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EVALUATION OF ASCORBIC ACID CONTENT AND TOTAL ANTIOXIDANT STATUS OF FRESH-SQUEEZED ORANGE JUICES

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ABSTRACT

This study aimed to evaluate i) consumer behaviour regarding fruit juices and ii) vitamin C content and total antioxidant status (TAS) of fresh-squeezed orange juices. To determine consumption habits of consumers regarding fruit juices, a questionnaire was applied. Fresh-squeezed orange juices were supplied from the stands, cafes and patisseries/bakeries/pastry shops. Ready-made orange juices were purchased from the markets. The pH, ascorbic acid (mg/100 g) and TAS (mmol/L) of orange juices were analyzed. This study found that there was a tendency towards fresh-squeezed juice types especially orange juice and the main motivational factors were naturalness/freshness, taste perceptions, vitamin C content and positive health effects. It was shown that ascorbic acid contents (42.4±6.71 vs. 17.0±11.09 mg) and TAS values (3.3±0.35 vs.1.2±0.90 mmol/L) of fresh-squeezed orange juices were higher compared to ready-made orange juices and vary depending on the place of purchase($p<0.05$). As a result of keeping the juices although they were freshly squeezed, their ascorbic acid contents decreased. Also, there was a positive correlation between ascorbic acid and TAS values in all orange juices ($r:0.902$, $p<0.05$). Supporting the perception of consumers, ascorbic acid content and antioxidant capacities of fresh-squeezed orange juices were higher compared to ready-made juices, and the fruit juices must be consumed without being kept even if they are freshly squeezed.

1.Introduction

Citrus, a genus belonging to the Rutaceae family, is one of the most demanded agricultural products in the world due to its rich bioactive content, and health benefits. Vitamin C, one of the component in citrus, is among the strongest antioxidant foods due to polyphenols, flavonoids and carotenoid compounds (Abudayeh *et al.,* 2019). Vitamin C, in particular, has been found to correlate with more than 65% of antioxidant activity in many fruits and fruit beverages (Mditshwa *et al.,* 2017).

Vitamin C is considered to be the most important water-soluble antioxidant and shows anti-atherogenic, anti-inflammatory, antimicrobial, anticancer, anti-epileptic and immune-enhancing effect in metabolism by directly cleaning the superoxide radical, single oxygen, hydrogen peroxide and hydroxyl radical (Abudayeh *et al.,* 2019; Kaur & Kapoor, 2001; Klimczak *et al.,* 2007). Due to such potential health benefits, the interest in consuming foods containing vitamin C is increasing day by day. Orange and orange juice is among the most important sources of vitamin C in the diet due to high consumption (Klimczak *et al.,* 2007). The vitamin C content of orange juice varies between 15-45 mg/100 mL and a glass of orange juice

(200 mL) can meet about 30-80% of the recommended daily intake of vitamin C (Klimczak *et al.,* 2007).

The vitamin C content of orange juice is affected by many factors. The type, variety, harvest time and post-harvest conditions of the orange used may affect the Vitamin C content of the final product (Mditshwa *et al.,* 2017). Post-harvest conditions include storage conditions and post-harvest stress, such as physiological disorders and mechanical damage. Conditions such as temperature, relative humidity, packaging material, and access to light and oxygen in the storage process of the orange used in the production of orange juice and orange juice offered for consumption affect the content of vitamin C (Klimczak *et al.,* 2007; Mditshwa *et al.,* 2017). In a study, an increase in anthocyanin, flavanone, hydroxycinnamic acid content and antioxidant capacity and decrease in vitamin C were observed in oranges stored at 6 °C for 65 days (Rapisarda *et al.,* 2008).

Nowadays, when grocery shopping, consumers take cognizance of the health benefits of the products besides their taste and content (Bech-Larsen & Grunert, 2003). So, they may tend to prefer fresh-squeezed fruit juices and consider them healthier than packaged ones. A study has shown that the most important factors for consumers when purchasing fruit juice are vitamin, mineral and antioxidant content, respectively (Oral *et al.,* 2016). It is extremely important to know the vitamin C and antioxidant levels of products that reach the end consumer, as orange juice is an important source of dietary vitamin C. Moreover, consumers' juice consumption has been increasing in recent years, and it is important to investigate the juice consumption trends and motivational factors in this regard.

