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Evaluation of the effects of storage time and temperature on the beverages

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Abstract
Beverages are an important part of the food sector their storage is also one of the most critical issues. This study investigated the effects of different storage temperatures and times on physicochemical properties, flavonoid and organic acid contents of soda, sherbet and ice tea produced by adding sour grape concentrate. Changes in the flavan-3-ol content of ice tea were also measured during the storage period. The beverages were stored in three different conditions, cold storage (~4°C), room temperature (~24°C) and controlled storage (20±1°C), for six months and analyzed every two months. Storage temperatures and time affected the total soluble solids and acidities of the beverages ($p \leq 0.05$). Tartaric acid decreased during storage, especially during the first two months in sherbets. Ice tea and soda drinks were found more stable than sherbets. The malic acid was found the major organic acid in beverages. Flavonoid content in ice tea was higher than others. The flavonoid concentrations of ice tea stored at 20 and 24°C and of sherbet at 4°C were statistically significant as a function of storage time while these values were not significant for ice tea stored at 4°C and of sherbet at 20 and 24°C ($p \leq 0.05$) . Concentrations of flavan-*3*-ols varied with storage conditions. The levels of epicatechin, epigallocatechin and epigallocatechin gallate in ice tea samples decreased between an average of 43.72 and 71.15% at the end of six months of storage. Principal component analysis separated two months storage from other storage periods and perfectly discriminated the studied flavan-3-ols except catechin. Soluble solid and brix-acid ratio also dissociated similarly to flavan-*3*-ols.

Keywords: Soft drinks, storage, Physico-chemical parameters, Flavonoids, Flavan-*3*-ols

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INTRODUCTION

Non-alcoholic drinks include bottled water, coffee and tea, soft drinks, juices and other similar products. The latest trends in this industry are consumer products that focus on health and wellness. Functional and fermented of non-alcoholic beverages goal to provide some benefit to the different consumer groups beyond thirst-quenching or flavour (Anonymous, 2024). In recent years, the increasing demand for functional beverages has accelerated product development efforts and the introduction of different products into the sector. In particular, several studies have been conducted on innovative functional drinks using different fruits and herbal extracts (Balaswamy et al., 2011; Jooyandeh, 2015; Nanasombat et al., 2019; Aguilar et al., 2018; Din et al., 2019; Idan et al., 2021; Bendaali et al., 2022; Paredes et al., 2022; Nikolaou et al., 2023).

Flavonoids having a protective role against human diseases such as inhibition of plasma platelet aggregation, radical scavenging activity and exhibiting antibacterial, antiviral and anti-allergenic effects are phenolic compounds and abundant in foods and beverages of herbal origin (Yang et al., 2009; de la Bastida et al., 2022;). The flavan-*3*-ols, flavonols and anthocyanins are in the flavonoid group. The sour grapes contain considerable amounts of polyphenols, as ripe grapes (Hayoğlu et al., 2009; Sabir et al., 2010). Polyphenol content varies depending on maturity, culture, soil and environmental conditions (Sabir et al., 2010). Accordingly, phenolic compounds can also be found in considerable amounts in ripe grape and sour grape-based beverages. Sour grape juice or concentrate has a distinctive tartaric flavour with high acidity, polyphenol content. It can be used to acidify, source polyphenols and flavour drinks. On the other hand, tea drinks are also an important source of phenolic compounds (Wang et al., 2000; Henning et al., 2003; Serpen et al., 2012; Korkmaz et al., 2019; Thennakoon et al., 2022).

Ingredient stability is one of the most critical parameters of stored beverages. These ingredients are generally carbohydrates, proteins, acids, vitamins and phenolics. In particular, parameters such as pH, acidity and total soluble solid (TSS) are monitored to provide information on whether there is any microbial change in the beverages both during processing and storage. However, it is necessary to analyze the relevant components when monitoring of reduction of nutritional compounds. The effects of storage conditions on physical, chemical, microbial and sensory parameters of soft drinks being fruit-based soft drinks and tea drinks have been investigated by many researchers (Spanos and Wrolstad, 1990; Wang et al., 2000; Balaswamy et al.,2011; Genova et al., 2012; Yadav et al., 2013; Jooyandeh, 2015; Din et al., 2019; Omokpariola, 2022; Kumar et al., 2023). However, studies on the storage stability and changes in carbonated drinks, sorbets and ice tea produced with the addition of sour grape concentrate are limited. Furthermore, there are no studies on the changes of flavan-*3*-ols, which are found in significant amounts in tea-based beverages, under different storage conditions.

The aim of this study was to reveal the impacts of different storage temperatures and times on the physico-chemical properties, flavonoid and organic acid contents of sodas, sherbets and ice tea prepared by adding sour grape concentrate. In addition, the study aims to determine the changes in flavan-*3*-ols, one of the most important components of tea drinks, as a consequence of storage conditions.

MATERIALS AND METHODS

Preparation of the samples

The material of this study consists of drinks produced with the addition of sour grape concentrate. These drinks were soda (carbonated drinks or gaseous), sherbet and ice tea. The brix-acid ratios were used to design the drinks. These ratios were determined to commercial beverages and previous literature (Plestenjak et al., 2001; Balaswamy et al., 2011; Jooyandeh, H., 2015). The TSS of the sour grape juice concentrate used for beverage production was 45°Brix. The pH and acidity of this concentrate were 2.50 and 19.3 g/L, respectively. The soluble solids values were set at 10°Brix for the soda and sherbet samples and 8°Brix for the ice tea samples. For the production of sherbet samples, the brix-acid ratio was adjusted to 20 by adding sour grape concentrate and sugar syrup. The samples were then filled into 250cc bottles, closed with crown caps and pasteurized (85°C/15 min). To produce soda, sugar syrup and sour grape concentrate were mixed with carbonated water and the final brix-acid ratio was adjusted to 30. These mixtures were then bottled and sealed. In accordance with the Turkish Food Codex Communiqué on Non-Alcoholic Beverages (2007/26), sorbic acid (0.25 g/L) and benzoic acid (0.15 g/L) were used as preservatives. To prepare ice tea, black tea (0.75 kg Çaykur Kamelya, Türkiye) was infused (15 min) and diluted 2-fold with boiled aqua. Then sugar syrup, infused black tea and sour grape concentrate were mixed and the final brix-acid ratio was adjusted to 35. The iced tea produced was bottled and closed. The bottles were pasteurized at 85°C for 15 minutes.

