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Changes in Vitamin C and Carbohydrate Contents of Commercially Supplied Orange Juice and Nectars during Storage

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HIGHLIGHTS

- This study will help raise consumers' awareness about the vitamin C and sugar contents in market orange juices and nectars.
- This study aims to inform the appropriate storage conditions for market orange juices and nectars, providing guidance on preserving their nutritional value.

Abstract

Orange juice and nectars are important sources of vitamin C for human health, and their consumption is becoming increasingly widespread due to ease of transportation. This study aimed to trace the quality change of orange juice and nectars from two different brands, focusing on vitamin C and sugar contents, after 3 days of storage at two different temperatures (4 °C, 20 °C). 100% orange juices and orange nectars were used as study materials. Fruit juice samples prepared from concentrates with a production year of 2022 and packaged in cardboard boxes with aseptic filling technique were provided from two different companies. Unpackaged orange juices and nectars were stored for 3 days in total, and vitamin C, invert sugar, total sugar, and sucrose contents were evaluated as quality change criteria on 1st, 2nd, and 3rd days of storage. One-way ANOVA test was used to compare all groups, and post hoc Tukey test was used for differences between pairwise comparisons. Statistically, a $p<0.05$ value indicates a level of significance. Statistically significant differences were observed in orange juices and nectars across all brands and temperatures, varying by storage time. The results showed that as storage time increased, vitamin C content decreased ($p<0.05$). In comparisons made according to storage temperature, it was determined that orange nectar and orange juices of brands A and B had a statistically significantly higher amount of invert sugar at 4 °C compared to 20 °C ($p=0.008$; $p=0.001$); ($p=0.018$; $p=0.039$). As a result, it was determined that as storage time and temperature increased, the vitamin C and sugar contents of orange juices and nectars decreased. It is thought that this study will be a useful resource in raising awareness in our society about the consumption of ready-made fruit juices, particularly orange juice and nectars, and their nutritional preferences.

Keywords: Carbohydrate; orange juice; orange nectar; vitamin C

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1. Introduction

Fruits are one of the main food groups in food pyramids and healthy plate models and are among the important food groups that should be included in our diet due to their taste and health benefits, as well as their nutritional values. Based on dietary recommendations from countries and organizations regarding fruit consumption, there is consensus that increasing fruit consumption can improve overall health and reduce the risk of major non-communicable diseases (Angelino et al. 2019; Sarac and Butnariu 2020). In recent years, with increasing chronic diseases, individuals' tendency towards fruit consumption has increased and an economic market area for fruit production has come to the fore worldwide (Sun et al. 2021; Wallace et al. 2020).

Fruit juice is a beverage made from the extraction or pressing of the natural liquid found in fruits and is one of the most efficient ways to evaluate fruit production (Nighojkar et al. 2019; Ruxton and Myers 2021). Fruits constitute essential components of a healthy diet (Jaglan et al. 2022). The World Health Organization (WHO) recommends consuming 400 g of fruits and vegetables per day to prevent cancer, cardiovascular diseases, diabetes, obesity and diseases caused by nutritional deficiencies, especially seen in underdeveloped countries (WHO 2005). In our country, based on nutritional guidelines, it is stated that at least 5 portions of vegetables and fruits should be consumed per day (at least 400 g / day), and 2-3 portions of these should be fruit. In addition to fresh, frozen and dried fruits, they can also be consumed by processing them into fruit juice. Fruit juice is a popular beverage option and the juice economy is a market area that has grown significantly in recent years (TSB 2016; Besler et al. 2015).

In addition to the increasing interest in fruit juice, we also encounter a conceptual confusion regarding fruit juice. When it comes to fruit juice in society, all fruit-derived drinks come to mind. However, beverages within the scope of fruit juice sold in the market are divided into 4 different groups in the Turkish Food Codex. The main differences between these groups stem from the difference in the fruit ratios they contain. While fruit juice is mainly obtained from the whole fruit, fruit nectar can be obtained from fruit concentrate, fruit juices, fruit powders, etc. It is also stated that fruit nectar should have a fruit content between 25-99%. Fruit juices below this ratio are included in the fruit juice concentrate category. In addition, fruit juices containing 100% fruit are expressed with the phrase "100% fruit juice" (Demir et al. 2020; TGK 2014).

