be formed as a consequence of citrate metabolism by yeasts and probably some heterofermentative *Lactobacillus* strains since yeasts such as *S. cerevisiae* disrupting in succinate dehydrogenase enzyme in the absence of oxygen can dramatically accumulate succinate via citrate [26]. As shown in Fig. 4b, succinic acid increased significantly ($P < 0.001$) from 383 mg/kg on day 7 to 643 mg/kg on day 21. No significant differences in succinic acid were observed between days 1 and 7, also days 21 and 28. Throughout the 21 days of storage, increases in succinic acid were complied with decreases in citric acid and glucose. High succinic acid concentration of strained compared with yogurt and kefir may be an important *Bacteroides* virulence factor for peoples [27].

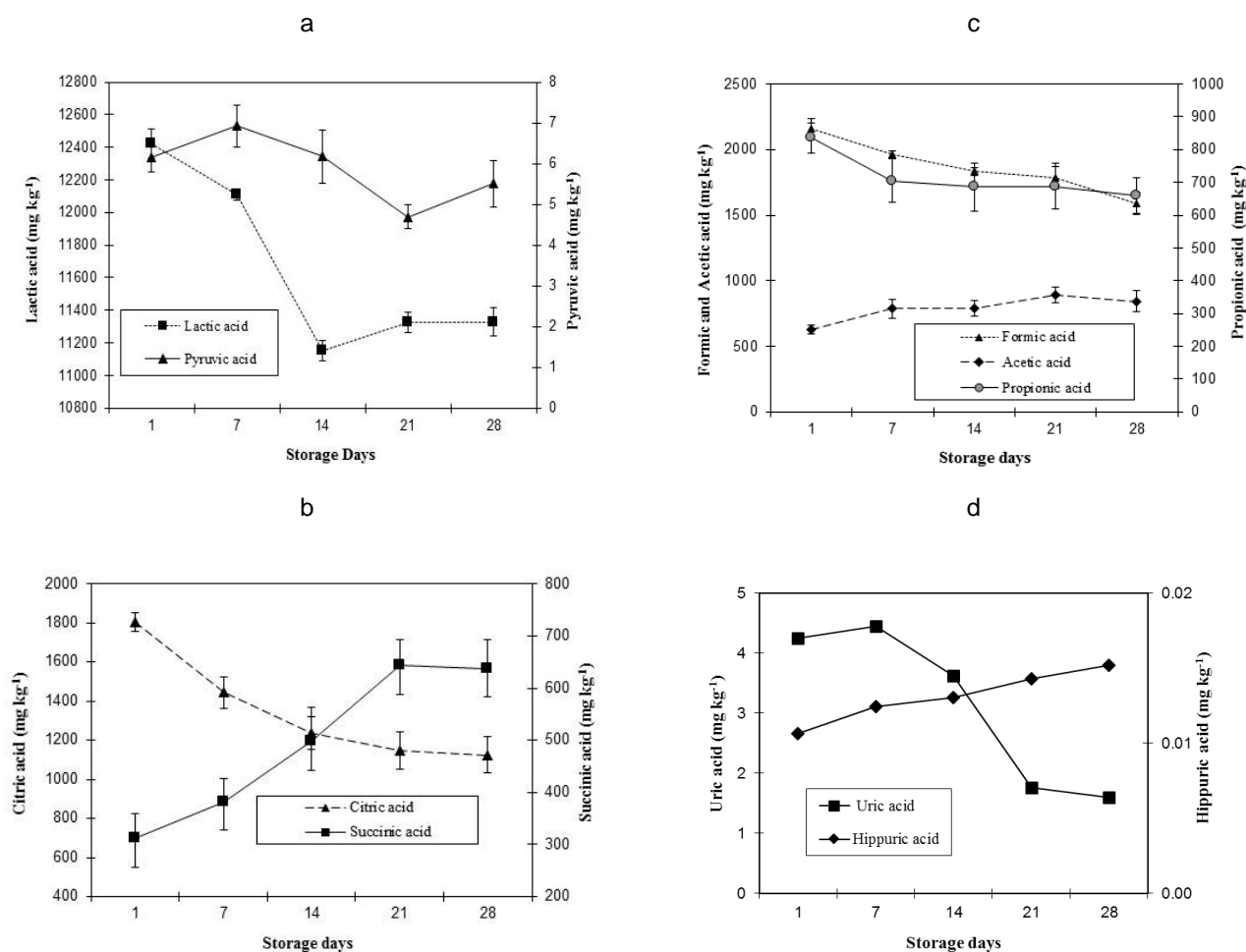


Figure 4. Variations in (a) lactic acid and pyruvic acid, (b) citric acid and succinic acid, (c) formic acid, acetic acid and propionic acid (d) uric acid and hippuric acid of strained product manufactured using kefir grains and yogurt culture during refrigerated storage. The error bars are indicated standart deviation. (n=3)