Measurement of soluble solids, acidity and brix-acid

The soluble solids values of the samples were measured directly using a refractometer (Hanna HI96801, Romania). Total acidity was determined by the titration method. 10 mL sample was titrated by 0.1 N NaOH to pH 8.1 and the acidity value was expressed as tartaric acid equivalent (Ough and Amerine, 1988). The brix-acid ratio was calculated by proportioning the soluble solids value to the total acid.

Determination of flavonoids

The aluminium chloride colorimetric method was used to analyze the total flavonoids in the samples (Zhishen et al., 1999). The 1 mL of beverage was diluted with 4 mL of distilled water, and 300 µL of sodium nitrite (%5) was added to the diluted sample. After incubation for 5 minutes, 300 µL of %10 aluminium chloride was added to the mixture and incubated for 6 minutes. Then 2 mL sodium hydroxide (1 molar) was added and the final volume was adjusted to 10 mL with distilled water. A spectrophotometer (Thermo Sci., Multiskango, Finland) was used to measure the absorbance of the samples at 510 nm. Flavonoid results were expressed as catechin equivalents (mg/L).

Determination of organic acids

The analysis of tartaric and malic acids in the beverages was carried out with slight modifications of the method previously described by Castellari et al. (2000). An Agilent 1260 HPLC system (Quat pump, degas unit, autosampler, column oven, DAD detector and Lab advisor chemstation software) was used for the analysis. The 10 mL diluted sample was filtered through a 0.45 PTFE syringe filter and injected directly into the system. The column temperature was set at 30 °C and the elution time was 12 minutes. The measuring wavelength was 210 nm. The flow rate was set to 1 mL per minute for isocratic flow. Separation was performed on an Agilent ODS C18 column (250 x 4.6 mm, 5 µm). The mobile phase was 0.05 N H_2SO_4. The concentrations of tartaric and malic acids in the samples were detected by comparing their retention times and spectra with those of analytical standard solutions. The analysis was conducted at a wavelength of 210 nm. Results were calculated using the calibration curves and presented as g/L.

Determination of flavan-*3*-ols

The HPLC analysis of flavan-3-ols was performed according to the method previously described by Porgalı and Büyüktuncel (2012) with slight modifications. The HPLC system (Agilent 1260, USA) consisted of a diode array detector (DAD), a quaternary pump, an autosampler, a degasser and a column oven. The software system was Agilent lab advisor chemstation. A C18 Inertsil ODS-3 reversed-phase column (250 x 4.6 mm, 5 µm) was used for the separation of flavan-3-ols. Beverage samples were adequately diluted with mobile phase and passed through a PTFE syringe filter (0.45 µm, Sartorius, Germany) before injection into the instrument. The mobile phase was methanol: HPLC water: formic acid (19.5:80.2:0.3) and the flow was isocratic. The column oven was set at 40 °C. The flow rate was 1 ml/min and the injection volume was 5 µL. Detection of flavan-3-ols was carried out at a wavelength of 280 nm. Chromatographic analysis was performed in triplicate and retention times and UV spectra of analytical phenolic standards were used for compound identification.

Storage conditions

The beverages were stored at different temperatures to determine the effect of storage conditions. Cold storage (~4°C), room conditions (~24°C) and temperature-controlled storage (20±1°C) were used for storage during six months. A refrigerator was used to create the cold storage conditions and the temperature was set at ~4°C. The beverages were stored a temperature-controlled storage and the temperature was set at 20±1°C. A normal temperature uncontrolled storage was used for storing beverages at room conditions (~24°C). Beverages were analyzed for physicochemical properties, organic acids, flavonoids and flavan-3-ols for every two months up to the end of storage.

Statistical analysis

In the current study, all analyses and treatments were carried out in triplicate. The mean values of the results were displayed with their standard deviations. The data were evaluated by one-way analysis of variance with Duncan's multiple comparison test using by SPSS IBM Statistics program ($p \leq 0.05$). Principal component analysis (PCA) was conducted to determine the relationships among the physicochemical parameters, organic acids and flavan-3-ols of the iced tea samples.

RESULTS AND DISCUSSION

In the current study, the effects of storage time and temperature on beverages were evaluated. The physico-chemical parameters of the soft drinks were presented in Table 1. Total acidity values ranged from 0.37 to 0.40% at 4°C, 0.38 to 0.51% at 20°C and 0.35 to 0.48% at 24°C for sodas depending on storage time. These values changed between 0.24 and 0.28% at 4°C, 0.24 to 0.26% at 20°C and 24°C for ice tea samples. Total acidity of sherbets was between 0.47 and 0.50%. The highest acidity variance with storage conditions was observed for sodas. The changes in acidity of the samples were also presented as a graphical on Figure 1.

