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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

The Physical, Chemical, Sensory Properties and Aromatic Organic Substance Profile of Kefir Added Citrus Fruits in Different Proportions

Farklı Oranlarda Turunçgil İlave Edilen Kefirin Fiziksel, Kimyasal, Duyusal ve Organik Madde Profili

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Abstract

This study aims to increase the functionality of plain kefir by adding citrus fruits. Dry matter ratios of kefir samples ranged from 11.04 % to 11.75 %. The addition of fruit to kefir reduced the milk-fat ratios. The pH values of kefir samples ranged from 3.37 to 4.08 depending on fruit concentration. pH values also ranged from 3.37 to 4.08 depending on fruit concentration. Kefir samples containing grapefruit (37.5 %) had the lowest pH value (3.37) among the kefir samples. The viscosity of kefir samples at 20 rpm and 50 rpm at sliding speed ranged from 0.42 Pa.s to 2.88 Pa.s and from 0.31 to 1.60 Pa.s, respectively. The addition of fruit to plain kefir was reduced its viscosity. DPPH[*] of samples was between 1.21 and 38.93 % DPPH of samples with citrus fruit were statistically ($p<0.01$) higher than that of plain kefir samples. While adding orange to plain kefir samples reduced the amount of ethanol, adding grapefruit increased its amount, conversely. Plain kefir samples had higher acetic acid, butanoic acid, hexanoic acid, octanoic acid, n-decanoic acid, benzoic acid, benzaldehyde, benzaldehyde (2,5 bis), silanediol dimethyl, and benzyl alcohol ratios than that of orange, mandarin and grapefruit samples. However, the d-limonene, 1-methyl benzene and benzene 2-ethyl-1,3-dimethyl ratios of kefir samples containing orange, mandarin and grapefruit increased significantly compared to plain kefir. Panelists preferred orange (23 % and 37.5 %) and mandarin (37.5 %) kefir samples more than the others. Panelists gave lower scores to grapefruit-added samples than the other kefir samples.

Keywords: Kefir, Citrus fruits, Aromatic organic matter, Viscosity, Sensory quality

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Öz

Bu çalışmanın amacı, kefirin işlevselliğinin artırılmak için turunçgil ilave edilmiştir. Kefir örneklerinin kuru madde oranları 11.04 % ile 11.75 % arasında değişmektedir. Meyve eklenmesi, kefirin süt yağı oranlarını azaltmıştır. Kefir örneklerinin pH değerleri, meyve konsantrasyonuna bağlı olarak 3.37 ile 4.08 arasında değişmektedir. Kefir örnekleri içinde greyfurt bulunanlar (%37.5), en düşük pH değerine (3.37) sahip olan kefir örnekleri arasında yer almıştır. Kefir örneklerinin 20 rpm ve 50 rpm'deki kayma hızında viskozitesi sırasıyla 0.42 Pa.s ile 2.88 Pa.s ve 0.31 ile 1.60 Pa.s arasında değişmektedir. Meyve eklennesi, kefirin viskozitesini azaltmıştır. Örneklerin DPPH* değerleri %1.21 ile %38.93 arasında değişmektedir. Turunçgiller içeren örneklerin DPPH değerleri istatistiksel olarak ($p<0.01$) kefir örneklerinden daha yüksektir. Portakal eklenen kefir örnekleri etanol miktarını azaltırken, greyfurt eklenmiş olan kefir örnekleri etanol miktarını artırmıştır. Düz kefir örnekleri, portakal, mandalina ve greyfurt örneklerinden daha yüksek asetik asit, butanoik asit, heksanoik asit, oktanoik asit, n-dekanoik asit, benzoik asit, benzaldehit, benzaldehit (2,5 bis), silanediyol dimetil ve benzil alkol oranlarına sahip olduğu görülmüştür. Bununla birlikte, portakal, mandalina ve greyfurt içeren kefir örneklerinin d-limonen, 1-metil benzen ve benzen 2-etil-1,3-dimetil oranları kefire göre önemli ölçüde artmıştır. Panelistler diğerlerine oranla daha çok portakal (%23 ve %37.5) ve mandalina (%37.5) kefir örneklerini tercih etmiştir. Panelistlerin greyfurt eklenmiş örnekleri diğer kefir örneklerine göre daha düşük puan vermiştir.