Like succinic acid, formic acid was detected for the first time in kefir. Insignificant changes in formic acid were observed up to 21 days of storage, but it decreased significantly ($P < 0.05$) at the end of storage (Fig. 4c). Formic acid may have been converted to other substances such as ether methoxy (Table 2). After one day of storage, propionic acid decreased significantly ($P < 0.05$). This could be attributed to increase in propionic acid ethyl ester. However, acetic acid together with acetate esters increased during storage up to 21 days. This could be explained by the ongoing acetic acid production via citrate, lactose or ethanol by *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, heterofermentative lactic acid bacteria or acetic acid bacteria, respectively [28]. As shown in Fig. 4d, hippuric acid, is consisting of benzoic acid, was found at the lowest level initially, but it increased significantly ($P < 0.05$) after 21 days. Uric acid decreased significantly ($P < 0.01$) from day 14 to the end of storage. This could be attributed to the utilization of uric acid as nitrogen source by microorganism in strained product since some gasses such as CO_2 and NH_3 are produced as a result of urea hydrolysis [1]. This finding was agreement with decreases in total solids and titratable acidity after 14 days of storage.

Volatile Organic Compounds (VOCs)

As shown in Table 2, a total of 42 volatile compounds were identified in strained product during storage, including 6 alcohols, 10 esters, 6 acids, 6 ketones, 10 compounds with benzene, 1 alkane, 1 alkene, 1 amine and 1 ether. Ethanol, 3-methyl butanol, benzenemethanol, ethyl acetate, ethyl octanoate, oxime-methoxy phenyl and n-ethyl-1,3-dithioisindoline were the most abundant compounds, accounting for 90% of the total VOCs identified in strained product (Fig. 5). The present product was different from yogurt with respect to distribution of VOCs since carbonyl compounds such as acetaldehyde, diacetyl, and acetone were no detected. It was probably that the high ethanol content of the product might have been suppressed the activity of yogurt bacteria. Mena and Aryana [29] reported that ethanol incorporation yogurt was significantly affected *Lactobacillus delbrueckii* subsp. *bulgaricus* counts, and the magnitude of cell death increased with increase in ethanol concentration. However, the strained product was similar to kefir in terms of the high ethanol, 3-methyl butanol and ethyl acetate contents [2, 6, 7] and to soft cheeses with respect to the most VOCs such as oxime-methoxy

phenyl, n-ethyl-1,3-dithioisindoline, ethyl octanoate, benzyl alcohol, p-cresol, vinyl benzene and dimethylamine [30]. As indicated in Table 2, 13 volatile compounds were identified for first time in strained product when compared to yogurt and kefir.

During storage, ethanol was principle VOCs in strained product as kefir [2, 7]. It increased significantly ($P < 0.01$) up to 14 days of storage, and it did not any change at the rest days of storage (Fig. 5). This could be attributed to the increase in percentage composition of ethyl ester compounds despite the formation of ethanol. Three-

methyl-1-butanol was the second most abundant alcohol. It decreased significantly ($P < 0.01$) during storage. Decreased 3-methyl-1-butanol would be due to decreased leucine uptake by yeasts and/or increased 3-methyl-1-butanol acetate during storage. Three-methyl-butanol also 2-methyl propanol can be formed by reduction of aldehydes produced from Leucine and Valine amino acids via Strecker degradation. Similarly, phenyl alcohols (benzene methanol) or phenyl derivatives can be formed from amino acids as phenylalanine and tyrosine [31].

Table 2. The percentage compositions of volatile compounds identified in strained product during refrigerated storage