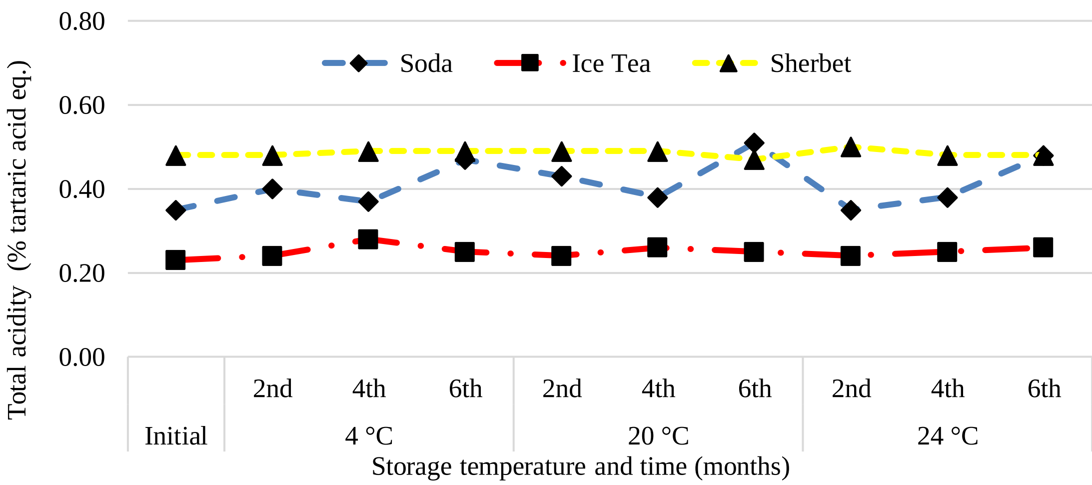

Figure 1. The changes in the total acidity of the soft drinks
*Cold storage: ~4 °C; temperature-controlled storage: 20°C; room conditions: ~24°C.

The TSS values varied between 10.67 and 11.30°Brix at 4°C, 10.77 and 11.47°Brix at 20°C and 10.80 and 11.80°Brix at 24°C for sodas during six months. These values ranged from 7.87 to 8.40°Brix for ice tea samples and 10.27 to 10.84°Brix for sherbets according to the storage temperature and time. There were no significant quantitative changes in TSS values among treatments for ice tea and sherbets, although the differences were statistically significant at the $p \leq 0.05$ level. Soda TSS values showed a higher variation than other samples. The TSS changes of the soft drinks were graphically illustrated on Figure 2 also.

Table 1. The changes in physico-chemical parameters with storage conditions.

Soft Drinks	Storage temperature	Storage time, months	Total acidity, %	TSS, °Brix	°Brix/Acid
Soda		*Initial*	0.35±0.01	10.33±0.60	29.51±0.74
	Cold storage, 4 ºC	*2nd*	0.40±0.01b	11.03±0.05b	27.44±0.13b
		4th	0.37±0.01c	10.67±0.05c	28.57±0.14a
		6th	0.47±0.01a	11.30±0.01a	24.13±0.39c
	Controlled storage, 20 ºC	*2nd*	0.43±0.01b	11.47±0.10a	26.95±0.01b
		4th	0.38±0.01c	11.10±0.17b	29.29±0.01a
		6th	0.51±0.01a	10.77±0.19c	21.30±0.01c
	Room conditions, 24 ºC	*2nd*	0.35±0.01c	11.80±0.08a	34.15±0.24a
		4th	0.38±0.01b	10.80±0.01c	28.47±0.31b
		6th	0.48±0.01a	11.67±0.05b	23.44±0.30c
Ice Tea		*Initial*	0.23±0.01	8.07±0.06	35.75±0.23
	Cold storage, 4 ºC	*2nd*	0.24±0.01c	8.40±0.05a	35.27±0.14a
		4th	0.28±0.01a	8.10±0.01c	29.27±0.18c
		6th	0.25±0.01b	8.20±0.01b	32.68±0.46b
	Controlled storage, 20 ºC	*2nd*	0.24±0.01c	8.30±0.01a	34.32±0.30a
		4th	0.26±0.01a	8.17±0.05b	31.53±0.10c
		6th	0.25±0.01b	8.20±0.01b	32.39±0.23b
	Room conditions, 24 ºC	*2nd*	0.24±0.01c	8.23±0.05a	34.68±0.25a
		4th	0.26±0.01a	7.87±0.05c	29.92±0.34c
		6th	0.25±0.01b	8.07±0.05b	31.95±0.28b
Sherbet		*Initial*	0.48±0.01	10.40±0.0.01	21.76±0.18
	Cold storage, 4 ºC	*2nd*	0.48±0.01b	10.87±0.05a	22.65±0.23a
		4th	0.49±0.01a	10.33±0.05c	21.12±0.11b
		6th	0.49±0.01a	10.57±0.05b	21.49±0.23b
	Controlled storage, 20 ºC	*2nd*	0.49±0.01a	10.80±0.14a	22.10±0.29
		4th	0.49±0.01a	10.50±0.01b	21.61±0.28
		6th	0.47±0.01b	10.27±0.05c	22.12±0.36
	Room conditions, 24 ºC	*2nd*	0.50±0.01a	10.67±0.05	21.23±0.53
		4th	0.48±0.01b	10.60±0.01	21.96±0.11
		6th	0.48±0.01b	10.57±0.05	21.94±0.19

* Means followed by different letters within each column and beverage are significantly different at $p \leq 0.05$. TSS: Total soluble solids.

The differences in brix-acid ratios were statistically significant for all samples and storage conditions ($p \leq 0.05$) except for sherbets stored at 20 and 24°C temperatures ($p > 0.05$). These scores varied from 21.30 to 34.15 in sodas, 29.27 to 35.27 in ice tea samples and 21.12 to 22.65 in sherbets.