Anahtar Kelimeler: Kefir, Citrus meyveleri, Aromatik organik madde, Viskozite, Sensör kalitesi

1. Introduction

The word "kefir" is associated with something enjoyable that gives pleasure in Turkish language. This milk product is made by soaking kefir grains in fresh milk and then fermenting it with alcohol and acid (Moltiva et al., 2013).

This product is known by different names such as Kefir, Kiapur, Kanapon, Kopi or Kipi in different parts of the World (Arslan, 2015). The origin of kefir is believed to be in the Caucasus (Özden, 2008). Kefir production has been popular in eastern and central Europe since the 19th century (Russia, Germany, Poland, Slovakia, Denmark, Switzerland, Norway and Hungary) (Karatepe et al., 2012). Kefir contains lactic acid, CO_2 and a small amount of ethanol as well as aromatic substances such as acetaldehyde, acetone and diacetyl, that give kefir its organoleptic properties (Arslan, 2005). The quality and sensory properties of kefir are affected by the type of milk consumed, type and ratios of microorganisms present in the kefir grain, incubation period, storage temperature and its duration (Yaygın, 1996).

Fruit juice is contains sugars, antioxidants, carotenoids, vitamins and polyphenols which are important for human health (Noğay, 2019). Since the fruit sugar present in fruit kefir is used by the kefir microbiota, causing their number to increase, the functional level of fruit kefir as a functional food increases. Citrus fruits have beneficial effects on health due to their components such as ascorbic acid, folic acid, dietary fiber, pectin, potassium, magnesium, carotenoids and flavonoids. Citrus flavonoids such as Naringin and hesperidin that are prominent components in citrus fruits have beneficial effects on hyperglycemia, hyperlipidemia, hypertension, inflammation and weight control. In a study conducted by Kök-Taş et al. (2013) kefir was produced with the addition of 10 % plum and 7.5 % molasses. They found that the total antioxidant content of control, and added kefir samples containing plum or molasses were 13.30 µmol mL^{-1}, 16.80 µmol mL^{-1} and 17.35 µmol mL^{-1} respectively. The control sample and kefir containing dried tangerine, orange or lemon peels had total phenolic content of 945.70 mg mL^{-1}, 2535.80 mg mL^{-1} and 2357.60 mg mL^{-1}, respectively. Dry matter, ash, oil content, pH values and titratable acidity were in the range of 8.64-10.38 %, 0.74-0.79 %, 2.50-3.10 %, 4.15-4.33 and 0.57-0.74 %, respectively. Harmankaya et al. (2019) found that apricot kefir had the highest acidity (0.73%) at the end of the incubation stage, and strawberry kefir and apricot kefir had the lowest pH (5.80). Apricot kefir had the highest acidity at the end of the storage period (+4 °C).

The objective of this study was to determine the quality characteristics of kefir with various citrus fruits added to increase its nutritional value and consumer acceptability Due to the fact that fruit kefir can attract the attention of children and all other age groups and improve the health of consumers, this study was conducted to produce functional kefir in order to improve dietary diversity. In this study evaluates the effect of citrus juice on the physical, chemical and sensory quality of kefir, and it has attempted to determine whether kefir could be combined with citrus juices. In this study, Kefirs containing citrus juices was compared to plain kefir in terms of antioxidant capacity and aroma component differences.

2. Materials and Methods

2.1. Production of plain and fruit kefir

Citrus juices (orange, tangerine and grapefruit) were obtained in a hygienic condition. Plain kefir was made with milk and powdered kefir grain (home kefir grain(vivo)). 1 g of powdered kefir grain was added to 1 kg of milk at 22-25 ºC and incubated for 1 day at the same temperature. The plain kefir was then refrigerated (4 ºC±2 ºC) for 1 day. Citrus kefir samples were made by combining 400 g plain kefir with 120 g (23 %) and 240 g (37.5 %) citrus juice (orange, tangerine and grapefruit). These kefir samples were kept in the refrigerator (4±2 ºC) and subjected to microbiological, physical and chemical analyses.

2.2. Phsical, chemical and biochemical analysis

Dry matter, ash and fat content were determined according to the methods of the Kurt et al. (2012). pH was measured using with a pH meter(Seven Compact pH/Ionmeter S220; Mettler Toledo, Switzerland) (Kurt et al., 2012). The color analyses were done by measuring L* (brightness, 0: black, 100: white), a* (+: red, −: green) and b* (+: yellow, −: blue) values were determined using a chroma meter (CR-300; Konica Minolta, Japan, (Karshenas et al., 2018). Mix viscosity was measured at 4°C using a viscometer (Model DV-II; Brookfield EngineeringLaboratories, USA) at 20 and 50 rpm (Soukoulis et al., 2014).