No	Compounds	RI	No	Compounds	RI
<i>Alcohols</i>			<i>Esters (continued)</i>		
1	Ethanol ^{§#}	941.45	22	Hexanoic acid, ethyl ester [°]	1257.14
2	2-Methyl-1-propanol [§]	1130.11	23	Propanoic acid, 2-hydroxy-, ethyl ester [°]	1380.90
3	3-Methyl-1-butanol ^{§#}	1236.36	24	Octanoic acid, ethyl ester [§]	1470.35
4	2-Heptanol [°]	1347.24	25	Nonanoic acid, ethyl ester	1577.18
5	2,3-Butanediol [°]	1615.79	26	Decanoic acid, ethyl ester [°]	1683.46
6	Furfural alcohol (2-Furan methanol) [§]	1710.66	27	Ethyl-9-decenoate	1739.34
<i>Ketones</i>			28	Iron, monocarbonyl-(1,3-butadiene-1,4-dicarboxylic acid, diethyl ester) a,a'-dipyridyl	2159.68
7	2-Heptanone [§]	1211.69	<i>Compounds containing benzene</i>		
8	3-Hydroxy-2-butanone (Acetoin) ^{§#}	1331.16	29	N-Ethyl-1,3-dithioisindoline	878.98
9	2-Nonanone [§]	1427.91	30	Styrene (Vinyl benzene) [§]	1293.07
10	9H-pyrrolo[3'4':3,4]pyrrolo[1,2-a]phthalazine-9,11(10H)dione, 10-ethyl-8-penhyll	1568.46	31	11H-Dibenzo[b,e] [1,4]diazepin-11-one, 5,10-dihydro-5-[3-(methylamino)propyl]	1495.93
11	δ-Amylvalerolactone	2261.43	32	8-Methylisothiazolo[4,5-c]-2,1,3-benzothiadiazole	1582.14
12	2H-Pyran-2-one, tetrahydro-6-pentyl- (δ-valerolactam)	>2200	33	Benzaldehyde [§]	1584.11
<i>Acids</i>			34	Oxime-, methoxy-phenyl [§]	1771.31
13	Acetic acid ^{§#}	1491.86	35	2-Phenylethyl acetate [°]	1890.27
14	Butanoic acid ^{§#}	1673.68	36	Benzenmethanol (Benzyl alcohol)	1992.16
15	Pentanoic acid ^{§#}	1715.57	37	β-Resorcylic acid (2,5-dihydroxy benzoic acid)	2048.41
16	Hexanoic acid ^{§#}	1895.58	38	p-Cresol	2111.29
17	Octanoic acid ^{§#}	2078.34	<i>Amines</i>		
18	Decanoic acid ^{§#}	2279.84	39	Dimethylamin	615.29
<i>Esters</i>			<i>Alkane-Alkene</i>		
19	Acetic acid, ethyl ester [§]	902.63	40	2-Methoxycarbonyl-2-(cis-2'pentenyl)-3-Methoxycarbonylcethylcyclopentene	1525.50
20	Butanoic acid ethyl ester [§]	1059.89	41	Pristane [§]	1532.89
21	1-Butanol, 3-methyl, acetate	1148.33	<i>Ether</i>		
			42	Diisopropyl ether [#]	712.24

RI= retention index based on identified compound retention times (RTs), calculated from linear equation between each pair straight alkanes (C₅-C₂₅). [§]Compounds were previously determined in yoghurt and kefir. [°]Compounds were previously determined in only kefir. [#]Compounds verified with authentic standards. All compounds were also considered to be tentative (based on the MS library Wiley7n.1/ Nist

Of ester compounds, 3-methyl butanol acetate, nonanoic acid ethyl ester, ethyl-9-decenoate and Iron, monocarbonyl-(1,3-butadiene-1,4-dicarboxylic acid, diethyl ester) a,a'-dipyridyl were found for the first time in yogurt and/or kefir. Ethyl acetate was the most abundant ester in strained product (Fig. 5). It increased significantly ($P < 0.01$) up to 21 days of storage and latter significantly decreased. The value of ethyl acetate was

similar to that reported by Aghlari et al. [6]. Unlike ethyl acetate, the other esters increased steadily up to the end of storage (data not shown). These findings were well agreement with the decreases in their corresponding acids except for acetic acid. As known, esters are formed by a reaction between alcohols and acids via alcohol acyl transferase enzyme.