Preserving of the freshness and taste of the food is one of the purposes of storing products. The ratio of soluble solids to the total acidity is one of the most important parameters affecting the edibility and taste of foods, especially beverages. Changes in TSS and acidity provide information not only about the brix-acid, but also about the microbial activities in the soft drinks. The acidity and TSS values of ice tea and sherbet samples were statistically significant depending on the different storage conditions. However, the differences between these results were relatively minor in quantitative terms. This situation also affected the brix-acid ratios in the same direction. However, these values varied more in soda samples than in other samples. In particular, total acidity and TSS in soda samples increased depending on the storage time, and brix-acid ratios were affected accordingly. It is thought that this change may be due to a change in carbonation in soda samples. The increase in the acidity values of soda beverages with the storage period was compatible with the results of previous studies (Umeocho et al., 2021; Jooyandeh, 2015). Furthermore, Jooyandeh (2015) stated that the increase in acidity and decrease in pH were caused by the production of CO_2 forming weak acids upon dissolution in naturally carbonated fruit juices. Balaswamy et al. (2011) expressed that the changes in acidity and TSS were negligible after 6 months of storage period at room temperature in sour grape-based carbonated and non-carbonated beverages. The TSS findings of the current study are in agreement with the results of Balaswamy et al. (2011). It is thought that the acidity and TSS values of sherbet and ice tea as they were pasteurized changed less depending on different storage conditions compared to soda samples.

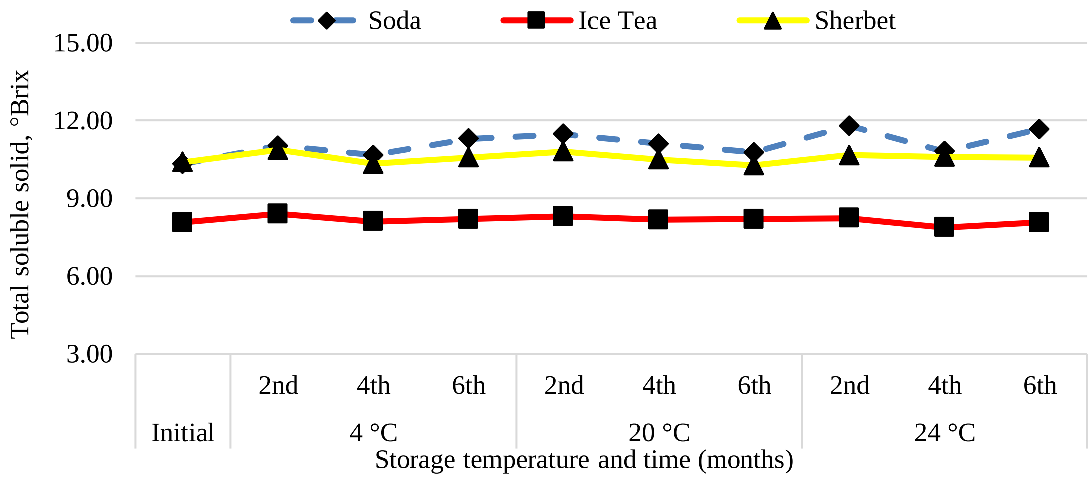

Figure 2. Total soluble solids change of the samples during storage
*Cold storage: ~4 °C; temperature-controlled storage: 20°C; room conditions: ~24°C.

The organic acids and total flavonoids of the samples were presented in Table 2. The Total flavonoid content in the sodas could not be detected because of the low concentration. Since the spectrophotometric method was used in the analysis of total flavonoids, low flavonoid values could no measured.

Table 2. Total flavonoids, malic and tartaric acids in soft drinks

Soft Drinks	Storage temperature	Storage time, months	Total flavonoid, mg/L	Tartaric acid, g/L	Malic acid, g/L
Soda		*Initial*	nd	0.12±0.05	3.00±0.34
	Cold storage, 4 ºC	*2^{nd}*	nd	0.12±0.01^{b}	3.37±0.01^{a}
		4^{th}	nd	0.14±0.01^{a}	2.89±0.01^{c}
		6^{th}	nd	0.08±0.01^{c}	2.98±0.01^{b}
	Controlled storage, 20 ºC	*2^{nd}*	nd	0.12±0.01^{b}	3.22±0.01^{a}
		4^{th}	nd	0.11±0.01^{c}	3.16±0.01^{b}
		6^{th}	nd	0.13±0.01^{a}	2.85±0.01^{c}
	Room conditions, 24 ºC	*2^{nd}*	nd	0.11±0.01^{b}	2.94±0.01^{b}
		4^{th}	nd	0.10±0.01^{b}	3.09±0.01^{a}
		6^{th}	nd	0.14±0.01^{a}	2.93±0.01^{b}
Ice Tea		*Initial*	123.97±13.62	0.08±0.01	1.87±0.10
	Cold storage, 4 ºC	*2^{nd}*	127.65±8.26	0.07±0.01^{a}	1.85±0.02
		4^{th}	120.59±6.76	0.04±0.01^{b}	1.98±0.02
		6^{th}	115.59±5.29	0.05±0.01^{b}	1.99±0.07
	Controlled storage, 20 ºC	*2^{nd}*	102.45±4.60^{b}	0.07±0.01^{a}	1.83±0.01^{c}
		4^{th}	117.35±10.81^{b}	0.04±0.01^{b}	1.97±0.01^{a}
		6^{th}	144.70±8.80^{a}	0.05±0.01^{b}	1.89±0.01^{b}
	Room conditions, 24 ºC	*2^{nd}*	120.10±2.37^{a}	0.10±0.04	1.79±0.04
		4^{th}	128.82±8.83^{a}	0.05±0.01	1.95±0.01
		6^{th}	98.38±9.26^{b}	0.04±0.01	1.79±0.15
Sherbet		*Initial*	4.34±0.25	0.42±0.01	4.13±0.03
	Cold storage, 4 ºC	*2^{nd}*	5.14±0.17^{ab}	0.29±0.01^{a}	5.00±0.01^{a}
		4^{th}	3.97±0.83^{b}	0.22±0.01^{b}	4.61±0.01^{b}
		6^{th}	7.01±0.97^{a}	0.23±0.01^{b}	4.48±0.01^{c}
	Controlled storage, 20 ºC	*2^{nd}*	5.76±0.66	0.30±0.01^{a}	4.77±0.01^{a}
		4^{th}	4.73±0.36	0.23±0.01^{b}	4.54±0.01^{b}
		6^{th}	6.54±0.71	0.23±0.01^{b}	4.37±0.01^{c}
	Room conditions, 24 ºC	*2^{nd}*	4.93±0.11	0.30±0.01^{a}	4.69±0.02^{a}
		4^{th}	6.01±1.01	0.22±0.01^{c}	4.59±0.01^{b}
		6^{th}	7.51±0.72	0.23±0.01^{b}	4.44±0.01^{c}