The antioxidant activity was analyzed according to DPPH* radical scavenging activity. DPPH* radical scavenging activity was determined, According to the methods modified by Binici et al. (2021). Briefly, DPPH* solution was prepared by dissolving 39 mg of DPPH* in 100 mL ethyl alchol. Sample extracts were mixed with 0.5 mL of the DPPH* solution and adjusted to a final volume of 3 mL with ethyl alchol. After 30 minutes in the dark, the absorbance value was measured at 517 nm. DPPH* values are calculated as a percentage. DPPH*% radical inhibition was calculated as follows: Flavor and aroma compounds were determined according to the modified methods of Grabarczyk and Korolczuk (2010). Briefly, 20 g was diluted from each sample with made up 30 mL of distilled water. Then, HCl was added until the pH was 2.5 and mixed for 1 hour. Samples were centrifuged (10 min, 4000 rpm) and defatted with hexane.

2.3. Sensory analysis

Acording to Nelson and Trout (1951), kefir samples were placed in special 150 mL odor-free containers with glass lids and presented to the panelists in a randomly coded manner at regular intervals. While the panelists were performing sensory analysis, water was placed in 100 ml glass bottle containers to clean their mouths before moving on to the other sample. Sensory evaluations were made by considering color and appearance, texture and fluency, taste and aroma, and general acceptability. Sensory evaluation was performed in a spaced seating arrangement in a room at an appropriate temperature (20±2°C). The 8 experts in the Department of Food Engineering were selected as panelists. Each panelist was experienced, trained and informed about sensory analysis methodology.

2.4. Statistical analysis

The data was analyzed using ANOVA procedures using SPSS (Statistical Software 10.0 for Windows, SPSS). Significant differences between parameters were calculated using the Duncan comparison test at ($p<0.05$) (Pripp, 2013).

3. Results and Discussion

3.1. The results of the dry matter and pH analysis of milk and fruit juices

The results of the drymatter and pH analysis of milk and fruit juices are shown in *Table 1*.

Table 1. Dry matter and pH analysis of milk and fruit juices

Samples	Dry Matter (%)	pH
Milk	12.70	6.75
Orange juice	9.18	3.54
Mandarin juice	10.96	3.20
Grapefruit juice	9.18	2.88

Dry matter and pH values of milk samples were found to be 12.70 % and 6.75 %, respectively. Sahin et al. (2014) found that the pH of the milk was between 6.55 and 6.57. Önal et al. (2021) found that the dry matter ratio of cow milk was between 12.35-13.50 %. Our results were similar to the findings of those studies.

The results of some physical and chemical analyses of fruit kefir samples are shown in *Table 2*.

The dry matter ratios of kefir samples ranged from 11.04 % to11.75 %. Kök-Taş et al. (2013) determined that the dry matter content of the control kefir sample was 11.91 % which is consistent with our findings. The addition of fruit juice reduced the fat ratio of samples (*Table 2*). In our study, pH values ranged from 3.37 to 4.08 depending on fruit concentration with lower pH values found when compared to the related study. Kefir samples containing grapefruit 37,5 % had the lowest pH value (3.37) compared to the other. In line with our findings, Harmankaya et al. (2019) determined that the pH level was lower in all fruit kefirs when compared to plain kefir. Dinç (2008) determined the pH level of plain kefir to be 4.26 which was higher than that of samples of our plain kefir. Uslu

(2010) determined the pH level of plain kefir to be 4.73 while fruit kefirs had an average pH of 4.65. The pH of plain and fruit kefir samples in this study was lower than that of Uslu (2010). It can stem from a difference in kefir grains, incubation periods and fruits at different acidity. The pH degree found by Yilmaz et al. (2006), Güzel-Seydim et al. (2005) and Öner et al. (2010) were higher than our findings. Garrote et al. (2001) found that the pH levels of plain kefir samples were between 3.5 and 4.0, which is similar to our results. In a study conducted by Al and Yıldız (2018), 3 different fruits (gojibery, blueberry and banana) were used, and the fruits used in the production of fruit kefir. They reported that the pH of blueberry kefir samples was 4.60 which was higher than our results.

