Impact of Storage Time on the Content of Kefir

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Abstract

Kefir is assumed as a functional beverage out of its probiotic content and its consumption has been increasing continually worldwide for few decades. This study was conducted to monitor the effect of storage time on the pH, acidity, total dry matter and total free amino acid content of kefir at cold storage (+4°C) for 7 days. The characteristics of kefir were analysed daily to observe the changes occurring during the storage. Total free amino acid content measurements were achieved via spectrophotometric method. The pH values of kefir inclined to decrease from 4.25 to 4.02 steadily during the storage. The acidity contents of kefir were within the range of 0.77- 0.92 g lactic acid/100 mL and have shown fluctuations in the storage duration. Levels of total dry matter augmented until the middle of the storage time and then showed fluctuations. While the highest amounts of total free amino acids were detected at the second day of storage, total free amino acid values were varying between 0.0214 and 0.0431 g/100 mL (as leucine equivalent) and storage caused significant differences on this trait of kefir.

Keywords: Kefir, fermented milk beverage, functional beverage, storage, content

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INTRODUCTION

Kefir is a probiotic fermented dairy product assumed as functional drink that is originated from Caucasus mountains of Russia and has an important role in the Mediterranean diet which is known in different names (such as kephir, knapan, kiaphur, kepi, kefer, kippi, knapon, kefyr) but mostly called "kefir" (Atalar, 2019; Rattray & O'Connell, 2011). It is produced as a result of both acidic and alcoholic fermentations, therefore it has a tart-acidic flavour with little alcohol content (about 0.5%) and creamy sparkling texture (Izquierdo-González et al., 2019). The production of kefir is traditionally based on the addition of kefir grains, yellowy tiny cauliflower-like shaped particles composed from principally lactic acid bacteria and yeasts, and sometimes acetic acid bacteria and moulds embedded in a complex polysaccharide, to cow milk (Wang, et al., 2021). However, as kefir grains from different origins contains different microorganisms, the standard production with kefir grains is hard. Herewith, in the industrial production, kefir is generally manufactured by starter cultures. The limiting factors with grains in commercial production can be listed as: contamination of grains, separation of kefir grains from the final product (Sarıca, 2019). Regarding the above-mentioned reasons, industrially, kefir is manufactured by the addition of lyophilized starter culture (combination of some bacteria and yeasts) to milk (Delgado-Fernández et al., 2019).

Consumption and popularity of kefir is constantly increasing worldwide because it has many health benefits such as lowering lactose content of milk, antimicrobial, antioxidative, anticarcinogenic, and therapeutic effects, and it also improves the absorption of nutrients, supports immune system, regulates digestion system, increases lifetime, lowers cholesterol and helps hormonal changes in the human body (De Oliveira Leite et al., 2013; John & Deeseenthum, 2015). Even though its consumption was limited with Middle East, Russia and Eastern Europe in the past, nowadays it is consumed in the most European, and Nordic countries and the United Kingdom (Delgado-Fernández et al., 2019). The first industrial level kefir production was made in Russia at the beginning of 1930s and the technique applied for kefir production today again was developed by a Russian research institute. In Turkey, industrial kefir production started in the middle of 1980s and the production was fluctuating, but after 2004 the production and consumption of kefir started to increase steadily (Alagöz Kabakcı et al., 2020; Sarıca, 2019). The characteristic of kefir changes depending on the type of milk, the starter culture used, and the conditions of fermentation process (Aiello et al., 2020, Atalar, 2019). Though kefir is generally produced from cow milk, it can also be produced from some other animal milks (goat, sheep, mare, and buffalo) or plant milks (soy, peanut, rice, coconut and almond) and lastly whey can be used for kefir production. Additionally, more recently, water kefir also has been produced (Atalar, 2019; Vimercati et al., 2020). In spite of its health benefits, kefir is not preferred by some consumers due to its acidic taste. Considering this, kefir with different flavours have been manufactured to make kefir drinking more desirable (De Roos, & De Vuyst, 2018).

Kefir is a fermented milk beverage that is produced by lactic acid and ethyl alcohol fermentation. As a result of fermentation process, lactose and proteins are degraded to less complex substances, and some vitamins and other bioactive substances like phenolic compounds are synthetized by microorganisms. The main products of the fermentation are lactic acid, ethanol and CO_2 while the secondary products are acetic acid, antibiotics, bacteriocins, amino acids and aromatic compounds such as diacetyl, acetaldehyde and acetoin (Liu et al., 2005; Sarıca, 2019). Lactic acid formed via fermentation and bioactive peptides produced by protein degradation underlie the antioxidant and antimicrobial activities of kefir (Shiby & Mishra, 2013). The vitamin (such as vitamin B_1, B_2, B_6, and B_{12},) essential amino acids and mineral content of kefir make it attractive for health-aware consumers (Alagöz Kabakcı et al., 2020).