Fruits are basically very rich sources of vitamins and are extremely important nutrients in the realization of many biochemical functions (Arias et al. 2022). Since most of them are not synthesized in the body, they must be taken externally through foods and nutritional supplements (Zhang et al. 2020). One of these vitamins is vitamin C (L-ascorbic acid), which can cause scurvy when not taken in sufficient amounts into the body which manifests itself as bleeding, including perifollicular bleeding, ecchymoses and gingival bleeding, as a result of fatigue, disorders in bone growth and improper formation of connective tissue. Vitamin C also has an important role in modulating neurotransmitter synthesis and release in the brain. The functions of vitamin C in the brain include it as a co-factor for dopamine beta-hydroxylase in the conversion of dopamine to noradrenaline, and it also regulates the release of catecholamine and acetylcholine from synaptic vesicles by taking part in the modulation of dopaminergic and glutamatergic neurotransmission. Vitamin C also has antioxidant properties, including limiting damage to the brain caused by ischemia-reperfusion injury and protecting against glutamate excitotoxicity (Carr and Rowe 2020; Plevin and Galletly 2020). The daily dietary intake (RDA) amount of vitamin C may vary depending on various factors such as age, gender and physiological state (Carr and Lykkesfeldt 2021). In Turkey, the recommended amount of vitamin C is 110 mg for adult men and 95 mg for adult women (TSB 2016).

Evaluating the vitamin C level in fruit juices and especially orange juices is considered one of the important quality criteria when determining the quality (Polydera et al. 2003). Among fruits and juices, oranges in particular are very popular, and the commercial value of orange juice is due to its sensory qualities and, above all, its high content of natural antioxidants such as vitamin C. Additionally, phenolic acids and flavanones are the two main groups of phenolic compounds found in orange juice. Orange juice contains significant amounts of hydroxycinnamic acid and antioxidant compounds including ferulic acid, p-Coumaric acid, sinapinic acid, caffeic acid and chlorogenic acids. In addition, vitamin C found in orange juice is a nutrient that is very sensitive to heat treatment and can easily lose its stability. Therefore, significant losses in vitamin C content occur during the processing and storage stages, especially in orange juice production. In addition, storage temperature and duration, oxygen contact, salt concentration, pH, sugar and various enzymes can cause significant losses in vitamin C in orange juice (Gomes et al. 2022; Romeo et al. 2020). In addition, the production of bad taste as a result of microbial spoilage, degradation of vitamin C causes quality loss and a decrease in the shelf life of orange juice (Kaddumukasa et al. 2017).

In addition to the vitamin C content of fruits and fruit juices, their carbohydrate content is also very important in terms of nutritional value and microbial properties (Li et al. 2020). Fruits mainly contain carbohydrate types such as glucose and fructose, but also contain some sucrose and mannose. The ratio of these components may vary depending on the type of fruit (Brizzolara et al. 2020). It is stated that carbohydrate content is an important indicator in evaluating the quality of fruits and fruit juices. Since fruit juices are very prone to adulteration and imitation due to their properties, carbohydrate analyzes are frequently used to detect fraud in the fruit juice industry and can prevent this situation. It is stated that temperature and time are very important on the carbohydrate content of fruit juices, especially on biochemical activity and microbial proliferation during storage and processing (Li et al. 2020; Tezcan et al. 2009; Tüfekci and Fenercioğlu 2010). In the light of this information, this study aimed to trace the quality change of orange juices and nectars of two different brands, which are widely consumed in our country, with vitamin C and carbohydrate contents after 3 days of storage and two different storage temperatures (4 °C, 20 °C).

2. Materials and Methods

2.1. Research Design

In the study, 100% orange juices and orange nectars were used as study materials. Fruit juice samples prepared from concentrates with a production year of 2022 and packaged in cardboard boxes with aseptic filling technique were provided from two different companies. Packaging in metal and glass boxes was not included in the study due to low usage prevalence according to consumer data. Care was taken to ensure that the expiration dates of the samples taken for all brands were similar. Based on the information that the recommended consumption period of fruit juices is 3 days after opening the packaging, the unpackaged samples were stored at two different temperature storage conditions (4 °C, 20 °C). In order to monitor the quality change, orange juice and nectars were analyzed every day, based on the 3-day storage period after the samples were put into use. All laboratory analyzes were conducted in duplicate. Vitamin C and carbohydrate contents of fruit juice and nectar samples were analyzed as quality change criteria.