This research was carried out to evaluate consumer behaviour regarding fruit juices and at the same time vitamin C content and total antioxidant status (TAS) of fresh-squeezed fruit juices.

2. Materials and methods

This study was conducted in two stages. While the tendencies of consumers towards fruit juices are determined in the first stage, some orange juice types (freshly squeezed and store-bought) were analysed in the second stage.

2.1. Determination of consumer behaviour regarding fruit juices

This stage of the study was conducted on a total of 246 adults (18-64 years), 128 of which were male and 118 were female. To determine individuals' consumption habits of fruit juices, a questionnaire was applied with a face-to-face interview technique. Thus, the types of fruit juice consumers often consume, the most frequently consumed fruit juices according to their fruit/aroma, and the reasons for consumption of fruit juice were determined.

2.2. Fruit juice analysis

2.2.1. Sample selection and supply

In line with the data obtained in the first stage of the study, it was determined that consumers mostly prefer fresh-squeezed orange juice. Therefore, fresh-squeezed orange juice was chosen as a basic sample in the second stage and further analysis of fresh-squeezed and store-bought orange juices were performed (Figure 1).

2.2.2. Fresh-squeezed orange juices

In Turkey, fresh squeezed juices are offered for sale mostly in municipal or private stands. Fruit juice is typically filled in transparent pet bottles immediately after the fruits are washed and squeezed using a manual extractor. In cases where freshly squeezed fruit juices are not purchased by consumers immediately, they are offered for sale in bottles kept in AHT coolers in ice.

In this study, fresh-squeezed orange juices in plastic bottles were purchased from the stands, cafes and patisseries/bakeries/pastry shops located in the 3 large central districts in Ankara province, Turkey by researchers and were analysed after brought to the laboratory by ensuring the cold chain. Fresh-squeezed orange

juices supplied from all points were distinguished as "freshly-squeezed/not kept" and "previously squeezed/kept".

2.2.3. Store-bought orange juices

Packaged orange juices in Tetra Pak were purchased from the big market chains in the districts where fresh-squeezed juices were supplied, and the samples brought to the laboratory for analysis were prepared for analysis in a dark environment isolated from UVs.