The composition and nutritional value of kefir alters according to the composition of milk, production method of kefir (with kefir grains or starter culture) and finally the time and conditions of the fermentation process. Although kefir can be manufactured from many types of milks in different ways, the most preferred industrial production type is cow milk fermentation by starter culture. Nowadays, related with consumer demand for production kefir at homes, kefir culture is also sold in the stores. The studies in the literature have not studied the changes in kefir content daily, therefore this study aimed to provide a close look to the changes in kefir content during the cold storage period. In this study, some chemical properties of kefir produced by starter culture obtained from a countrywide market chain monitored daily during the storage (7 days at +4°C). Even though the shelf life of kefir has been specified differently, the most appropriate duration is generally given as 7 days, therefore this study focused on the changes of content and pH values of kefir for 7 days of storage.

MATERIALS and METHODS

Materials

Pasteurised milk was bought from the stores in İzmir, Turkey. Kefir starter culture in powder form was obtained from Doğadan Bizim Gıda ve Süt Ürünleri Ind.Trade. Co.Ltd. (İstanbul/Turkey) which is sold in a countrywide market chain.

Chemicals

L-leucine and ethanol were supplied from Sigma (St. Louis, MO, USA); phenolphthalein and glacial acetic acid were from Pancreac (Barselona, Spain); hydrochloric acid (HCI), sodium hydroxide (NaOH) and ninhydrin were from Merck (Darmstadt, Germany).

Production of Kefir

Kefir was produced in plain form independently twice and analysed during 7 days of storage. The analyses carried out for 7 days as the most suggested time for kefir consumption is the first 7 days. To produce kefir, pasteurised milk (2 L) was heated to 90°C for 5 min and subsequently cooled to 25 °C. After that, kefir culture (2 g) was added to the milk and fermented in an incubator at 25°C for 48 hours and then stored at +4°C. For each analysis, two samples have been taken from each replicate at the 1st, 2nd, 3rd, 4th, 5th, 6th, and 7th days of fermentation, and total dry matter, total titratable acidity, pH value and total free amino acid content has been determined for each sampling time. All experiments were repeated two times.

Measurement of pH Values

The pH values of samples were detected at approximately 15°C via the immersion of a digital pH meter (Isolab Laborgeräte GmbH, Germany), which adjusted with pH=4 and 7 standard buffer solutions, to kefir samples (Kebede et al., 2007).

Determination of Total Dry Matter Content

Total dry matter content of kefir samples were estimated gravimetrically by drying the samples in the oven at 105°C until obtain a constant weight and the results were given as % (w/w).

Estimation of Titratable Acidity

Total titratable acidities were performed via titration of samples with 0.1 N NaOH by addition of phenolphthalein to the sample mixture and results were expressed as % of lactic acid (Gül et al., 2015).

Determination of Total Free Amino Acid Content

Quantification of total free amino acid content of samples were achieved following the procedure of Folkertsma & Fox (1992) with slight modifications. 30 mL of 0.1 M HCI added to 30 mL of kefir sample and homogenized. The final blend was mixed by using a magnetic stirrer for 15 min, then allowed to stand at 40°C (1 hour), and then centrifuged (Hettich Universal 320 R, Tuttlingen, Germany) at 460*g* (30 min). Pursuant to filtration of the aqueous phase, 0.3 mL of supernatant was diluted to 2 mL by using ultrapure water, then 4 mL of Cd-ninhydrin reagent (0.8 g ninhydrin + 80 mL of ethanol (HPLC grade) + 10 mL of glacial acetic acid + 1 mL of Cd solution (1 g/mL) added and the resulting solution was vortex-mixed. After keeping it at 84°C (5 min), the mixture was cooled, centrifuged and the absorbance values were recorded at 507 nm by using a spectrometer (Agilent Cary 60 UV-Visible, Santa Clara, CA, USA).

To increase the reliability of the experiment, two additional blank solutions were used. The first solution was prepared by following the same procedure with the exception of addition 4 mL of Cd reagent without ninydrin and this solution was utilized for prevention of colour errors related with samples. The second blank solution was prepared with the same procedure by replacing 4 mL of ultrapure water in place of sample. The results were represented as leucine equivalent (g/100 ml kefir) by using calibration graph prepared with L-leucine as standard.

