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INVESTIGATION OF THERMOSONICATED STRAWBERRY NECTAR QUALITY DURING STORAGE AND KINETIC MODELLING

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ABSTRACT
The changes in ascorbic acid-(AA) content, hydroxymethylfurfural-(HMF) content, total color difference-(Δ*E**) and browning index-(BI) of optimally thermosonicated (59°C-455 J/g) cloudy strawberry nectar during storage (3 months at 4°C) were evaluated. Also, the data obtained were modelled. The initial AA content (104.86 mg/L) decreased ~99% after storage, while HMF level increased from 20.46 to 494.44 μg/L. The significant increases in BI-(0.45-0.56) and Δ*E**-(5.21–11.23) were consistent with each other and over storage time. The changes in HMF content-(R^2=0.849), BI-(R^2=0.942) and Δ*E** were best fitted to zero-order kinetic model, while decrease in AA content-(R^2=0.9755) was described with the first-order kinetic model. The reaction rate constants of AA, HMF, Δ*E** and BI were determined as 5.58x10^{-2} 1/day, 4.34 mg/L.day, 6.40x10^{-2} mg/L.day and 14x10^{-2} mg/L.day, respectively. The highest correlation with the change in Δ*E** was observed in AA-(R^2=0.955) followed by BI-(R^2=0.859). Therefore, the greatest effect on color might be caused by AA degradation.
Keywords: Strawberry nectar, Kinetic modelling, Hydroxymethylfurfural, Ascorbic acid, Total color difference, Browning index, Storage

DEPOLAMA BOYUNCA TERMOSONİKASYON UYGULANAN ÇİLEK NEKTARI KALİTE ÖZELLİKLERİNİN ARAŞTIRILMASI VE KİNETİK MODELLEME

ÖZ
Bulanık çilek nektarında depolama (4°C-3 ay) boyunca askorbik asit-(AA) içeriği, hidroksimetilfurfural-(HMF) içeriği, toplam renk farkı-(Δ*E**) ve esmerleşme indeksi-(BI) değerlerindeki değişimler değerlendirilmiş ve modellenmiştir. AA içeriği (104.86 mg/L) depolamadan sonra ~%99 azalırken, HMF içeriği artmıştır (20.46 μg/L-494.44 μg/L). Depolama boyunca, BI (0.45-0.56) ve Δ*E** (5.21–11.23) değerlerinde meydana gelen önemli artışlar birbiri ile ve depolama süresince tutarlılık göstermiştir. HMF içeriği (R^2=0.849), BI (R^2=0.942) ve Δ*E** değişimleri sıfırıncı dereceden kinetic modele uyum gösterirken, AA içeriğindeki azalma birinci dereceden kinetik model ile açıklanmıştır (R^2=0.9755). AA, HMF, Δ*E** ve BI reaksiyon hız sabitleri sırasıyla 5.58x10^{-2} 1/gün,

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4.34 mg/L.gün, 6.40x10^{-2} mg/L.gün ve 14x10^{-2} mg/L.gün olarak belirlenmiştir. ΔE* değişimiyle en yüksek korelasyona sahip olan AA içeriğini (R^2=0.955), BI (R^2=0.859) takip etmiştir. Bu sebeple renk üzerindeki en yüksek etkiye AA parçalanmasının neden olması söz konusu olabilir.
Anahtar kelimeler: Çilek nektarı, Kinetik modelleme, Hidroksimetil furfural, Askorbik asit, Toplam renk farkı, Esmerleşme indeksi, Depolama

INTRODUCTION

Strawberries (*Fragaria* x *ananassa*) have a bright red color directly affects the consumer perception, besides nutrients which are effective against cardiovascular diseases, some cancer types and neurodegenerative diseases (Giampieri, 2012). Browning of fruit juices including citrus and strawberry juice during storage is one of the major quality deteriorations, which cause a big challenge in the juice industry (Paravisini et al., 2016; Wibowo et al., 2015; Buve et al., 2018). Juice browning can occur through enzymatic and/or non-enzymatic browning pathways. Ascorbic acid (AA) degradation and Maillard reaction have been proposed as the reaction pathways responsible for non-enzymatic browning of fruit juices. Therefore, the most important precursors for browning reactions can be AA and reactive carbonyl products that may polymerize and react with amino acids of the juice to form brown compounds. The high acidic conditions of juices (pH < 4.0) may not favor the classical type of Maillard reactions occurring between reducing sugars and the free amino group of amino acids and/or proteins. However, a Maillard-associated reaction in which reactive carbonyl compounds resulting from AA condensation with amino acids could still play an important role in the browning of juice during storage (Pham et al., 2020; Bharate and Bharate, 2014).