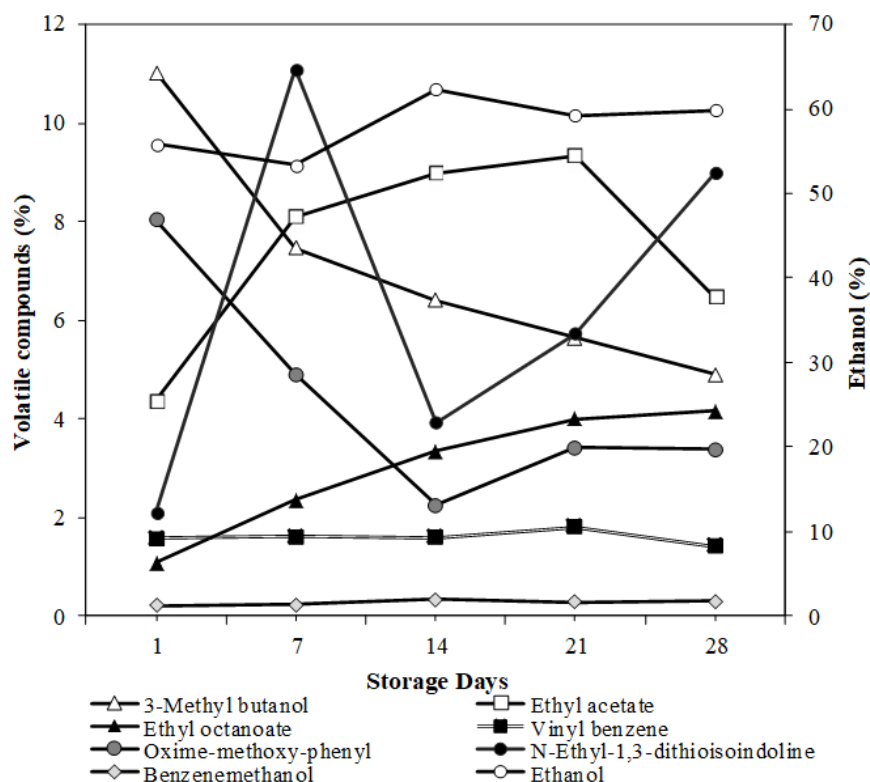


Figure 5. The main volatile compounds in strained product during refrigerated storage (n=3)

Of benzene-containing compounds oxime methoxy phenyl, n-ethyl-1,3-dithioisindoline, benzenmethanol (benzyl alcohol) and styrene (vinyl benzene) were the most plentiful compounds identified in strained product (Fig. 5). These compounds exhibited fluctuations as increase and decrease during storage. This could be attributed to the microbial flora in strained product. Compounds n-ethyl-1,3-dithioisindoline, 11H-dibenzo[b,e] [1,4]diazepin-11-one, 5,10-dihydro-5-[3-(methylamino)propyl], 8-methylisothiazolo[4,5-c]-2,1,3-benzothiadiazole, benzenmethanol (benzyl alcohol), β -resorcylic acid (2,5-dihydroxy benzoic acid), *p*-cresol were identified for the first time in yogurt and/or kefir. The other compounds with benzene were previously found in kefir [6] and yogurt [32]. High levels of benzene derivatives could be attributed to the yeast activation since some yeasts have extracellular enzymes which are accelerated the cyclocondensation of 2-aminothiophenol and aldehydes [33]. The presence of inorganic compounds such as NaCl in medium may catalysis this cyclocondensation.

Apparent Viscosity

During the storage, the apparent viscosity of strained product decreased significantly ($P < 0.05$) from 5754 mPa to 1050 mPa (Fig. 6). This result was different from findings of Guler [10] for yogurt. However, decrease in apparent viscosity with storage time was similar to that reported by Irigoyen et al. [34] for kefir. Decrease in

apparent viscosity would be explained by the decrease in strength and number of bonds between casein micelles in strained product. Apparent viscosity values found for strained product during storage were higher than that in kefir [34, 35] and in yogurt [10]. This could be attributed to the high total solids content of strained product compared to kefir and yogurt.

Sensory Analysis

Sensory analyses scores are shown in Figure 7. As the strained product was unacceptable by panelists at day 28, it is shown in Figure 7. Scores of sensory properties for taste, odor, whey separation, spreadable and also overall acceptability increased up to 14 days of storage and decreased significantly ($P < 0.01$) on day 21. According to panelists' reports, they perceived intensively "fruity", "bitter", "salty" and "sour" taste attributes after 14 days of storage and a decrease in "milky" taste intensity on day 21. These results were consistent with chemical and physical findings since a considerable increase in ethyl octanoate and ethyl decanoate characterizing by "apricot", "wine" and "fruity", "grape" flavor notes, and 2-phenylethyl acetate and *p*-cresol characterizing by "medicinal" and "heavy" flavor [30] was observed after 14 days of storage. Viscosity also decreased significantly ($P < 0.01$) on day 28.

