*Araştırma Makalesi / Research Article*

# The Effect of Zivzik Pomegranate Juice on the Physico-chemical and Sensorial Properties of Kefir Produced by Conventional Method

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**Abstract**
This research was carried out to investigate the synbiotic effects of various concentrations of Siirt Zivzik pomegranate juicy kefir at different times. In this study, sterile cow's milk of 7 L (1.5% fatty), Zivzik pomegranate juice of 2 L and kefir grains of 100 g were used. Physio-chemical and sensorial analyses were conducted by conventional methods. The initial pH values of the samples were adjusted to 4.00 for the purpose of decontamination and increased slightly during the storage period. Depending on the storage period, the highest $a_w$ value was determined as 6.25% (0.973) on the zeroth day; whereas, the lowest $a_w$ value was determined in the sample in 50% concentration (0.949) on the 7$^{th}$ day. The highest dry matter amount in the samples was found in the kefir with 50% concentration (10.9%) and it was observed that the most important parameter affecting the dry matter amount was the fruit amount added as additive. It was determined that depending on the increase of the concentrations with Zivzik pomegranate juice, color measurement values (Red-Green-Blue; RGB) decreased. As a result of the sensorial analyses, the lowest general liking values according to hedonic scale of the ready-to-drink kefir was found in 6.25% concentration (10.12) and its highest general liking values according to hedonic scale were found in 50% concentration (15.94). A statistically significant negative correlation was determined between $a_w$ ($p < 0.05$) and color ($p < 0.01$) with dry matter in the samples. As a result of this study, it was concluded that new products with high functional properties were obtained, the shelf life of kefir extended partly, and Zivzik pomegranate juice may have an effect increasing the consuming level of kefir. Furthermore, it was considered that Zivzik pomegranate juice would be a reference for the studies on development of the new products to be obtained by being adding into kefir and similar other products.

**Keywords:** Kefir, zivzik pomegranate, physio-chemical, sensorial, shelf life

# Zivzik Narı Suyunun Geleneksel Yöntemle Üretilen Kefirin Fiziko-kimyasal ve Duyusal Özellikleri Üzerine Etkisi

**Öz**
Bu araştırma, Siirt Zivzik nar sulu kefirin çeşitli konsantrasyonlarının farklı zamanlardaki sinbiyotik etkilerini araştırmak için yapılmıştır. Araştırmada 7 L yarım yağlı (%1.5) steril inek sütü, 2 L Zivzik narı suyu ve 100 g kefir tanesi kullanılmıştır. Fiziko-kimyasal ve duyusal analizler konvansiyonel yöntemlerle gerçekleştirilmiştir. Örneklerin başlangıç pH değerleri dekontaminasyon amacıyla 4.00'a ayarlanmış ve depolama sürecince az da olsa artış göstermiştir. Depolama süresine bağlı olarak $a_w$ değeri en yüksek 0. gün %6.25 (0.973), en düşük ise 7. gün %50 konsantrasyonlu örnekte (0.949) belirlenmiştir. Örneklerdeki en fazla kurumadde miktarı %50 konsantrasyonlu kefirde (%10.9) bulunmuş ve kurumadde miktarını etkileyen en önemli parametrenin katkı olarak ilave edilen meyve miktarı olduğu görülmüştür. Zivzik nar sulu konsantrasyonların artışına bağlı olarak, renk ölçüm değerlerinin (Red-Green-Blue; RGB) azaldığı saptanmıştır. Duyusal analizler sonucunda içime hazır kefirlerin genel beğeni değerleri hedonik skalaya göre en az %6.25 (10.12) ve en yüksek %50 konsantrasyonda (15.94) bulunmuştur. Örneklerde negatif yönlü olarak kurumadde ile $a_w$ ($p < 0.05$) ve renk ($p < 0.01$) arasında istatistiksel olarak önemli bir ilişki belirlenmiştir. Bu araştırma sonucunda yeni ve fonksiyonel özellikleri yüksek ürün elde edildiği, kefirin raf ömrünün kısmen de olsa uzadığı ve Zivzik narı suyunun kefirin tüketim düzeyini arttırıcı yönde etkisinin olabileceği kanaatine varılmıştır. Ayrıca Zivzik narı suyunun kefir ve benzeri diğer ürünlere katılarak elde edilecek yeni ürün geliştirme çalışmalarına referans niteliği taşıyacağı düşünülmektedir.