Statistical Evaluation of the Results

The findings of all experiments were reported as mean values and standard deviations of 4 measurements. The obtained data were compared by means of SPSS 20.00 statistical package program (IBM Corp., New York, USA). ANOVA test was applied to detect the differences and Duncan's multiple range test ($P<0.05$) was used to detect the degree of significance between the storage days, and finally Pearson's correlation test was performed to find out the relationship between pH, acidity, total dry matter and total free amino acid values of kefir samples ($P<0.05$).

RESULTS and DISCUSSION

The changes of pH values in kefir during 7 days of storage have been given in Fig. 1. The pH values of kefir tended to decrease continually with increasing storage time and were in the range of 4.02-4.35 (Table 1). The highest pH decline was observed between the 5th and 6th days of storage. Öner et al. (2010) determined the pH of kefir samples produced from cow milk with kefir grains and starter culture, and reported that the pH values of kefir with starter culture was 4.47 at the 0th day and 4.48 at the 7th day of storage while there were no significant difference due to the storage time. Similarly, Yüksel-Bilsel, & Şahin-Yeşilçubuk (2019) reported pH values of kefir as 4.26, 4.21, and 4.13 at the 0th, 5th, and 10th days of storage, respectively. Contrary to our results, Alagöz Kabakcı et al. (2020) indicated the pH values of kefir as 4.31 at the 0th day and 4.38 at the 12th day of storage which were rising with time. Regarding these studies, it can be concluded that the type of milk, type of starter culture and fermentation conditions affects the pH changes of kefir during storage.

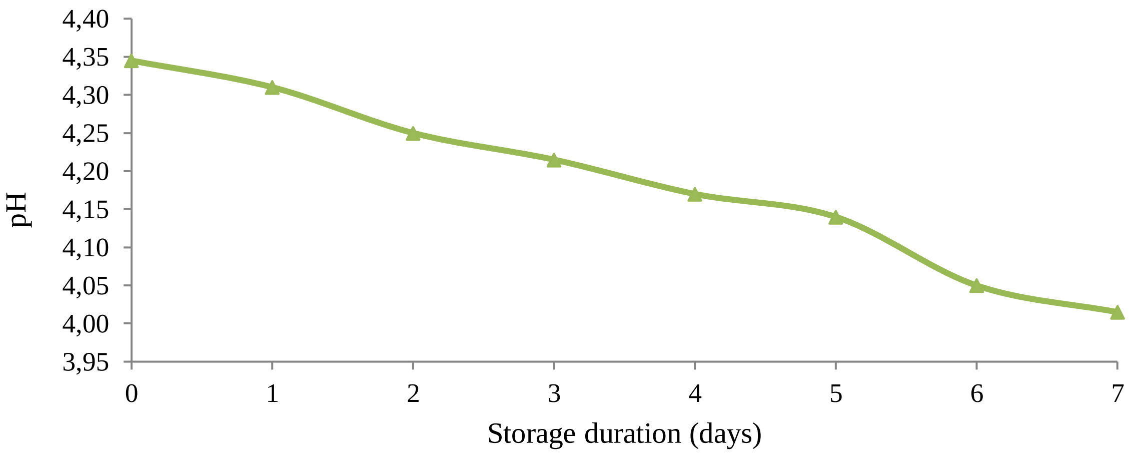

Figure 1. pH values of kefir during the storage at +4°C for 7 days

Total titratable acidities of kefir variations during the storage have been illustrated in Fig. 2. The acidity showed a downward tendency until the 5th day and then the values increased gradually. As can be seen from Fig. 2, acidity values of kefir were showing a fluctuating change during the storage. The acidity of kefir was changing between 0.77 and 0.92 g lactic acid/ 100, and significant changes (P<0.05) were recorded between the beginning and the end of the storage time. Sarıca (2019) pointed out that the acidity values of kefir remained the same between the 1st and 7th days of storage while Alagöz Kabakcı et al. (2020) and Yüksel-Bilsel, & Şahin-Yeşilçubuk (2019) notified steadily increments in the acidity of kefir during storage.