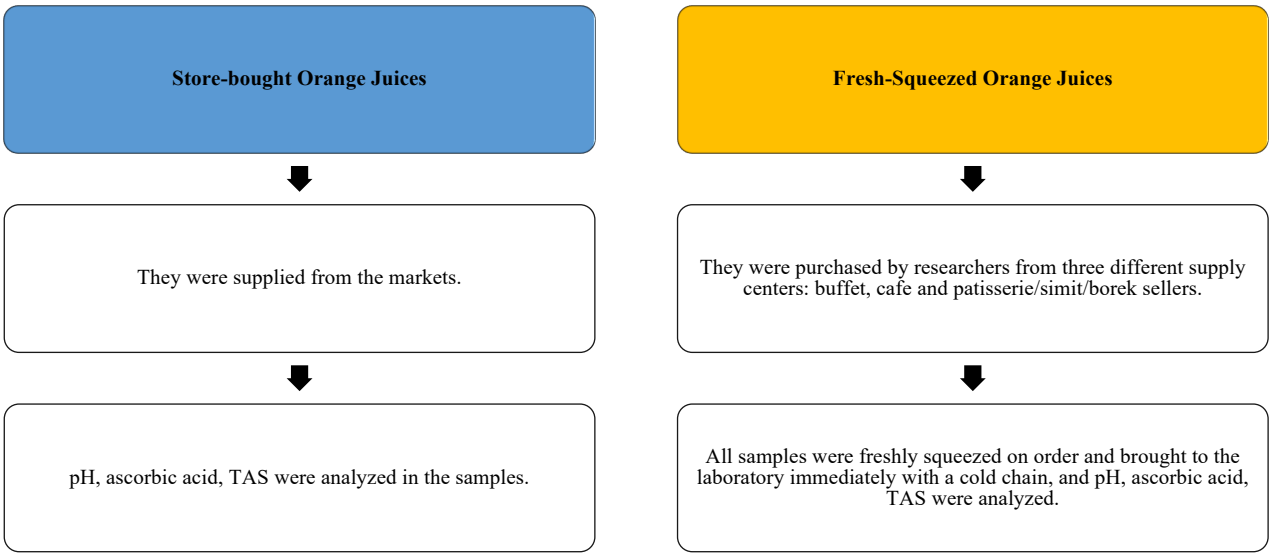

Figure 1. The flow of orange juices analysis

2.3. Analysis of samples

2.3.1. Determination of pH

Commercial pH meter (SelectaTM) was used for the pH determination of samples. The pH meter was calibrated before starting the analysis. For this, the pH meter was adjusted with buffer solutions of pH 4, 7 and 10 at 21 0C. Then, the samples were mixed until they were homogenized and the pH values were measured by taking them into a clean beaker.

2.3.2. Ascorbic acid analysis

In this study, the titrimetric method and Tillman reagent (2,6-dichlorophenolindophenol) were used for the determination of ascorbic acid in orange juices. The principle of this method is based on the principle that ascorbic acid is a strong reducing agent that bleaches the 2,6-dichlorophenolindophenol dye. In the last phase of the titration, the pink colour of the acid medium in 2,6-dichlorophenolindophenol, which can no longer react, appears due to the complete depletion of ascorbic acid in the medium.

All chemicals used in the analysis were provided before the study and all solutions were prepared fresh. Metaphosphoric acid solution (3%) was used as an extraction solution. Standard ascorbic acid solution (0.02%) was dissolved in 3% metaphosphoric acid solution and its volume was increased to 250 mL and titrated with 2,6-dichlorophenolindophenol and the amount of ascorbic acid in the samples was calculated as follows:

Ascorbic acid mg/100 g = [(V.f)÷m]x100 (1)

V: Amount of 2,6-dichlorophenolindophenol solution spent in titration (mL)
f: factor of 2,6-dichlorophenolindophenol solution
m: the amount of sample taken for titration

2.3.3. Determination of Total Antioxidant Status

All samples were centrifuged at 3000 rpm for 3 minutes at +4 °C and their supernatant fractions were separated. TAS levels were measured using commercially available kits (Relassay, Turkey). The novel automated method is based on the bleaching of characteristic colour of a more stable ABTS (2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox equivalent/L. All experiments and analyses were carried out in duplicate.

2.4. Statistical Analysis

The data were analysed using SPSS 22.0. Percentage (%) and arithmetic mean±standard deviation ($\bar{x}$±SD) values were given as descriptive statistics for variables. "Mann-Whitney U Test" or "Kruskal-Wallis Test" was used to compare pH, ascorbic acid (mg/100 g) and TAS (mmol/L) values in orange juices according to the type, place provided and holding state. "Spearman Correlation Test" was used to determine the relationships between the variables. Level of significance was determined as α=0.05 in all analyses.

3.Results and discussions

Orange juices have been accepted as a part of healthy nutrition with their vitamin and bioactive compounds, and therefore antioxidant content for many years. Orange juices have types such as concentrated, frozen and pasteurized (Lee & Coates, 1999). In recent years, unpasteurized-hand and industrial fresh squeezed types have increased in line with the increasing health awareness of consumers all over the world (Gul *et al.,* 2011). In this context, this study was conducted to evaluate consumer trends regarding freshly squeezed orange juices and vitamin C and antioxidant contents which serve as hypothesis for these trends.

3.1. Determination of Consumer Behaviour Regarding Fruit Juices

87.0% of individuals consume fruit juices at least once a week. The factors that individuals pay attention to when purchasing fruit juices include production and expiration date (86.5%), contents (76.4%), price (59.3%) and nutritional value (45.1%), respectively (Data not shown in the table).