**Anahtar kelimeler:** Kefir, zivzik narı, fiziko-kimyasal, duyusal, raf ömrü

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## 1. Introduction

The recognition of fermented foods dates back to the first periods of humanity [1] and these products are generally produced by conventional methods. The consumption rates of the fermented products with long storage periods and high nutritional values increase day by day. One of these products is kefir, the name of which is heard frequently recently and which has high nutritional value and functional properties.

Kefir is described in the Turkish Food Codex Communique on Fermented Milk Products [2] as "*Lactobacillus* kefir, different strains of *Leuconostoc*, *Lactococcus* and *Acetobacter* species (*Kluyveromyces marxianus*), starter cultures containing fermented and non-fermented yeasts (*Saccharomyces unisporus*, *Saccharomyces cerevisiae* and *Saccharomyces exiguus*) or fermented milk product using kefir grains are used specifically in fermentation". Kefir, produced from sheep, goat and cow milk, had been produced in Russia and Southwest Asia at earlier times, it has been produced mostly from cow milk industrially in many locations of the world recently. The nutritional value of fermented milk products is high and they have a longer shelf life compared to the unfermented ones; therefore, the demand for kefir has increased [1, 3]. The microorganisms included in the natural flora of kefir have an antibiotic effect on many pathogens and it has more probiotics compared to yogurt. Additionally, it is rich in vitamins K and B and it is a beverage preventing lactose intolerance [1, 3, 4].

The conventional kefir is produced by directly adding kefir grains into milk. Raw milk is heated at 85-95°C for 5-20 min, cooled up to 20-25°C and kefir grains are added at the rate of 2-10%. Then, it is fermented for 14-24 h at fermentation temperature, milk is filtered, kefir grains are separated, and kefir is stored at 4-8°C [1, 5, 6].

The studies on the nutritional and functional properties of kefir have been ongoing [7-9]. Different innovative studies are conducted on fruit flavored kefir based on the tastes of consumers from many different parts of the world [10-14]. Pomegranate contains a significant amounts of acid, polysaccharide, vitamin and polyphenol, it is also rich in anthocyanins and is antioxidative [7, 10]. *Punicalagin* is the most important phenolic material with antioxidant capacity in pomegranate juice [15]. Depending on the bio-active characteristic of phenolic materials, pomegranate juice has been used in the conventional therapy methods for centuries and it has been found to have positive effects on human health due to its antioxidant and anti-tumor characteristics [15, 16]. In the recent years, the attention of the consumers and the businesses for kefir has increased in Turkey and kefir has started to be included among the important beverages, today. As a result of the literature review, no study on adding Zivzik pomegranate into kefir was found. The aim of this study is to examine the physico-chemical changes and the sensorial properties in the product that may form upon addition of Zivzik pomegranate juice in kefir. It is thought that Zivzik pomegranate juicy kefir can be used for healthy nutrition, companies may be willing to release to the market and provide added value to the regional economy.

## 2. Material and Methods

### 2.1. Kefir production and preparing samples

In this study, semi-skimmed (1.5%) sterile cow's milk for consumption of 7 L, fresh squeezed Zivzik pomegranate juice of 2 L and kefir grains obtained from producers in Eskisehir of 100 g were used. Kefir grains were added into sterile milk reaching approximately 25°C by being kept at ambient temperature in conventional kefir production and incubated for 24 h at 25°C. At the end of the incubation, after deciding that fermentation was completed, kefir grains were taken by being filtered [1, 6]. Then, fresh-squeezed Zivzik pomegranate juice at different rates (control, 6.25%, 12.5%, 25%, 50%) was added to the obtained kefir under aseptic conditions. Afterwards, the pH values of the kefir samples with pomegranate juice samples were adjusted to 4.00 by lactic acid against possible infection and intoxication [17, 18]. After this process, physico-chemical and sensorial analyses were performed on the zeroth, $7^{th}$, $14^{th}$, $21^{th}$ and $28^{th}$ days and all the samples 200 ml in sterile glass jars were kept at +4°C.

### 2.2. Physico-chemical analyses

pH values of the samples at pH-meter (Mettler Toledo SevenCompact™ S220, Switzerland) [19], water activity ($a_w$) values in $a_w$ device (Novasina LabTouch® CH8863, Switzerland) [20], dry matter amounts in refractometer (Hanna® HI 96801, Romania) and color properties (R, G, B) in the color measurement device (Artoxy, Pencolor art 1l, Turkey) were determined [19].