Table 2. Some phsical and chemical analysis of fruit kefir

Kefir Samples	Dry Matter (%)	Milk Fat (%)	pH	Viscosity (Pa. s)20 RPM	Viscosity (Pa. s)50 RPM	DPPH* (%)
Plain (Control)	11.25±0.45[a]	3.20±0.14[a]	4.08±0.03[a]	2.88±0.04[a]	1.60±0.0[a]	1.28±0.10[e]
Orange (23%)	11.34±0.78[a]	2.60±0.14[b]	3.90±0.33[a]	0.95±0.04[c]	0.64±0.03[c]	32.59±0.10[c]
Orange (37.5%)	11.04±0.37[a]	2.33±0.18[b]	3.82±0.01[a]	0.52±0.04[d]	0.27±0.01[e]	32.86±0.10[c]
Mandarin (23%)	11.75±0.21[a]	2.50±0.14[b]	3.78±0.20[a]	0.99±0.03[c]	0.70±0.01[c]	18.69±0.29[d]
Mandarin (37.5%)	11.73±0.23[a]	2.35±0.07[b]	3.68±0.04[a]	0.52±0.03[d]	0.35±0.04[d]	38.26±0.10[b]
Grapefruit (23%)	11.73±0.31[a]	2.55±0.28[b]	3.60±0.28[a]	1.31±0.03[b]	0.88±0.03[b]	38.67±0.02[ab]
Grapefruit (37.5%)	11.05±0.23[a]	2.30±0.14[b]	3.37±0.18[a]	0.42±0.04[e]	0.31±0.03[de]	38.93±0.48[a]
Sig.	ns	*	ns	**	**	**

Note: Data are the average of two replicates, [a,b,c, d] means shown with different letters are statistically different from each other, *: p<0.05 **: p<0.01

The viscosity of kefir samples at 20 rpm and 50 rpm ranged from 0.42 Pa.s to 2.88 Pa.s and from 0.31 and to 1.60 Pa.s, respectively. The addition of fruit to plain kefir reduced its viscosity. Kök-Taş et al. (2013) who made kefir samples found that the viscosity of samples was between 0.225 Pa.s and 0.315 Pa.s. DPPH* % of samples ranged from 1.28 to 38.93. The addition of citrus fruits to kefir caused their antioxidant activity to increase significantly ($p< 0.01$). Randazzo et al. (2016) made the fruit kefir by adding apple, grape, kivifruit, pomegranate, prickly pear and quince. They reported that the DPPH* values of the samples ranged from 34.21 % to 94.70 %. These values were higher than the findings of Randazzo et al. (2016) and our study results. The DPPH* radical scavenging activities of 6 kefir samples collected from the markets were between 58.35 %-94.08 %. The findings of Taşkın (2011) were higher than that of our study results.

The aromatic matter amounts of plain and fruit kefir samples are shown in *Table 3* and continuation of *Table 3*. The ethanol ratio of kefir samples ranged from 0.89 % to 5.67 % (*Table 3*). As the orange amount added to kefir samples increased, the ethanol amount decreased, but the grapefruit addition caused the ethanol level to increase. Randazzo et al. (2016) reported that samples of apple, grape, kiwifruit, pomegranate, prickly pear, and quince kefir contain ethanol at 2.67 %, 4.44 %, 1.03 %, 4.96 %, 2.31 % and 4.51 % ratios, respectively. The results of Randazzo et al. (2016) were consistent with our study results.

The acetic acid, butanoic acid, hexanoic acid, octanoic acid, n-decanoic acid, benzoic acid, benzaldehyde, benzaldehyde (2,5 bis), silanediol dimethyl and benzyl alcohol ratio of plain kefir samples were higher than orange, mandarin and grapefruit. The benzaldehyde content of orange juice was low as 6.3 µg L^{-1} (Erdoğan, 2019), so the benzaldehyde content of the orange kefir samples was also very low. The benzaldehyde ratio decreased as the amount of fruit added to kefir increased (*Table 3*). However, the d-limonene, 1-methyl benzene and benzene 2-ethyl-1,3-dimethyl ratios of kefir samples containing orange, mandarin and grapefruit increased at a significantly higher level than that of plain kefir (*Table 3*). Because a large amount (90.4 %) of the terpene compounds in orange juice is composed of DL-limonene (Erdoğan, 2019), the kefir samples containing citrus fruit had D- limonene at a higher ratio. Randazzo et al. (2016) found that the acetic acid ratio of fruit kefir samples ranged from 3.34 % to 9.77 %. But, we found that the acetic acid ratio of fruit kefir samples ranged between 1.81 % and 6.69 %. Randazzo et al. (2016) discovered that the benzyl alcohol ratio of fruit kefir samples was below a detectable level, which was consistent with our findings. Randazzo et al. (2016) found that the benzaldehyde ratio of various fruit kefir samples ranged from 1.57 % to11.69 % which was higher than our research findings. The organoleptic analysis results of kefir samples are shown in *Table 4*.