2.2. Determination of Vitamin C (Ascorbic Acid)

Vitamin C content in orange juice and nectar was determined using the iodine titration method. This method is based on two formulations. In the first formulation, iodine production is carried out in an aqueous medium with potassium iodate, and in the second reaction, oxidized vitamin C is obtained as a result of the interaction of the produced iodine with the sample. Both reactions were carried out with dilute sulfuric acid. In the first reaction stage, potassium iodide solution was added for the dissolved iodide ion source (Dioha et al. 2011). By adapting the method to this study, vitamin C analyzes in orange juices and nectars were carried out based on 0.005 mol L^{-1} iodine solution and 5% starch indicator solution. The values obtained as a result of the analysis are expressed as mg/100 mL.

$$KIO_3 + 6H+ + 5I- \rightarrow 3I_2 + 3H_2O(l) + K+ \text{ (}I_2\text{ production) (aq)}, \quad (1)$$

$$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2I + 2H+ \text{ (C vitamin oxidation) (aq)}, \quad (2)$$

2.3. Determination of Total Carbohydrate, Invert Sugar

It was determined using the Lane-Eynon method and colorimetric method for the determination of carbohydrates in orange juice and nectar according to storage time and temperature Carbohydrate determination in the method is based on the reduction of Copper-II oxide in the Fehling solution to water-insoluble Copper-I-oxide. According to the method, since copper oxide is insoluble in water, the water-soluble complex salt of copper (synthetic salt) is used in the determination. NaOH provides the basicity of the medium. The soluble copper tartrate ion is blue in this solution. Since the copper ions formed by the reduction of copper do not form complex ions with tartrate, they separate and precipitate as copper-1 oxide. Methylene blue, used as an indicator, is blue in basic media and colorless in the presence of sugar. Therefore, it becomes colorless at the end point of the titration and the medium becomes copper red (Sewwandi et al. 2020). The values obtained as a result of the analysis are expressed as g/100 mL.

$$2CuO \rightarrow Cu_2O + O, \tag{3}$$

2.4. Determination of Sucrose Amount

Sucrose content was obtained by multiplying the difference between the total carbohydrate and reducing sugar amounts obtained as a result of the analysis by 0.95.

2.5. Statistical Analysis

All analyses carried out in the study were performed in duplicate. In the statistical evaluation of the study, the normality distributions of the samples to be used were evaluated with the Shapiro–Wilk Test in terms of their suitability for the number of samples. One-way ANOVA test was used to compare all groups, and post hoc Tukey test was used for differences between pairwise comparisons. As a result of the analyses, quantitative variables are shown with mean and standard deviation (SD) values. The data obtained was analyzed using SPSS (Statistical Package for the Social Sciences) 26 package program. Statistically, $p<0.05$ value indicates the level of significance.

3. Results and Discussion

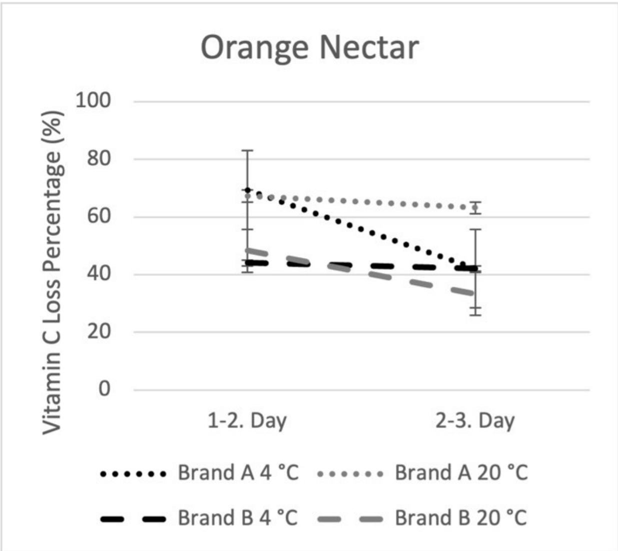
The change in the amount of vitamin C in orange juices and nectars according to storage time, temperature and brands is summarized in Table 1. Statistically significant differences were obtained in orange juices and nectars in all brands and temperatures, according to storage times, and it was determined that as storage time increased, vitamin C content decreased ($p<0.05$). No significant results were obtained in pairwise comparisons between storage temperatures and brands ($p>0.05$). The change in vitamin C loss percentages of orange juices and nectars according to storage time, temperature and brands is shown in Figure 1.