Ultrasound energy has been identified as a potential technology to meet the need of US Food and Drug Administration for a 5-log reduction in pertinent microorganisms found in fruit juices (Salleh-Mack and Roberts, 2007). However, thermosonication, the combination of heat and high frequency sound waves (>16 kHz), can be preferred to eliminate or reduce the unfavourable effects of thermal treatment on strawberry juice quality properties (Lafarga et al., 2019). Thermosonicated juices may have better quality properties when compared to pasteurized juices (Nayak et al., 2018; Oladunjoye et al., 2021). Regarding the PPO inactivation, change in color and some bioactive components (ascorbic acid, total phenolic, monomeric anthocyanin), the optimum thermosonication parameters was reported as 59°C and 455 J/g (Dündar et al., 2019). There are some other process optimization studies in the literature. However, to the best of our knowledge, there is no study focused deeply on the changing kinetics of ascorbic acid, HMF contents or color of cloudy strawberry juice/nectar during storage.

The objective of the study is to determine the changing kinetics of ascorbic acid content, hydroxymethylfurfural content, total color difference and browning index of optimally thermosonicated strawberry nectar during 3 months of storage at 4°C and to evaluate the relationship between these quality parameters.

MATERIAL AND METHODS

Strawberries

Strawberries (*Fragaria* x *ananassa* Duch.) cv. Rubygem (10 kg of strawberries harvested in May 2017) at similar maturity and size were used for producing nectar.

Strawberry nectar production

Strawberry nectar (50% fruit puree content, 0.5 g/L titrable acidity and 10°Brix total soluble solids) was prepared after determining the initial acidity level of strawberries and adjusting it with citric acid solution (50%), sugar and water additions. The thermosonication of nectar was conducted at 59°C and 455 J/g ultrasound energy density according to the results of an optimization study completed before (Dündar et al., 2019). A laboratory-scale ultrasonic device (S14 standard sonotrode, UP200S, Hielscher, Germany), a temperature controller, a digital power-meter and digital timer to apply optimum processing at a constant temperature and time were used during thermosonication treatment (Supplementary File 1). Strawberry nectar (for each run 250 mL) was placed in a double-walled glass beaker with a cooling/heating system with a sonotrode at the point 1 cm upper than the bottom. Temperature was controlled by a water-circulator, and the treated nectar was cooled to room temperature immediately. All of the thermosonicated nectars (5 L) were mixed to eliminate the difference from the material, before filling the dark-colored glass bottles.

Ultrasound energy density parameter (*UED, J/g*) and its relationship with ultrasonic power (*P, W*), treatment time (*t, s*) and sample amount (*m, g*) were explained with following equation (Eq. 1);

$$UED = \frac{P.t}{m} \quad (1)$$

The mean power value was determined as 150 W during treatment.

Analyses

All the analyses were performed at the 0, 10, 20, 30, 60 and 90. days of storage with at least three repetitions. Five mL of the strawberry nectar samples and 5 mL of 2.5% meta-phosphoric acid (Merck, Germany) centrifuged (4000 rpm at 4 °C for 10 min) according to the procedure of Lee and Coates (1999) to determine ascorbic acid (AA) content. A 0.5 mL aliquot of the supernatant was completed to 10 mL with 2.5% metaphosphoric acid. The mixture was filtered using a 0.45 µm nylon filter (Millipore, Munich, Germany). HPLC (Shimadzu LC-20AT, Japan) system was used for quantification of AA (Sigma-Aldrich, USA) with UV detector at 244 nm using an external ascorbic acid standard method (R^2=0.998). The identified peaks were quantified by a calibration curve (5−50 mg/L AA concentrations, R^2 = 0.998). The statistical limit of detection (LOD) and limit of quantification (LOQ) values were calculated as 0.124 and 0.155 ppm, respectively (Akyıldız et al., 2021).