### 2.3. Sensorial analyses

General liking evaluation of taste, appearance, consistency and smell of the kefir obtained on the zeroth, 7$^{th}$, 14$^{th}$, 21$^{th}$ and 28$^{th}$ days was made by 10 panelists according to the hedonic scale. In the assessment; 0.00-3.99 (very bad); 4.00-7.99 (bad); 8.00-11.99 (moderate); 12.00-15.99 (good) and 16.00-20.00 (very good) scores were used [21].

### 2.4. Statistical analyses

In the statistical assessment of the results obtained, SPSS 23.0 statistical packaged software was used and correlation analyses were performed in determining the between-group difference [22].

## 3. Results and Discussion

The average pH values at Figure 1, $a_w$ values at Figure 2, the dry matter amounts at Figure 3, RGB values at Figure 4, the general liking values based on time together with concentration at Figure 5, and the general liking values based on only concentration at Figure 6 of Zivzik pomegranate juice added samples (control, 6.25%, 12.5%, 25%, and 50%) obtained on the zeroth, 7$^{th}$, 14$^{th}$, 21$^{th}$ and 28$^{th}$ days are shown.

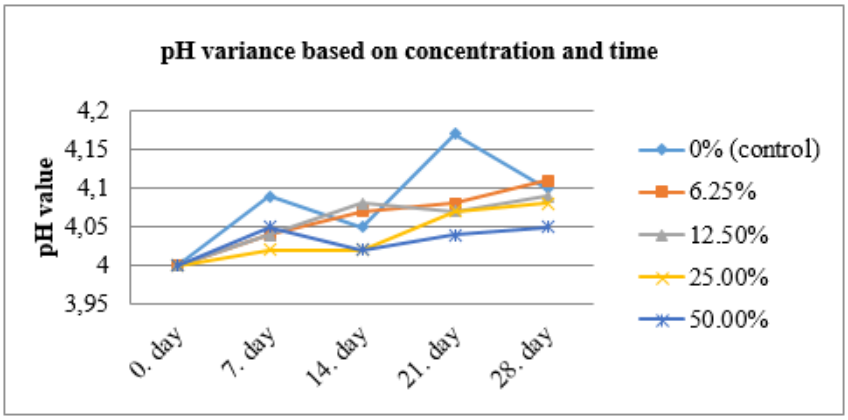


**Figure 1.** pH variance graph based on concentration and time

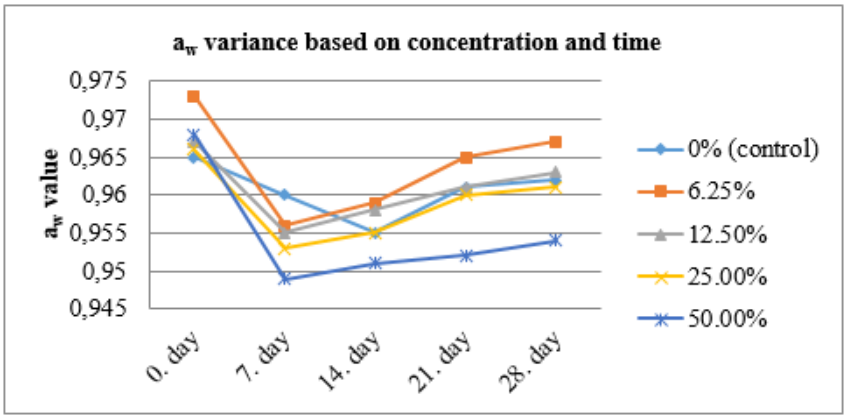


**Figure 2.** $a_w$ variance graph based on concentration and time

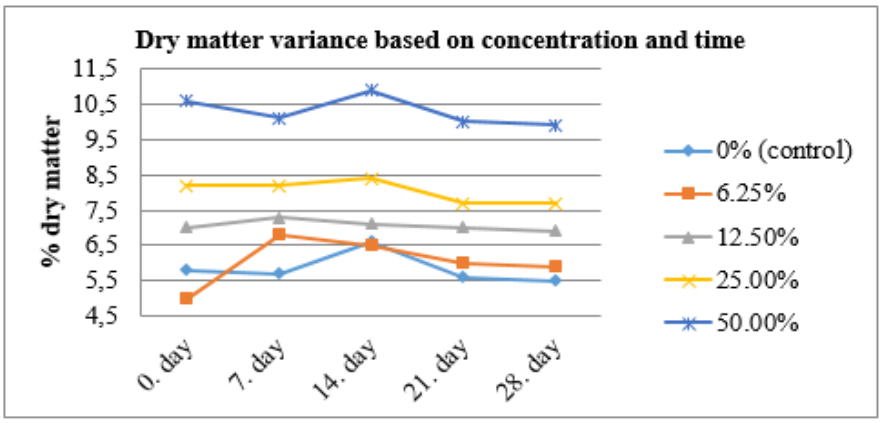


**Figure 3.** Dry matter variance graph based on concentration and time

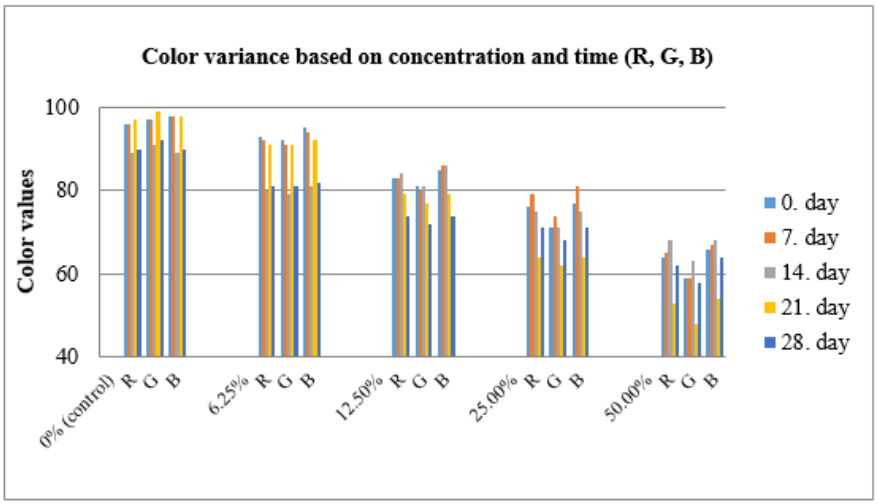


**Figure 4.** Color variance graph of the samples based on concentration and time
(R: Red; G: Green; B: Blue)

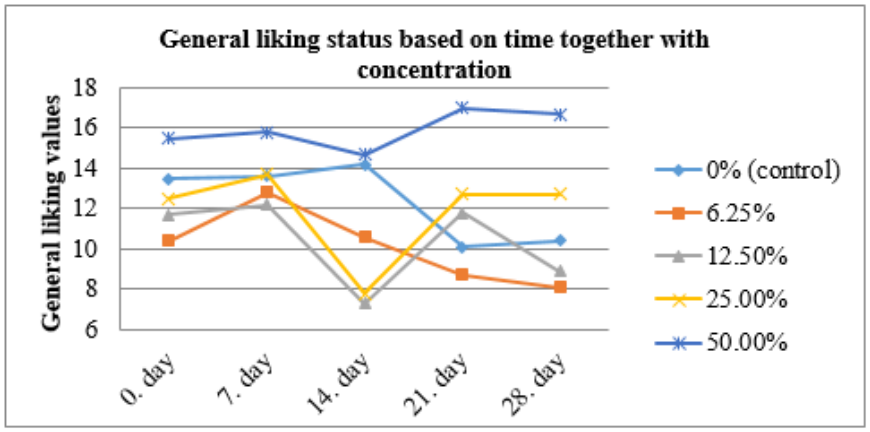


**Figure 5.** General liking variance graph of the samples based on concentration and time

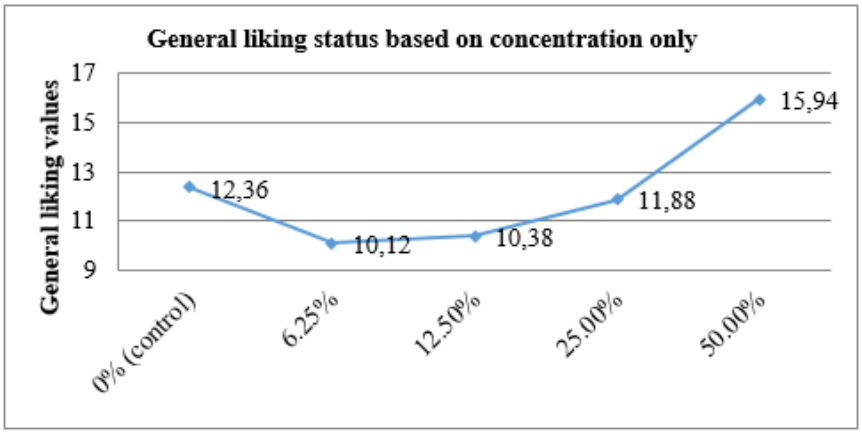


**Figure 6.** General liking variance graph of the samples based on concentration only

Determining pH value in foods indicates that the relevant food is acidic or basic [18]. In a previous study [7], it was stated that the fruit juice added to kefir decreased pH value depending on the storage time, the highest pH value was determined in the kefir without any additives, and the lowest pH value was determined in the grapefruit added kefir. In another study on kefir [10], pH value decreased depending on the storage period. It was stated that the highest pH value was obtained in kefir in the control group in the 1st storage day (4.33±0.09) and the lowest pH value was obtained in the dried