In a study conducted in Poland, it was determined that the vitamin C content of orange juices, regardless of their type, decreased within 24 hours (Kujawińska et al. 2022). In similar studies, it was determined that the vitamin C content of orange juices decreased as the storage time increased (Kabasakalis et al. 2000; Klimczak et al. 2007). In a study examining the effect of storage temperature on vitamin C levels in citrus juices, it was found that vitamin C levels decreased as storage temperature increased. It has been determined that as vitamin C loss increases, hydroxydimethylfurfural (HMF) levels in fruit juices increase (Burdurlu et al. 2006). In this study, similar to the literature, it was determined that there were decreases in the vitamin C values of orange juice and nectars from the first day of storage. In addition to storage time and conditions, it is thought that vitamin C losses in orange juice and nectar may be due to the heat produced by the juicer device, including during production and packaging. In this study, vitamin C levels were lower at 20 °C compared to 4 °C, confirming the possible thermal instability of vitamin C. In addition, it can be stated that vitamin C losses occur even during the production phase through the oxidation of ascorbic acid by catalyzing trace amounts of iron from the cutting parts of the juicer. Orange juice is one of the sources of vitamin C containing strong antioxidant properties, and vitamin C concentration is an important indicator of orange juice quality. When the literature is reviewed, it is known that storage temperature and duration, oxygen contact, salt concentration, pH, sugar and various enzymes cause significant losses in vitamin C in orange juice. In addition, citrus variety, fruit maturity, climate, juice processing and storage conditions can affect the amount of vitamin C, carbohydrate composition and other characteristic properties of the juice (Gomes et al. 2022; Kujawińska et al. 2022; Romeo et al. 2020).

Table 1. Change in vitamin C amounts (mg/100 mL) in orange juices and nectars according to storage time, temperature and brands

		A Brand Orange Nectar	p	A Brand 100% Orange Juice	p	B Brand Orange Nectar	p	B Brand 100% Orange Juice	p
4 °C	1st day	0.62 ± 0.03[a]	**<0.001**	0.53 ± 0.04[a]	**0.006**	0.34 ± 0.05[a]	**0.013**	0.72 ± 0.03[a]	**0.003**
	2nd day	0.19 ± 0.01[b]		0.34 ± 0.05[b]		0.19 ± 0.01[b]		0.53 ± 0.04[b]	
	3rd day	0.11 ± 0.02[b]		0.19 ± 0.01[b]		0.11 ± 0.02[c]		0.39 ± 0.02[c]	
20 °C	1st day	0.58 ± 0.04[a]	**0.001**	0.43 ± 0.04[a]	**0.007**	0.29 ± 0.01[a]	**0.001**	0.58 ± 0.04[a]	**0.001**
	2nd day	0.19 ± 0.01[b]		0.29 ± 0.01[b]		0.15 ± 0.01[b]		0.39 ± 0.02[b]	
	3rd day	0.07 ± 0.02[c]		0.19 ± 0.01[c]		0.1 ± 0.01[c]		0.19 ± 0.01[c]	

Results are expressed as the mean±SD of two replicates. a,b,c: Different letters in the same column indicate statistically significant difference between samples (p<0.05).



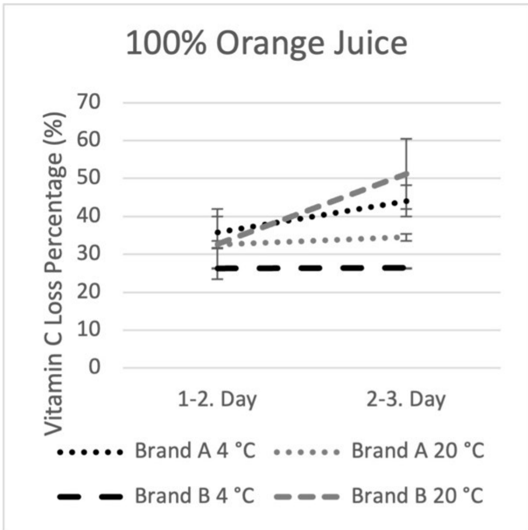


Figure 1. Change in vitamin C loss percentages of orange juices and nectars according to storage time, temperature and brands

The change in the amount of invert sugar in orange juices and nectars according to storage time, temperature and brands is summarized in Table 2. Although no statistically significant differences were observed in orange juices and nectars in all brands and temperatures according to storage times, it was determined that as storage time increased, decreases in invert sugar content were observed (p>0.05). In addition, in comparisons made according to storage temperature, it was determined that orange nectar and orange juices of brands A and B had a statistically significantly higher invert sugar content at 4 °C compared to 20 °C (p=0.008; p= 0.001); (p=0.018; p=0.039). No significant results were obtained in comparisons made according to brands (p>0.05).