Figure 2 shows the types of fruit juice most frequently consumed by individuals. Accordingly, the most frequently consumed fruit juices are freshly squeezed juices (84.9%), 100% fruit juices (60.9%), nectars (39.0%) and flavoured juices (30.9%), respectively (Figure 2).

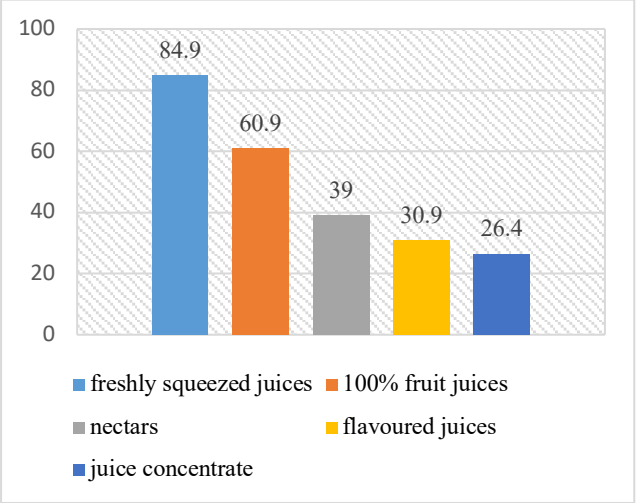

Figure 2. Fruit juices most frequently consumed by individuals

Figure 3 shows the most frequently consumed juice types according to fruit/flavour. Accordingly, the most frequently consumed fruit juices according to their fruit/flavour are orange juice (77.6%), cherry juice (58.9%), mixed juice (54.0%), peach juice (51.2%), pomegranate juice (36.1%), apple juice (32.5%), pineapple juice (24.3%) and mango juice (19.9%), respectively (Figure 3).

Individuals stated that they purchase juices mostly from the grocery stores (51.6%), while 36.6% stated that they prepare juices at home through their own means. The places where

individuals consume fruit juices most frequently are houses (84.5%), cafes (48.3%) and bakeries (24.8%).

74.7% stated that they consume fruit juices since they like the taste, 58.9% stated that they consume fruit juices since juices are healthy, 53.6% stated that they consume fruit juices since juices are rich in vitamin content, and 17.4% stated that they consume fruit juices since juices are affordable. 65.4 percent stated that they believe that freshly squeezed fruit juices are healthier because of being fresh (69.4%) and natural (77.6%), not containing added sugar (49.4%), and richness in vitamin C (44.7%) (Figure 4).

This study found that the majority of individuals purchase/consume fruit juices, and there is a tendency towards freshly squeezed juice types (Figure 3 and Figure 4). It was determined that naturalness/freshness, taste perceptions, vitamin C content and the thought that they have positive effects on health are the main motivational factors in fruit juice consumption (Figure 4).

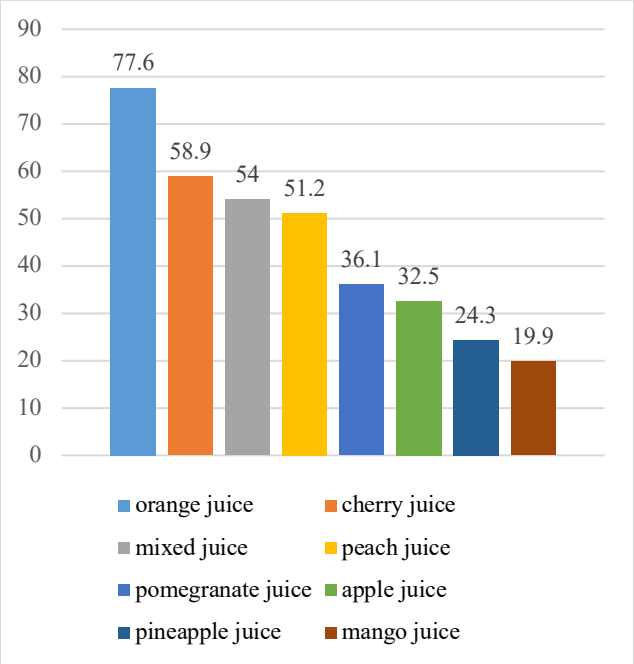

Figure 3. The most frequently consumed fruit juices according to their fruit/flavour