lemon peel added kefir on the 27th storage day (4.15±0.03). Garrote et al. [23], stated that the pH values of the kefir produced by adding kefir grains of different types into milk varied between 3.50-4.00. Irigoyen et al. [5], stated that there was no significant change in the pH values and dry matter amounts until the 14th day of the storage in kefir they produced by adding kefir grains at the rates of 1% and 5% experimentally. In this study, no statistical difference was determined between groups and within group in terms of pH value. In a study conducted in Ankara on kefir produced commercially and conventionally [24], it was determined that while the pH value of the conventionally produced kefir varied between 4.00-6.00, the pH value of commercial ones varied between 5.35-5.80. It was reported that *Enterococcus* bacteria was determined in one of the commercial kefir samples; whereas, coliform bacteria has been determined in one of the conventionally produced kefir samples. In this study, the pH values increased slightly during the storage (Figure 1). Slight increase in the pH values was considered as an expected situation, this change was considered to be caused by the adjustment of the initial pH values and also the increase in the organic acid amounts forming in the fermentation. The differences in pH values among the studies are associated with the grains used in the production and the fact that their rates of being added to milk have affected acidity value, as stated by Garrote et al. [23] and Uslu [25]. Also, it is considered that the chemical compositions of the fruits used in the studies are effective in the variance of the pH values during the ripening process. As a matter of fact, Januário et al. [9] reported that the matters added to kefir in fruit and honey-flavored kefir ice-cream affected the acceptability level of the products.

The $a_w$ value of foodstuff is among the important parameters used to determine shelf life [18]. In terms of its microorganisms, kefir should have the minimum $a_w$ value required for these microorganisms to perform their vital activities. Lucey and Singh [26] reported that the process parameters affecting the structural characteristics of fermented milk products were fat rate and homogenization conditions, heat treatments applied on milk, the amount and nature of the starter cultures, incubation temperature, the last pH value in a product and the storage conditions. In