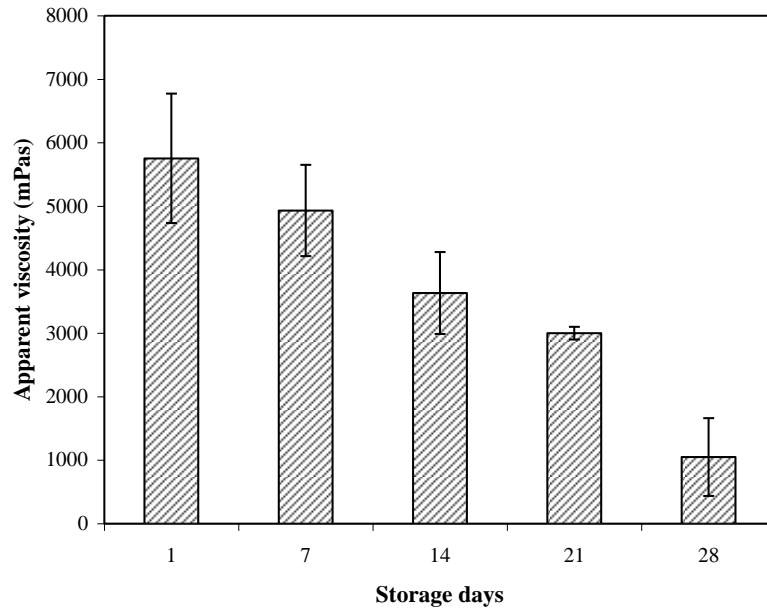


Figure 6. Variations in viscosity values of strained product manufactured using kefir grains and yogurt culture during refrigerated storage. The error bars are indicated standart deviation (n=3)

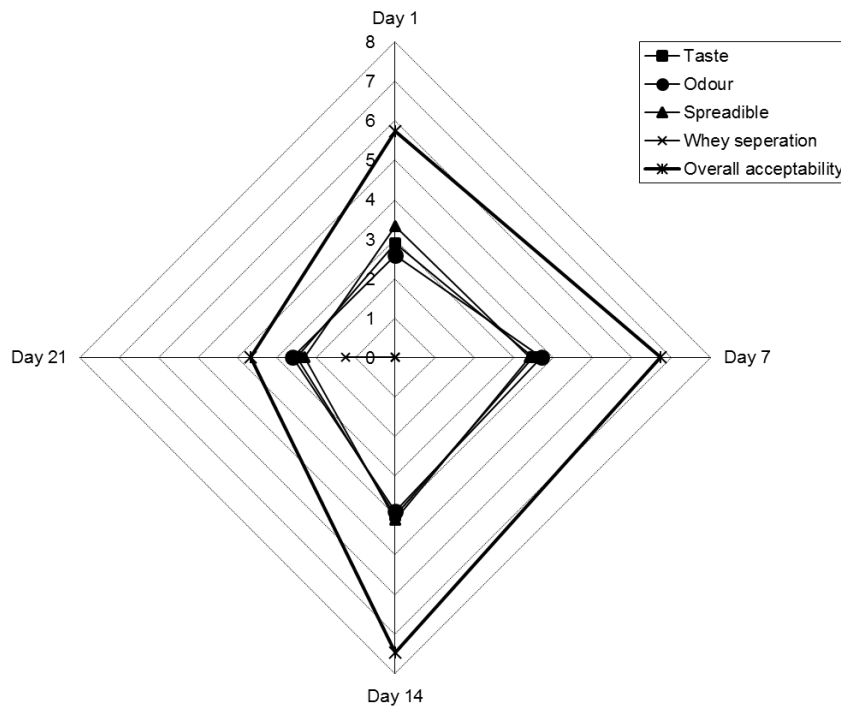


Figure 7. Scores of taste, odour, spreadible and whey separation (out of 5), and overall acceptability (out of 9) for sensory evaluation of strained product manufactured using kefir grains and yogurt culture during refrigerated storage (Except for day 28)

Succinic acid may have resulted in an unacceptable flavor in strained product, reaching a maximum level of 643 mg/kg on day 21 since it is characterized by a strong salty flavor and acidic taste [36]. At the end of storage, the occurrence of diisopropyl ether might also have been caused unacceptance of strained product by panelists. It is noteworthy that the panelists have liked

product with a pronounced milky taste and a certain viscosity level.

CONCLUSION

We concluded that strained product using kefir grains and yogurt culture may be produced. With respect to

changes in organic acids and the distribution of most VOCs, strained product showed a different trend from yogurt and kefir according to available literature. Significant changes in physicochemical properties of strained product were observed after 14 day of storage. Decreases in ketones and acids and also increases in esters and some benzene derivatives towards the end of storage may have adversely affected product acceptability. Shelf-life of this product was shorter than strained yogurt. However, shelf-life of product could be extended by changing of ratios of grains and/or yogurt culture used as inoculate. When compared to yogurt and kefir, such a product may advice for lactose intolerance humans due to its lactose content at trace level.

In the further studies, the main objects will be to determine the both microbial count and to make their identification, to detect CO₂ content and to produce using the different ratios of kefir grains and yogurt culture.

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