* Means followed by different letters within each column and soft drink are significantly different at $p \leq 0.05$. nd: not detected.

The tartaric and malic acid concentrations of the soft drinks during the storage period were analyzed, and their changes were evaluated. The tartaric acid values were 0.08-0.14 g/L for sodas, 0.05-0.10 g/L for ice tea samples and 0.23-0.30 g/L for sherbets. While the tartaric acid content of the soda samples stored at 4°C decreased with increasing storage time, this decrease was not observed in the samples stored at 20 and 24°C. On the other hand, with increasing storage time, tartaric acid content decreased slightly in iced tea and sherbets. The changes in tartaric acid of the soft drinks were shown on Figure 3. The tartaric acid concentration of sherbet decreased dramatically during the storage period, in particular during the first two months. The ice tea and soda drink samples were more stable than sherbets in terms of tartaric acid contents. The changes in the tartaric acid composition of the beverages were no statistically significant as a function of storage temperature. However, the storage durations affected these values. In particular, the tartaric acid concentration of the sherbet samples decreased significantly with increasing storage time. This was due to the crystallization and sedimentation of tartaric acid as a consequence of storage duration.

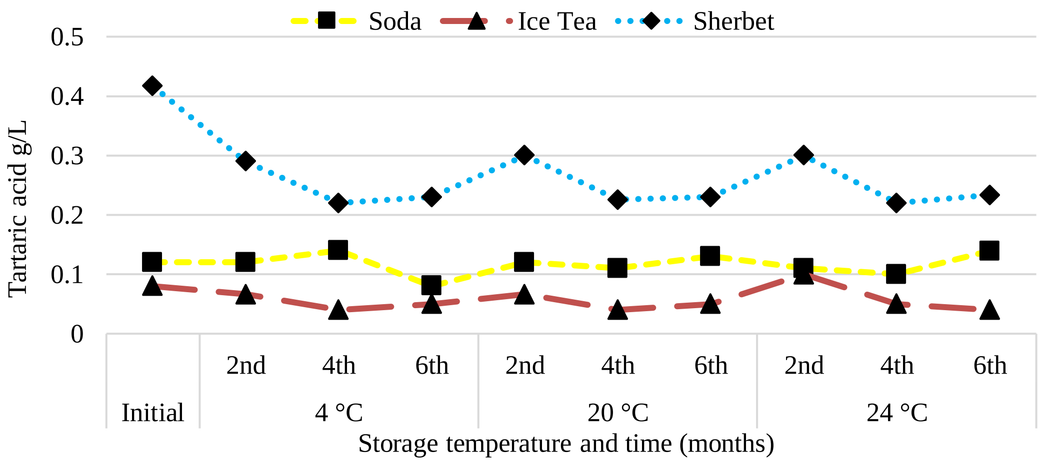

Figure 3. Tartaric acid changes in soft drinks during storage
*Cold storage: ~4 °C; temperature-controlled storage: 20°C; room conditions: ~24°C.

The main organic acid was malic acid in sodas, sherbets and ice tea samples produced by adding sour grape concentrate. The concentration of tartaric acid was lower than malic acid. Malic acid is a carboxylic acid and is found in many fruits and vegetables as the L-isomer form. It has a smooth taste and its DL-form is used as a flavouring and preservative in foods (Marques et al.,2020). The concentration of malic acid in each soft drink varied with storage temperature and time. The malic acid concentrations ranged from 2.98 to 3.37 g/L at 4ºC, 2.85 to 3.16 g/L at 20ºC and 2.93 to 3.04 g/L at 24ºC for sodas during storage period. These concentrations varied between 1.85 and 1.99 g/L at 4ºC, 1.83 and 1.97 g/L at 20ºC and 1.79 and 1.95 g/L at 24ºC for ice tea samples. While the change in the malic acid content of the soda samples was statistically significant for all three storage temperatures as a function of the storage time, it was only found to be statistically significant for the iced tea samples at the 20 ºC condition ($p \leq 0.05$). On the other hand, the malic acid content of the sherbets decreased depending on the storage time, especially after two-months storage. The malic acid concentation of ice tea was more stable than that of other soft drinks.

The flavonoid contents in ice tea samples were higher than in other soft drinks. In ice tea samples, the flavonoid contents ranged from 115.59 to 127.65 mg/L at 4 ºC, 117.35 to 144.70 mg/L at 20 ºC and 98.38 to 128.82 mg/L at 24 ºC. In sherbets, these values changed between 3.97 and 7.01 mg/L at 4 ºC, 4.73 and 6.54 mg/L at 20 ºC and 4.93 and 7.51 mg/L at 24 ºC. The changes in flavonoid content of the ice tea samples were showed on Figure 4. The differences in flavonoid content of ice tea stored at 20 and 24ºC were statistically significant according to storage temperatures ($p > 0.05$). In additon, the differences between sherbet flavonoid concentrations for 4ºC storage temperatures were statistically significant at the $p \leq 0.05$ level also. It is thought that the flavonoid content of ice tea samples decreased slightly with the precipitation occurring depending on the time stored at 4 ºC. This difference was no significant as statistical. However, storage in room conditions at 24 °C caused a time-dependent decrease in flavonoids, probably due to temperature changes under uncontrolled conditions. Flavonoids were better preserved in storage at 20ºC, which was determined as a controlled storage temperature. The increase of flavonoids depending on the duration may have been caused by the collapse of the turbidity factor components over time and accordingly, a percentage increase in the concentration of flavonoids in the unit dry matter.