this study, it was determined that depending on storage duration, the highest $a_w$ value (0.973) was obtained on the zeroth day of the sample with 6.25% Zivzik pomegranate juice concentration; whereas, the lowest $a_w$ value (0.949) was obtained on the 7th day of the sample with 50% concentration. High $a_w$ value may be explained as the low osmotic pressure and high microbial activity. However, low $a_w$ value may be associated with the increase of the antimicrobial activity of phenolic compounds and dry matter amount.

The total dry matter in milk and milk products is composed of fat, protein, lactose, vitamin and minerals and these nutrition components are important parameters in terms of determining the nutritional value of the product and processing the products. Determining amounts of the nutritional elements included in food is one of the criteria required to be applied in the determination of the possible tricks in the products [3, 26, 27]. Tonguç [4] reported that the dry matter amounts varied between 9.52-12.20%

in probiotic ayran, Kesenkaş et al. [28] reported that the dry matter amounts of the kefir produced using soy milk varied between 10.10-11.60%. It was reported in another study [7] conducted on adding citrus peel into kefir that total dry matter amount increased depending on the storage period and the maximum amount of fat-free dry matter was obtained at level of 13.88% on the 21$^{st}$ day. It was determined in this study that total dry matter amount decreased depending on the storage period and the minimum dry matter amount was determined in the control group on the 21$^{th}$ day (5.6%) and the maximum dry matter amount was determined in the 50% pomegranate juice concentration added kefir on the 14$^{th}$ day (10.9%). The most important parameter affecting the dry matter amount was the amount of fruit added as an additive. As a result of the between-group and within-group statistical tests were revealed that there was a difference in dry matter amounts. A general decrease was determined in dry matter as the vital activities of the nutritional elements in the composition of kefir are continued by bacteria during the storage period and they are used for the continuation of the reproduction. This was considered to be due to the fact that some microorganisms passed to death phase on the 21$^{st}$ day and there were the yeast and mold reproducing in the spoilage stage of the production.