Table 3. Aromatic organic matter ratios of plain and citrus fruit kefir samples

Kefir Samples	Ethanol (%)	Acetic acid (%)	Butanoic acid (%)	Hexanoic acid (%)	Octanoic acid (%)	n-decanoic acid (%)	Benzoic acid (%)
Plain (Control)	4.35	14.69	5.79	14.08	12.59	5.25	3.18
Orange (23%)	1.12	3.96	1.20	3.11	2.98	1.33	0.83
Orange (37.5%)	0.89	1.81	0.62	1.51	1.56	0.74	0.50
Mandarin (23%)	2.40	4.70	1.60	3.80	3.55	1.43	1.19
Mandarin (37.5%)	4.40	3.43	1.41	3.47	3.29	1.62	1.03
Grapefruit (23%)	5.67	6.69	2.36	5.57	5.25	2.23	1.93
Grapefruit (37.5%)	5.01	3.72	1.12	2.83	2.82	1.26	0.97

Table 3. (Continued)

Kefir Samples	Benzaldehyde (%)	Benzaldehyde (2,5 bis) (%)	Silanediol dimethyl (%)	Benzyl alcohol (%)	d-limonene (%)	1-methyl benzene (%)	Eugenol % (%)	Benzene 2-ethyl-1,3-dimethyl (%)
Plain (Control)	1.34	3.32	2.07	1.36	nd	nd	nd	nd
Orange (23%)	0.47	0.99	0.90	nd	38.17	3.84	1.82	2.47
Orange (37.5%)	0.44	0.54	0.41	nd	49.30	2.61	nd	2.69
Mandarin (23%)	0.40	0.99	0.84	nd	39.64	2.77	nd	7.10
Mandarin (37.5%)	0.69	1.01	0.64	nd	38.41	4.13	nd	3.15
Grapefruit (23%)	0.71	1.48	0.73	nd	28.34	2.32	nd	3.52
Grapefruit (37.5%)	0.83	0.92	1.18	nd	39.57	2.77	nd	4.88

Table 4. Sensory analysis results of Kefir samples

Kefir Samples	Color and apperance	Texture and fluency	Taste and aroma	General acceptability
Plain (Control)	8.20	7.30	7.15	7.20
Orange (23%)	8.25	7.20	8.10	8.30
Orange (37.5%)	8.10	7.15	8.45	8.80
Mandarin (23%)	7.05	6.10	7.15	7.10
Mandarin (37.5%)	8.05	7.15	8.10	8.50
Grapefruit (23%)	6.90	6.15	6.05	6.20
Grapefruit (37.5%)	6.70	5.10	6.05	6.05

Panelists favored the orange (23 % and 37.5 %) and mandarin (37.5 %) kefir samples. Panelists gave lower score to grapefruit kefir samples than the others. Generally, all samples were found to have poor texture and fluency scores (*Table 4*). Harmankaya et al. (2019) determined that panelists favored banana and plain kefir more compared to the other samples. In this investigation, plain samples had greater texture and fluency scores than the other samples (*Table 4*). Kök-Taş et al. (2014) found that fruit kefir samples had higher sensory scores than plain kefir sample. The similar results were obtained for orange samples in this study too.

4. Conclusions

The addition of citrus fruit to plain kefir reduced the viscosity of kefir samples. Considering that the viscosity of kefir is an important quality factor, some stabilizers can be added to kefirs containing fruits to increase their viscosity. As orange was added to kefir samples, the ethanol level decreased, but the grapefruit addition caused the ethanol level to increase. The addition of citrus fruit enhanced the aromatic organic matter content, but it caused a decrease in the aromatic organic matter ratios of plain kefir. Considering the ideal aroma level of fruit and kefir, the fruit ratio must be adjusted. As a result, 23 % orange juice and mandarin juice can be added to plain kefir. The addition of 37.5 % citrus fruit juice significantly reduced the viscosity, so this state was not considered as a good result.

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