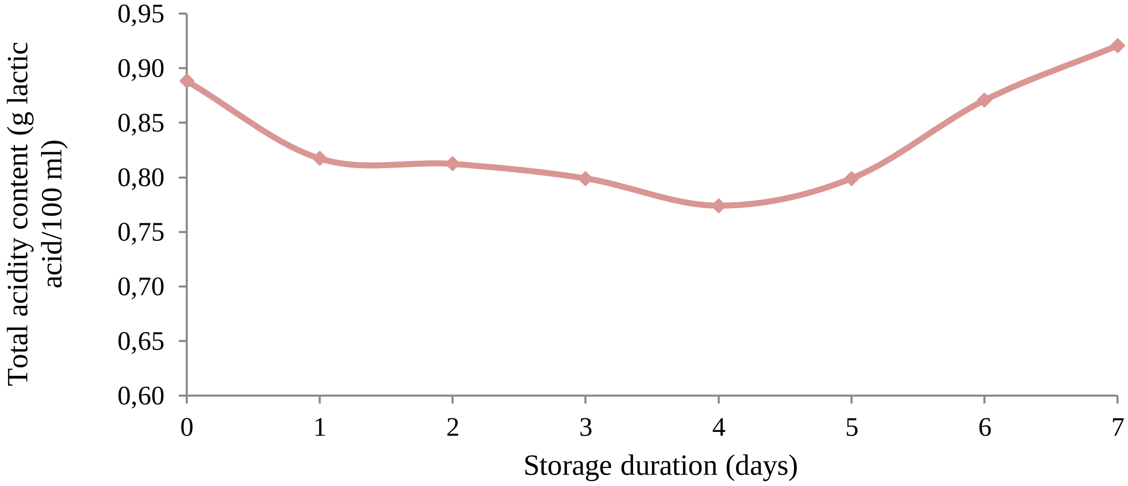

Figure 2. The alteration of total dry matter content with the increasing time at +4°C storage

The observations of total dry matter content of kefir for 7 days of storage have been exhibited in Fig. 3. According to the findings of the study, total dry matter content interval of kefir values were 10.41 (6th day) and 11.11 g/100 g (0th day). Storage time had variable effects on the total dry matter content of kefir and no significant differences (P>0.05) were detected between the values at the beginning and the end of storage (Table 1). The total dry matter contents of the current study were slightly different than the intervals stated by Atalar (2019) and Öner et al. (2010) and this variation is thought to be related with the characteristic of milk and the activity of starter cultures used in the production. Eventually, while the correlations between total dry matter and acidity, and total free amino acids were not statistically significant (P>0.05), the correlations of total dry matter and pH were positive significant (r= 0.738, P<0.05).

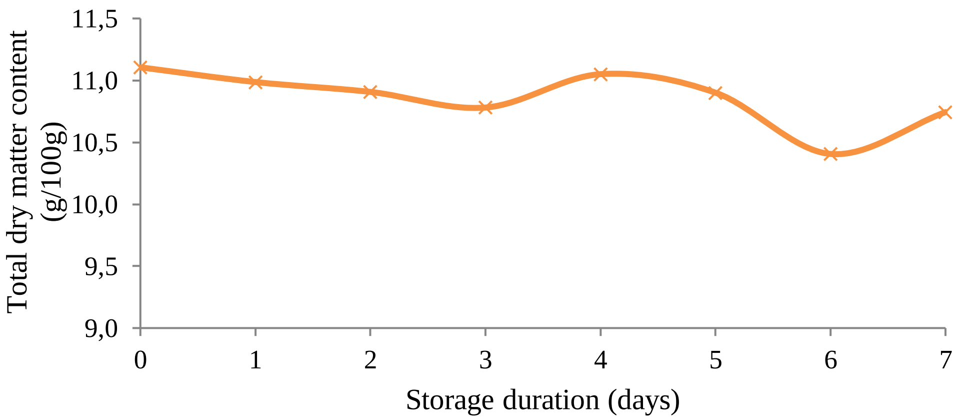

Figure 3. The effect of time on the total dry matter content of kefir during the cold storage (+4°C)

Free amino acids are nutritious components which are produced by proteolysis reaction and have noteworthy role in aroma formation, but they can also cause formation of several undesired toxic compounds. Thereof, determination of total free amino acid content of food products is of importance because free amino acids are precursors of some toxic compounds e.g. biogenic amines, acrylamide, nitrosamines and heterocyclic amines (Akan & Özdestan Ocak, 2019; De Mey at al., 2014; Keşkekoğlu & Üren, 2014; Lingnert et al., 2002).

During the storage of kefir, the fluctuations in the amounts of kefir have been displayed in Fig. 4. Related with the high protein content of milk and fermentation, total amino acid content of kefir samples showed increments and decrements during the 7 days of storage due to the actions of active microorganisms at the storage temperature (+4°C).