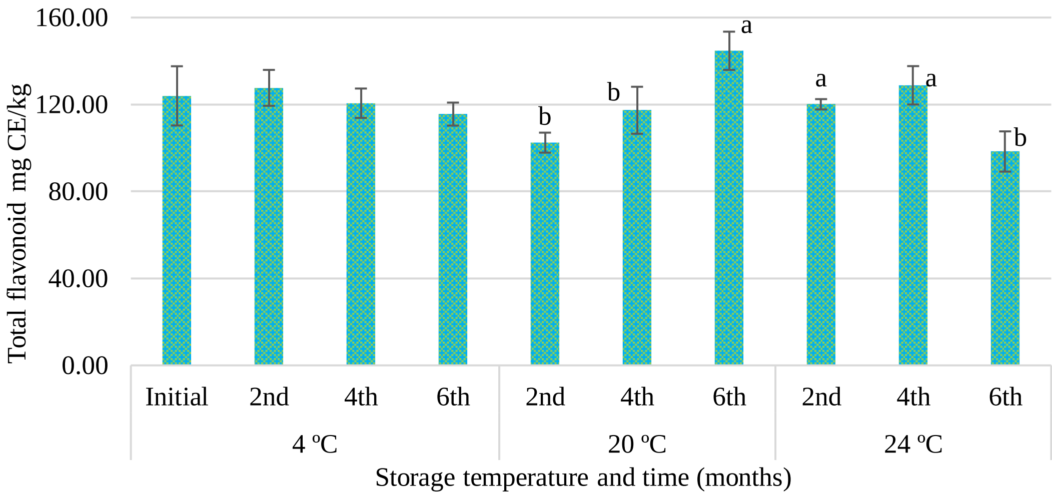

Figure 4. Total flavonoid changes in the iced tea samples during storage
*Cold storage: ~4 °C; temperature-controlled storage: 20°C; room conditions: ~24°C.

Flavonoids, consisting of flavan-*3*-ols, flavonols, and anthocyanins, are active polyphenols as biological and have high antioxidant power (Thennakoon et al., 2022). The flavonoid content of sherbet and soda were no remarkable compared to the ice tea when considering the soft drinks examined. According to these findings, almost all of the flavonoids in the produced beverages were originated by tea and its very low part by sour grape concentrate. In addition, the flavonoid content of iced tea was statistically more stable during storage than that of the sherbet. In a study comparing the properties of black and green tea, the flavonoid content varied between 0.47-5.15 mg QE/g in brewed black tea and 0.31-2.68 mg QE/g in green tea (Thennakoon ve ark., 2022). Aydemir et al (2023) found that the total flavonoid concentrations of 79 different black tea samples varied from 10.5 to 90.2 mg QE/g DW depending on the brewing time. In this study, the flavonoid findings obtained from ice tea samples were lower than in previous literature. It is thought that this situation may be caused by the tea variety and growing area, as well as the processing technique. In another study, Din et al. (2019) stated that the phenolic content of carbonated ice tea samples made from tea brewed in different proportions decreased significantly during the 90-day storage period. The results of the current study were no compatible with this literature. This difference may be caused by the process differences and the use of preservatives.

The variation of flavan-*3*-ols in iced tea samples as a consequence of storage condition was presented in Table 3. The flavan-3-ols in the ice tea samples were analyzed every two months during the storage period. The concentrations of (+)-catechin (CA), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG) altered between 27.10 and 48.50 µg/mL, 36.81 and 60.15 µg/mL, 5.25 and 8.43 µg/mL and 6.87 and 19.11 µg/mL, respectively, depending on storage conditions.

Table 3. Alterations of flavan-*3*-ols in ice tea samples depending on storage conditions

Soft Drinks	Storage temperature	Storage time, months	CA, *µ*g/mL	EC, *µ*g/mL	ECG, *µ*g/mL	EGCG, *µ*g/mL
Ice Tea		*Initial*	33.78±2.21	78.81±4.98	14.67±2.90	30.54±0.76
	Cold storage, 4 ºC	*2nd*	28.65±2.25^b	60.15±6.59	6.85±1.65	15.90±2.15
		4th	48.50±1.70^a	36.83±4.71	6.64±1.23	7.29±1.70
		6th	46.36±1.74^a	45.17±6.63	6.39±0.84	7.26±1.74
	Controlled storage, 20 ºC	*2nd*	28.21±0.51^b	51.91±7.90	8.43±0.65^a	19.11±1.19^a
		4th	46.38±1.10^a	36.81±4.05	5.40±0.91^b	12.04±2.19^b
		6th	45.29±0.78^a	47.43±5.43	5.25±0.75^b	12.03±2.22^b
	Room conditions, 24 ºC	*2nd*	27.10±0.21^b	59.45±6.42	6.22±0.05	10.83±1.01^a
		4th	45.02±5.26^a	41.02±7.67	6.39±0.59	6.87±2.03^b
		6th	44.45±4.76^a	40.35±7.42	6.31±0.45	7.14±2.17^b

* Means followed by different letters within each column are significantly different at $p \leq 0.05$. nd: CA: (+)-Catechin, EC: (-)-Epicatechin, ECG: (-)-Epicatechin gallate, EGCG: (-)-Epigallocatechin gallate.