It is stated in the literature that invert sugar contents may change with storage (Schnepel and Hoffmann 2013; El-Geddawy et al. 2024). According to the results of this study, it is thought that there may be microbial increases depending on the storage time and temperature, and that the invert sugar content may be converted into smaller particles by being used by these microbial components. It should be stated as one of the important results of this study that there is no need for very long periods of time in the storage period for microbial degradation and as can be understood from the invert sugar values in this study, it has been observed that decreases may occur in a short period of time such as 3 days. While this result emphasizes the importance of storage time and temperature, it may indicate that such rapid degradation does not provide sufficient microbiological protection not only after the packaging is opened but even before the packaging is opened.

Table 2. Change in invert sugar amounts (g/100 mL) in orange juices and nectars according to storage time, temperature and brands

		A Brand Orange Nectar	p	A Brand 100% Orange Juice	p	B Brand Orange Nectar	p	B Brand 100% Orange Juice	p
4 °C	1st day	5.61 ± 1.13	0.115	4.95 ± 1.00	0.209	5.21 ± 1.05	0.054	3.91 ± 0.78	0.137
	2nd day	3.94 ± 0.79		3.49 ± 0.70		4.07 ± 0.82		2.66 ± 0.54	
	3rd day	2.94 ± 0.59		3.2 ± 0.65		1.86 ± 0.38		2.23 ± 0.45	
20 °C	1st day	2.73 ± 0.55	0.169	2.13 ± 0.43	0.189	2.07 ± 0.42	0.253	2.39 ± 0.48	0.167
	2nd day	1.99 ± 0.40		2.04 ± 0.42		1.85 ± 0.37		1.94 ± 0.39	
	3rd day	1.61 ± 0.33		1.28 ± 0.26		1.33 ± 0.27		1.37 ± 0.28	

Results are expressed as the mean±SD of two replicates. a,b,c: Different letters in the same column indicate statistically significant difference between samples ($p<0.05$).

The change in the total carbohydrate amount in orange juices and nectars according to storage time, temperature and brands is summarized in Table 3. Total carbohydrate content could not be determined as storage time increased and in all samples at 20 °C. The carbohydrates found in orange are basically glucose, fructose and sucrose (Cywińska-Antonik et al. 2023). During processing and storage, sucrose can turn into glucose and fructose under the influence of acidic conditions. The average total carbohydrate content in orange juice is approximately 8.9 g/100 mL (Zhang and Ritenour 2016). In addition, it has been stated that the total sugar composition, concentration and ratios in orange fruit can be affected by many factors such as the citrus varieties used, the maturity of the fruit, climatic conditions, juice processing and storage conditions (Cancalon 1994; Grierson 2006). For example, in one study, the effect of climatic conditions was determined that glucose, fructose and sucrose had a ratio of 1:1:2 (by weight) from December to April, but the ratios changed to 1:1:3 from May to June of the same season (Cancalon 1994). It was stated that similar results were obtained in another study (Lee and Coates 2006). In this study, the total sugar contents between commercial brands may have differed due to similar factors.

Table 3. Change in total carbohydrate amounts (g/100 mL) in orange juices and nectars according to storage time, temperature and brands

		A Brand Orange Nectar	A Brand 100% Orange Juice	B Brand Orange Nectar	B Brand 100% Orange Juice
4 °C	1st day	8.71 ± 1.76	9.02 ± 1.82	12.16 ± 2.45	7.48 ± 1.51
	2nd day	6.58 ± 1.33	7.42 ± 1.49	6.56 ± 1.32	-
	3rd day	-	-	-	-

Results are expressed as the mean±SD of two replicates. a,b,c: Different letters in the same column indicate statistically significant difference between samples ($p<0.05$). "-": No content detected.