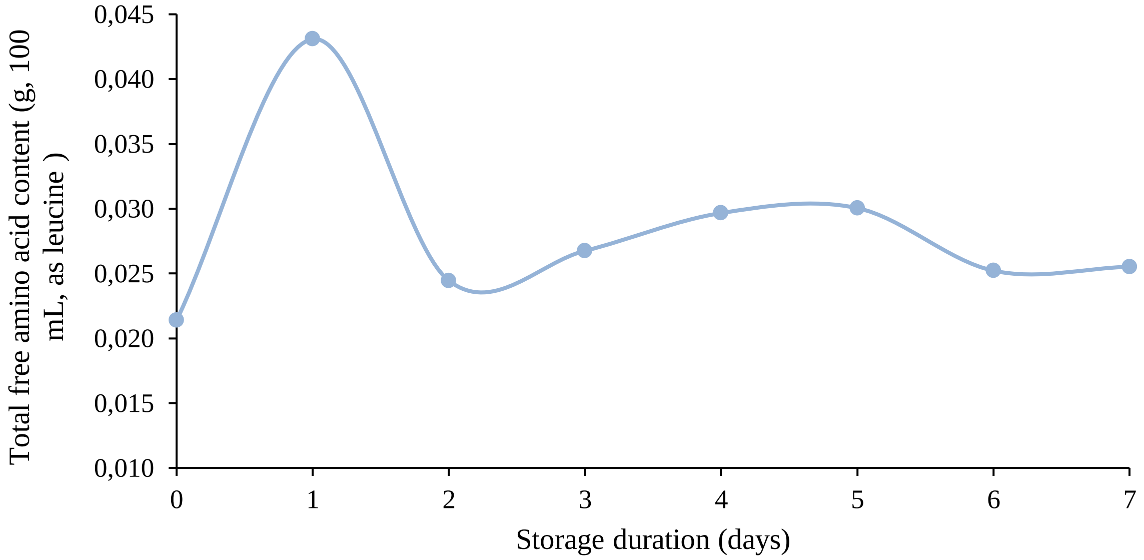

Figure 4. Changes of total free amino acid content during 7 days of storage at +4°C

The levels of total free amino acids of kefir were in the range of 0.0214 and 0.0431 g/100 mL (as leucine equivalent) which were compatible with the results (0.0070-0.0206 g leucine/100 mL) of Özdestan & Üren (2010). Contradictory with the current research, total free amino acid content of kefir reported as 0.00063 and 0.00072 g/100 g by Güler et al. (2016), and as 0.00057 g/100 g by Simova et al. (2006).

The variation in the total free amino acid content of kefir is thought to be originated from the type and composition of milk and the duration of fermentation. Özdestan and Üren (2010) found significant correlations between the amounts of total free amino acid and total biogenic amine of different kefir samples. In line with this idea, total free amino acid content of kefir also might be an indicator of total biogenic amine content.

Table 1. The alterations in the content and pH values of kefir during the storage at +4°C

Storage duration (days)	Total dry matter (g/100 g)	pH	Acidity (g lactic acid/100 mL)	Total free amino acids (g/100 mL, as leucine equivalent)
0	11.11±0.09[a]	4.35±0.01[a]	0.89±0.01[a,b]	0.0214±0.0003[d]
1	10.99±0.11[a,b]	4.31±0.01[a,b]	0.82±0.01[c]	0.0431±0.0007[a]
2	10.91±0.01[a,b]	4.25±0.00[b,c]	0.81±0.01[c,d]	0.0245±0.0005[c,d]
3	10.78±0.02[a,b]	4.22±0.01[c,d]	0.80±0.01[c,d]	0.0268±0.0010[b,c]
4	11.05±0.36[a]	4.17±0.01[d]	0.77±0.01[d]	0.0297±0.0025[b]
5	10.90±0.03[a,b]	4.14±0.06[d]	0.80±0.02[c,d]	0.0300±0.0002[b]
6	10.41±0.24[b]	4.05±0.01[e]	0.87±0.01[b]	0.0252±0.0005[c]
7	10.74±0.08[a,b]	4.02±0.00[e]	0.92±0.01[a]	0.0255±0.0003[c]

a-e; Expresses that dissimilar letters within the same column shows significant differences (P<0.05).

CONCLUSION

Kefir is a functional beverage that has been popular for few decades. The demand for kefir consumption is increasing day by day as it has many health benefits and therapeutic effects. Nowadays, preparation of food products at homes is an increasing trend because consumers think that production foods and beverages at home conditions is more hygienic, nutritious and healthy. Due to the request of consumers, nowadays kefir starter culture is sold at stores and consumers can produce kefir at home easily. This study has been conducted to determine the chemical traits of kefir prepared with starter culture obtained from a countrywide market chain. The investigation disclosed that the pH values and content of kefir changes daily at +4°C cold storage while acidity, total dry matter and total free amino acid content values were showing fluctuations.

Conflict of Interest Statement

The author indicates that there is no personal or commercial relationship that could influence the results of the study reported in this article.

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