The alterations of flavan-3-ols in the ice tea samples were indicated graphically on Figure 5. EC, ECG and EGCG concentrations decreased with increased storage time, but CA slightly increased. These results revealed that the concentrations of flavan-3-ols having high antioxidant activity in ice tea could vary depending on the storage conditions. As particular, the concentration of epicatechin derivatives decreased dramatically with storage conditions in the present study. EC concentrations decreased by 42.68% for 4 ºC, 39.81% for 20 ºC and 48.80% for 24 ºC at the end of the storage. These declines were 56.44%, 64.21% and 56.99% for ECG cocentrations, respectively. In addition, EGCG contents decreased between 60.61 and 76.62% during storage durations. The EGCG was the most sensitive component to storage conditions among the epicatechin derivatives investigated. The current study reveals that there may be a dramatic decrease in epicatechin derivates in the storage of ice tea beverages and this decrement rises from the second month of storage when all findings are taken into consideration. Furthermore, the increase in storage temperatures may influence the changes in their concentrations.

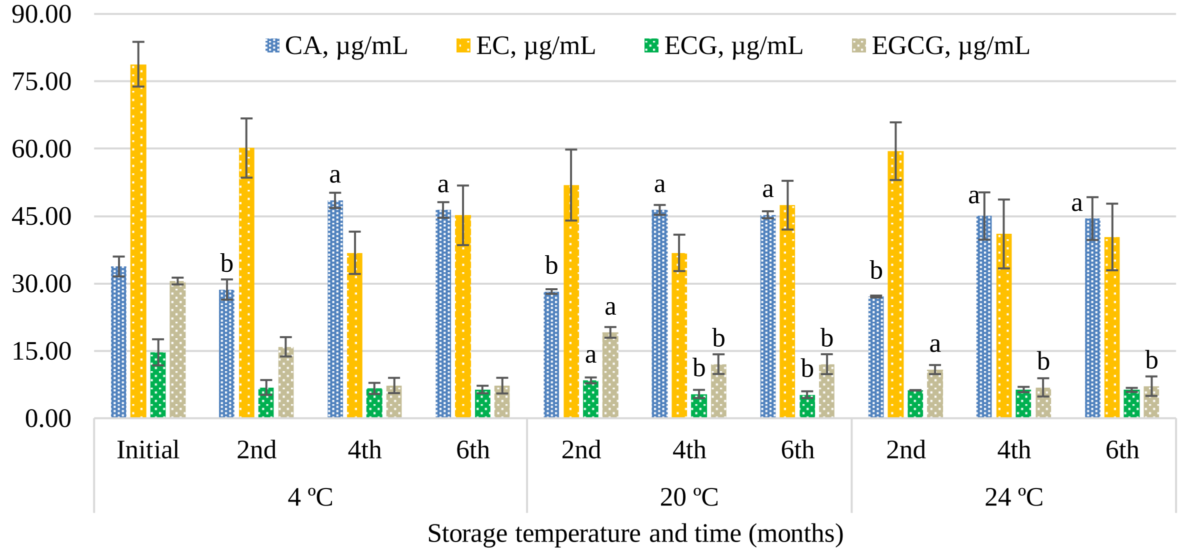

Figure 5. The changes in flavan-*3*-ols in the ice tea during storage (*µ*g/mL)
*Cold storage: ~4 °C; temperature-controlled storage: 20°C; room conditions: ~24°C.

This study investigated the flavan-*3*-ols (CA, EC, ECG and EGCG) in ice tea samples and their changes with storage conditions. The flavan-*3*-ols in tea have biological benefits for human health. On average, 36 % of fresh tea leaves are polyphenols, of which approximately 90 % are catechin phenolics (Luczaj and Skrzydlewska, 2005; Skotnicka et al., 2011; Xu et al., 2023). The main flavan-3-ols in tea are CA, EC, ECG, EGCG and (-)-epigallocatechin (EGC) (Henning et al., 2003; Skotnicka et al., 2011). In a study investigating the catechin phenolics in 11 black tea samples CA was found to be between 2.7 and 15.4 mg/100 mL, EC 1.1 and 9.0 mg/100 mL, ECG 1.4 and 21.3 mg/100 mL and EGCG 3.8 and 74.5 mg/100 mL. These compounds could be undetected in the 2 ice tea samples investigated (Henning et al., 2003). EGCG and ECG concentrations in 7 different Turkish black tea grades ranged from 1.06 to 3.16 mg/g DW and 0.73 to 2.54 mg/g DW, respectively (Erol et al., 2010). In another study, the CA, EC, ECG and EGCG contents of black tea were reported to be 20 µg/mL, 37 µg/mL, 763 µg/mL and 128 µg/mL, respectively (Skotnicka et al., 2011). Serpen et al. (2012) determined the nutritional and functional properties of seven grades of black tea produced in Türkiye. They reported that CA varied from 59.3 to 98.3 mg/100 mL, EC from 61.9 to 83.9 mg/100 mL, ECG from 89.5 to 115 mg/100 mL and EGCG from 102 to 155 mg/100 mL in seven different grades of black tea. CA, EC, ECG and EGCG were found to be 0.012-0.156 µg/g, 0.579-4841 µg/g, 1087-5578 µg/g and 3.27-4376 µg/g, respectively in 79 black tea samples from different origins (Aydemir et al., 2023). These references indicated that the concentration of flavan-3-ols in tea could be varied dramatically depending on the variety, brewing conditions, production conditions and various parameters such as origin. The results of this study regarding flavan-3-ols were compatible with the results of Xu et al. (2023). They revealed that EGCG values were 4.5-182.7 mg/L, ECG values were 2.6-38.1 mg/L, EC values were 2.3-20.4 mg/L and CA were 2.0-44.9 mg/L in 18 canned or bottled tea drinks.