The change in the amount of sucrose in orange juices and nectars according to storage time, temperature, and brands is summarized in Table 3. When the storage period was more than 24 hours, sucrose content could not be detected in all samples at 20 °C. In this study, it was determined that the sucrose content varied between 2.36-6.6 g/100 mL. In a study, it was determined that sucrose levels in the orange juices examined varied between 3.3 and 5.3 g/100 mL (average 4.4 g/100 mL) (Zhang and Ritenour 2016). It is thought that the reason for these differences in sucrose content in the literature may be due to differences in the orange variety used to obtain orange juice and nectar. Additionally, in this study, it was determined that carbohydrate contents decreased as storage time increased, and this could be attributed to possible microbial formations. HMF formations, which have been proven to be a cancer agent and have been found in fruit juices through literature review, are an issue that should not be ignored. It is obvious that beneficial nutrients in fruit juices decrease and harmful nutrients increase due to the effect of microbial proliferation. For this reason, it may be important to be careful about consuming ready-made fruit juices, including orange juice and nectars.

Table 4. Change in sucrose amounts (g/100 mL) in orange juices and nectars according to storage time, temperature and brands

		A Brand Orange Nectar	A Brand 100% Orange Juice	B Brand Orange Nectar	B Brand 100% Orange Juice
4 °C	1st day	2.94 ± 0.59	3.88 ± 0.78	6.6 ± 1.34	3.4 ± 0.69
	2nd day	2.51 ± 0.5	3.74 ± 0.76	2.36 ± 0.48	-
	3rd day	-	-	-	-

Results are expressed as the mean±SD of two replicates. a,b,c: Different letters in the same column indicate statistically significant difference between samples ($p<0.05$). “-”: No content detected.

3.1. Limitations and Strengths

The strengths of this study lie in the authors' consideration of vitamin C and carbohydrate contents in orange juices and nectars, examining not only their analytical significance but also their nutritional role. There is very little research in the literature on the content of fruit juices and nectars, but it is thought that this research will make important contributions to nutrition and society. In addition, this study has an important practical dimension as it provides information to the consumer about which storage condition and duration will be best in terms of the bioavailability of vitamin C. A Short storage period can be shown as a study limitation. However, the experiment planning was made considering that the recommended consumption period for the consumer is a maximum of two days after opening the package.

4. Conclusions

Considering the results of this study, in terms of consuming orange juice and nectar, opening the packaging and consuming the product will be more beneficial in terms of benefiting from the nutrients it contains. In addition, when evaluated in terms of vitamin C content, orange juices and nectars are thought to be rich in vitamin C throughout our society, and these products are frequently included in children's lunchboxes. However, the results of this study also showed that many factors, from the processing stage to the consumption stage, can cause vitamin C loss when obtaining juice from fruits in these products. Turkey's Food and Nutrition Guide accepts 95 mg/day vitamin C intake for adult women and 110 mg/day vitamin C intake for adult men as the recommended adequate intake level, although it varies in different age groups and during pregnancy and lactation periods (TSB 2016). In children, the need for vitamin C varies depending on the developmental period: preschool, school age and adolescence. While the maximum vitamin C value was determined as 0.7 mg/100 mL in the measurements made, vitamin C values as high as 95-110 mg/day cannot be met with these products. For this reason, it is a misconception that fruit juices, especially orange juices and nectars, in our society are thought to be rich in vitamin C. Therefore, it is very important to inform and raise awareness of the society in this regard by expert dietitians. Foods such as citrus fruits, strawberries, kiwi, tomatoes, cabbage and green leafy vegetables are rich in vitamin C and are additionally a source of fiber. In fruit juice production, in addition to vitamin C, one of the important nutrients, pulp is also lost. For this reason, consuming foods with their pulp is important for health and to benefit from vitamins. In addition, this study shows that orange juices and nectars have very high sugar content in their label value. Considering that these products are mostly consumed by children in their developmental age, this much sugar intake will be quite harmful. Market fruit juices may be economically affordable, ready-made and easily accessible, but it should not be forgotten that many factors such as storage temperature and storage time can change the nutritional contents in fruit juices. It is thought that this study will be a useful resource in terms of raising awareness in our society regarding the consumption of ready-made fruit juices, especially orange juice and nectars, in their nutritional preferences.

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