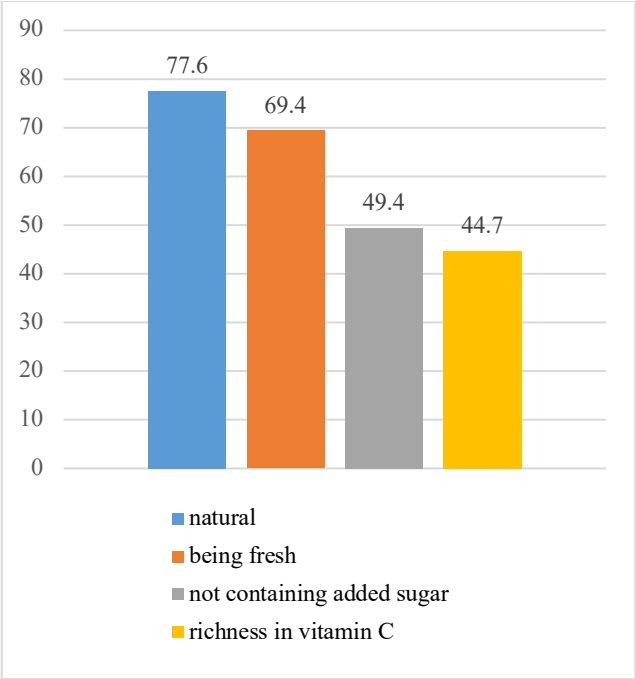

Figure 4. Reasons for consuming freshly squeezed fruit juices

Freshly squeezed unpasteurized fruit juices are often preferred by consumers because of their taste and nutritional value, and although they are typically costlier than pasteurized fruit juices, they are effectively marketed in many countries (Bagci & Temiz, 2011). It is reported in sensorial analyses that the most preferred juice is hand-squeezed orange juice compared to other juice extraction technologies (Baldwin *et al.,* 2012). Supporting the results of this (present) study, another study conducted in Turkey found that the opinion that the juice is healthy is widespread and especially vitamin content makes juices healthy (Bagci & Temiz, 2011). The aforementioned study reported that the opinion that fruit juices squeezed at home are healthier than packaged juices is very common (Oral *et al.,* 2016.). Moreover, another study determined that as the socioeconomic and cultural levels of adolescents increase, the consumption frequency of freshly squeezed fruit juice increases (22%), but the frequency of ready-made fruit juice consumption decreases (7%) (Gürel & Hisar, 2018). Another study reported that moderate consumption of 100% orange and grapefruit juices contributed to

nutrition because they contain vitamin C and other important nutrients such as potassium, folate, magnesium, and vitamin A, but it is not associated with anthropometric measurements in either children or adults (Rampersaud & Valim, 2017). Similarly, consumption of fruit juice and citrus fruits was found to be an important predictor of plasma vitamin C concentration in smokers and individuals with high body mass index (BMI) (Langlois *et al.,* 2016).

3.2. Fruit Juices Analysis

The pH, ascorbic acid (mg/100 g) and TAS (mmol/L) values of orange juice types are shown in Table 1. Accordingly, this study determined that ascorbic acid contents of freshly squeezed orange juices were higher (2.5 times) compared to ready orange juices (42.4±6.71 vs. 17.0±11.09 mg) regardless of pH levels ($p<0.05$). Similar to the findings of this study, Turkomp-Turkish National Food Composition Database reports that the average amount of L-ascorbic acid in orange juices are 46.8 mg/100 g (Turkomp Database, 2020). It is reported that there are many pre- and post-harvest factors affecting the content of vitamin C in foods as ascorbic acid and dehydroascorbic acid (Lee & Kader, 2000). Genotypic differences, climatic conditions, cultivation and processing techniques are among the effective factors (Lee & Kader, 2000). Furthermore, supporting the findings of this study, pasteurization/thermal processing in production of fruit juices is reported to cause significant losses in vitamin C. In the literature, it is suggested that fruit juices with no thermal processing have higher vitamin C content, but the shelf life of such juices is shorter and vitamin C losses may occur (Martí *et al.,* 2009).