PCA was used to provide additional information on the influence of the storage conditions on the soft drink quality parameters and their relations with each other. Two main principal components accounted for 73.04 % of the total variance. Principal component 1 (C1) and component 2 (C2) accounted for 59.21 and 13.83 % of the total variance, respectively. C1 discriminated two months of storage from other storage times and clearly distinguished the investigated flavan-*3*-ols except CA. In addition, TSS and brix-acid ratio dissociated similarly to flavan-3-ols. PCA is generally used to simplify a large and complex set of data into a smaller set that is easier to understand. The variables of PCA are defined as linear combinations of the original (Jayasuriya and Edirisinghe, 2016). For this purpose, in this study, both the storage time and temperature of the investigated parameters and their relationships with each other were evaluated together. This method was used to determine multidirectional relationships between flavan-*3*-ols, organic acids and physico-chemical parameters in beverages depending on storage conditions. The score plot and correlation scatterplots of the variables for PCA were indicated on Figure 6. PC1 discriminated the second month of storage from the other storage durations and perfectly separated epicatechin derivatives (EC, ECG and EGCG) of ice tea samples by this storage condition. In addition, TSS, brix-acid ratio and tartaric acid were also similarly separated by PC1. PC2 was associated with the EC, EGCG, TA, total flavonoid and acidity of soft drinks stored at 24°C for all storage times. Taking into account the results of the PCA, a strong correlation was found between the two-month storage treatment and EC, ECG, EGCG, TA, brix-acid ratio and TSS. Furthermore, four and six-month treatments at 4 and 20 °C were strongly correlated with the total flavonoids, CA, MA and acidity. These findings provided us with information about on the relationship between epicatechin derivatives and the physico-chemical properties of beverages and on how these components can be better protected during storage.

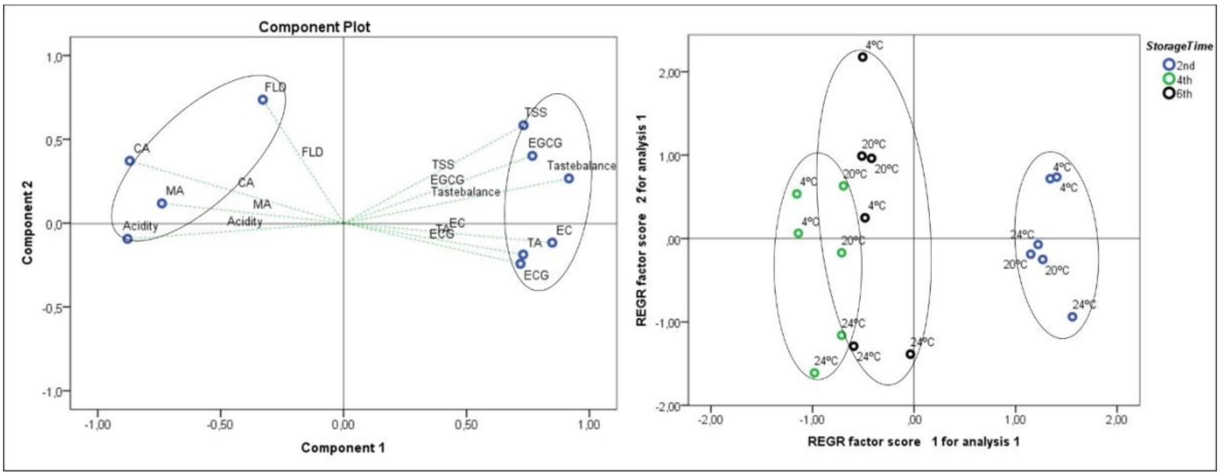

Figure 6. The score plot and correlation scatterplots of the variables for principal component analysis, with C1 and C2 as a function of quality parameters from different storage conditions. TSS: Total soluble solid, Taste balance: brix-acid ratio, FLD: Flavonoids, TA: Tartaric acid, MA: Malic acid, CA: (+)-Catechin, EC: (-)-Epicatechin, ECG: (-)-Epicatechin gallate, EGCG: (-)-Epigallocatechin gallate, Storage temperatures: 4, 20 and 24 ºC, Storage time: 2nd, 4th and 6th months.

CONCLUSION

This study revealed the effects of different storage conditions on the physico-chemical properties, flavonoid and organic acid contents and flaval-*3*-ols concentrations of beverages produced by adding sour grape concentrate. The acidity and TSS values of sherbet and ice tea samples changed less with different storage temperatures than soda samples because they were pasteurized. Tartaric acid concentrations of ice tea and soda were more stable during storage than sherbet. Malic acid was the main organic acid in the beverages. The flavonoid content of ice tea was higher than that of other soft drinks.

The concentration of flavan-*3*-ols in ice tea varied significantly with the storage conditions and duration. The levels of epicatechin, epigallocatechin and epigallocatechin gallate in ice tea samples decreased between an average of 43.72 and 71.15% at the end of six months of storage. Epigallocatechin gallate concentrations decreased by approximately 60-76% at the end of the storage compared to initial. There was a dramatic decrease in epicatechin derivatives during storage of ice tea beverages and this decrease increased from the second month of storage. Furthermore, increases in storage temperatures influenced the changes in their concentrations. Principal component analysis showed that storage conditions affected the epicatechin derivatives in ice tea and the physicochemical properties of beverages. The two-month storage was distinguished from other storage periods by principal component analysis. In future studies, it is recommended that studies be carried out on the usability of sour grape juice concentrate in different beverage and food products.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

The authors declare they have no conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. The authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

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