The color of foodstuffs is among the important criteria for consumers while buying a product and are the distinctive characteristics in determining product quality. The color values of red and black mulberry samples of different varieties grown in Turkey were examined and it was found that L* (lightness-darkness), a* (greenness-redness), and b* (blueness-yellowness) values of black mulberries were higher compared to red mulberries [29]. In parallel with the results of Özgen et al. [29], in this study R (red), G (green) and B (blue) color measurement values which are the mathematical expression of color decreased depending on the increasing concentrations. The color measurement values increased again after a serious decrease on the 14$^{th}$ day during the storage in 6.25% concentration compared to the control group. The same situation occurred on the 21$^{th}$ day in the 50% Zivzik pomegranate juice added concentration (Figure 4). It may be asserted that this increase was due to the anthocyanin-flavonoids in the pomegranate juice and the coloring agents were reduced by the microorganisms based on time. Indeed, it was stated in the study by Jiménez-Aguilar et al. [30] that the high a* color value of the samples was directly proportional to the total anthocyanin capacity. Besides, it was reported that the samples with the lowest anthocyanin loss had also the lowest color loss, after the encapsulation process.

In the general sensorial analysis of the Zivzik pomegranate juice added kefir performed by the panelists, the kefir with 50% concentration has had the most liking (15.94) and the kefir with 6.25% concentration had the least liking (10.12) (Figure 6). This may be due to the fact that kefir is sweeter beyond its usual taste and panelists tend to consume kefir quite low. The fruited kefir was preferred more, which was similarly determined in the studies of Uslu [25] and Kök Taş et al. [11] conducted with different kefir samples. In other words, in the study by Uslu [25], plain kefir samples were scored by the panelists as maximum 7.38 (avg. 6.99) in terms of general liking status and the fruited kefir samples were scored as maximum 7.66 (avg. 7.42). In such a way that in the study by Kök Taş et al. [11], it was stated that the plum added kefir was preferred more by the panelists. In a different study, it was reported that 3% whey protein concentrate (WPC) added fermented goat milk was the most preferred product among the fermented milks produced by adding 3-5% WPC to goat milk and cow milk [31]. The reason of the differences between the studies may be associated with the fact that the sugar and coloring agents in pomegranate juice increase the appreciation of the product by consumers and whey affects consumers negatively in terms of taste due to salty matters included in its composition.

## 4. Conclusion and Recommendations

The parameter results of Zivzik pomegranate juice added kefir such as pH-$a_w$ values and dry matter amount-color showed that this product was drinkable. As a result of the sensorial analyses, it was determined that the Zivzik pomegranate juice added kefir to be produced with the most liking was the one with 50% concentration and it was concluded that to produce for consumption in this concentration and putting it on the market would be more effective. It was found that the variances of the pH values among the samples was positive ($p < 0.05$) and there was a negative correlation between $a_w$ and dry matter ($p < 0.05$) and between color and dry matter ($p < 0.01$). It is thought that kefir with different concentrations of Zivzik pomegranate juice may have a positive effect on the shelf life in terms of the parameters examined.

Consequently, a new product was produced in this study that may be named as the kefir with pomegranate juice. The importance of kefir in terms of health and the characteristics of pomegranate juice such as having antioxidant activity should be taken into consideration and detailed studies should be conducted on the industrial production of the kefir with pomegranate juice and initiatives must be made in order to be placed on the market. Thus, providing that it is produced under hygienic conditions, it is thought that developing innovative products other than Zivzik pomegranate will make an economic contribution to the region and it would create a field providing employment opportunities for people of the region.

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**Author's Contributions**

All authors contributed equally to the study.

**Statement of Conflicts of Interest**

There is no conflict of interest among the authors.

**Statement of Research and Publication Ethics**

The authors declares that this study complies with Research and Publication Ethics.

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