When freshly squeezed orange juices were evaluated according to the place of purchase, the pH values of orange juices purchased from stands, cafes and patisseries/bakeries/pastry shops were statistically different ($p<0.05$). Ascorbic acid contents (39.8±8.78 mg/100 g) and TAS values (3.1±0.39 mmol/L) of freshly squeezed orange juices purchased from cafes were lower compared to those purchased from other places ($p<0.05$). It was determined that the pH (3.32±0.27 vs. 3.63±0.19), ascorbic acid (40.3±8.83 vs. 43.3±5.42 mg/100 g) and TAS values (3.2±0.45 vs. 3.3±0.29 mmol/L) of freshly squeezed orange juices kept in bottle for a while were lower compared to those instantly consumed ($p <0.05$) (Table 1).

In the present study, it was determined that the place of purchase was also important in terms of ascorbic acid content in freshly squeezed orange juices, but as a result of keeping the juices although they were freshly squeezed, their ascorbic acid contents decrease and pH levels change (Table 1). Storage conditions (temperature, pH, oxygen density of the environment, metal content, exposure to UV beam, etc.) are also important determinants of vitamin C (Martí *et al.,* 2009). A study evaluated the losses in vitamin C after storage of some juice types in plastic bottles at room temperature (29±1°C) or refrigerator temperature (4±1°C) for 4 weeks (Ajibola *et al.,* 2009). It was found that losses of vitamin C vary according to fruit type and storage method, but citrus juices such as orange, lemon and lime experience similar losses due to oxidation in vitamin C regardless of pH factor (Ajibola *et al.,* 2009). Another study found that there may be losses in vitamin C in fruits exposed to light by 10% when stored for 6 days at 5°C (Gil *et al.,* 2006). Moreover, it was reported that 0.34 mg/100 mL of vitamin C is lost per month, which was initially 32.8-40.6 mg/100 mL, even after freezing the freshly squeezed unpasteurized orange juices sold in polyethylene bottles (Lee & Coates, 1999). In this study, it is thought that keeping the orange juices especially in translucent containers, even if they are freshly squeezed, is the reason for the decrease in ascorbic acid content. Therefore, if consumers are to consume freshly squeezed juices in terms of vitamin C, it is believed that these juices must be consumed without being kept.

Table 1. pH, ascorbic acid (mg/100 g) and TAS (mmol/L) values of orange juices

	pH	Ascorbic Acid (mg/100 g)	TAS (mmol/L)
Type of fruit juice			
Freshly squeezed (n=45)	3.53±0.26	42.4±6.71	3.3±0.35
Ready-Made (n=7)	3.37±0.24	17.0±11.09	1.2±0.90
	p>0.05	**p<0.05**	**p<0.05**
Freshly squeezed fruit juices			
Place of Purchase			
Stands (n=13)	3.32±0.27	43.4±4.07	3.4±0.16
Café (n=18)	3.68±0.23	39.8±8.78	3.1±0.39
Patisseries/Bakeries/Pastry Shops (n=14)	3.54±0.15	44.8±4.45	3.3±0.38
	p<0.05	**p<0.05**	**p<0.05**
Holding state			
Freshly squeezed (n=31)	3.63±0.19	43.3±5.42	3.3±0.29
Previously squeezed/Kept (n=14)	3.32±0.27	40.3±8.83	3.2±0.45
	p<0.05	**p<0.05**	**p<0.05**

TAS: total antioxidant status

This study no statistically significant correlation was found between pH values and ascorbic acid (r: 0.078, p>0.05) and TAS (r: 0.031, p>0.05) values in all orange juices (p>0.05). In fact, ascorbic acid is very stable between pH 2 and 4. However; as pH level exceeds pK1 value 4.04, ascorbic acid loses its stability between pH 4 and 6 at the maximum level and irreversibly and swiftly gets hydrolysed to 2,3-diketo-L-gulonic acid, which has no vitamin activity (Gregory III, 1996). In this study, it is thought that the pH values of orange juices being acidic may be the reason for not detecting this relationship and therefore, it is not related to antioxidant capacity.

Moreover, there was a positive correlation (r:0.902, p<0.05) between ascorbic acid (mg/100 g) and TAS values (mmol/L) in all orange juices. A correlation (p<0.05) was found between ascorbic acid (mg/100 g) and TAS values (mmol/L) in both freshly squeezed (r:0.856, p<0.05) and ready-made orange juices (r:0.955, p<0.05). There is a positive correlation (p<0.05) between ascorbic acid (mg/100 g) and TAS values (mmol/L) in freshly squeezed orange juices purchased from stands (r:0.951, p<0.05), cafés (r:0.904, p<0.05) and patisseries/bakeries/pastry shops (r:0.697, p<0.05). In freshly squeezed orange juices; TAS values (mmol/L) are positively correlated (p<0.05) with ascorbic acid (mg/100 g) in both freshly squeezed juices (r:0.777, p<0.05) and kept juices (r:0.986, p<0.05) (Data not shown in the table).

In this study, similar to the vitamin C results, antioxidant capacities of freshly squeezed orange juices were found to be higher compared to ready orange juices (3.3±0.35 vs.1.2±0.90 mmol/L) and vary depending on the place of purchase (p<0.05) (Table 1). The antioxidant capacities of the freshly squeezed but kept orange juices were also found to be lower (p<0.05) (Table 1). Citrus fruits and juices contain many phytochemical/bioactive compounds that contribute to antioxidant capacity in addition to vitamin C. In a study evaluating the total antioxidant potential of beverages, it was determined that red grapes juice> mango juice> guava juice> cocktail juice> pineapple juice> orange juice> cherry juice> apple juice have antioxidant potentials, respectively (Ramadan-Hassanien, 2008). However, it is reported that fruit juice production processes have important effects on antioxidant potential, and contrary to manual squeezing, industrial production techniques can cause significant losses in bioactive compounds such as caffeic acid derivatives, vicenin 2 (apigenin 6,8-di-C-glucoside), and narirutin (5,7,4'-trihydroxyflavanone-7-rutinoside).

However, it was determined that such changes have no significant effect on the total antioxidant capacity of fruit juices, and pulp and L-ascorbic acid contribute to the antioxidant capacity of orange juices (77-96%) (Gil-Izquierdo *et al.,* 2002). Similarly, the present study also found a correlation between the vitamin C content and TAS values of orange juices. In a similar study, daily consumption of 500 mL of orange juice increased plasma C vitamin levels and decreases the concentration of 8-epi-PGF (2 alpha) and improves antioxidant status especially in smokers (Sánchez-Moreno *et al.,* 2003). Moreover, 8 fl. oz. doses of orange juice are reported to reduce lipid peroxidation, similar to 70 mg daily dose of vitamin C supplements, in healthy women (Johnston *et al.,* 2003). In terms of antioxidant activity, the bioavailability of bioactive compounds and antioxidant components in fruit juices is also an issue under discussion. A study found that the bioactive compounds of orange pulp and juice show high bioavailability, and especially the fruit itself contributes to antioxidant activity (De Ancos *et al.,* 2017). However, another study reports that the vitamin C bioavailability and antioxidant activity of freshly squeezed orange juices may vary depending on the orange type rather than the processing method (Mennah-Govela & Bornhorst, 2017). In this study, no characterization study was performed for bioactive compounds other than vitamin C, which may exhibit antioxidant activity, and it is thought that it will be beneficial in future studies.

4. Conclusions

In summary, this study determined that the majority of consumers have a tendency especially towards freshly squeezed fruit juice types, and the important motivation factors include nutritional value and health perception. Supporting the perception of consumers, ascorbic acid content and antioxidant capacities of freshly squeezed orange juices were determined to be higher compared to ready-made juices, and it was emphasized that the fruit juices must be consumed without being kept even if they are freshly squeezed.

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