20 µL of supernatant obtained by using the method of Gökmen and Acar (1996) were injected into a C18 ACE (4.6mm×250 mm) HPLC column (Shimadzu LC-20AT, Japan) at 30 °C for HMF analysis. Mobile phase was methanol/water/acetic acid (20/79/1, v/v/v), while the flow rate was 0.5 mL/min. Quantification of HMF was carried out at 285 nm using an external standard (Merck, Italy) method (R^2=0.995). The statistical limit of LOD and LOQ values were calculated as 1.36 and 1.51 ppb (Akyıldız et al., 2021).

Five mL of strawberry nectar and the same amount of ethyl alcohol (96%, Merck, Germany) centrifuged (6000 rpm, 10 min, at 4 °C) for the determination of browning index (BI). The supernatant filtered with a 0.45 µm teflon membrane filter (Millipore, Munich, Germany), and the absorbance of the supernatant was determined with a spectrophotometer (Perkin Elmer Lambda 25-UV/vis, USA) at 420 nm (Meydav et al., 1977).

The ColorQuest XE Hunter Lab (Virginia, USA) with 20mm Glass Optical Cell Light Path used for determination of lightness (L^*), redness (a^*) and yellowness (b^*) just after mixing the sample with vortex for a minute. Then total color difference values were calculated with following equation (Eq. 2).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

Kinetic Modelling of Changes in Strawberry Nectar

The changing kinetics of ascorbic acid, HMF, ΔE^* and BI of thermosonicated strawberry nectar were investigated. The obtained data during the 90 days of storage were used to determine the best fitted mathematical kinetic model to the changing (degradation of AA or formation HMF etc.) kinetics by using Curve Fitting Toolbox, Matlab ver. 7.10 (The MathWorks Inc., USA, 2010). The goodness of model fitting to the experimental data was determined with the aid of correlation coefficient (R^2), adjusted coefficients of determination (R^2_{adj}), confidence intervals (*ConfI*) and root mean square error (RMSE) values (Remini et al., 2015).

The solutions of differential equations for reaction kinetics with different order (zero (Eq. (3)), half (Eq. (4)), first (Eq. (5)) and second (Eq. (6))) were given below, where C_0 is initial concentration (mg/L), C is the concentration of some certain compound which is found in a certain time (mg/L), t is storage time (day), $t_{1/2}$ is half-life (day) and k is the reaction rate coefficient (Labuza, 1984).

$$\frac{dC}{dt} = -kC^0 \quad C = C_o - kt \quad t_{\frac{1}{2}} = \frac{C_o}{2k} \quad (k, \frac{mg}{L.day}) \quad (3)$$

$$\frac{dC}{dt} = -kC^{0.5} \quad 2\sqrt{C} = \sqrt{C_0} - kt \quad t_{\frac{1}{2}} = \frac{-4.142}{k} \quad (k, 1/day) \quad (4)$$

$$\frac{dC}{dt} = -kC^1 \quad \ln(C) = \ln(C_o) - kt \quad t_{\frac{1}{2}} = \frac{0.693}{k} \quad (k, 1/day) \quad (5)$$

$$\frac{dC}{dt} = -kC^2 \quad \frac{1}{C} = \frac{1}{C_o} + kt \quad t_{\frac{1}{2}} = \frac{1}{C_o k} \quad (k, \frac{L}{mg.day}) \quad (6)$$

Sensory Evaluation

The control (non-processed) and optimally thermosonicated strawberry nectars were evaluated according to their taste, aroma, color, viscosity and overall quality at the first day of storage by 14 panelists to determine the effect of optimum thermosonication treatment on sensorial acceptance. The point of the sensory evaluation was to remove the concerns of consumers about the sensory properties of thermosonicated nectar. Sensory analysis was conducted in laboratory by using a graphic scale test (between 0 and 10).

Statistical Analysis

The variance and Duncan's multiple tests of obtained values during storage were conducted with SPSS (PASW Statistic 18 for Windows, Chicago, IL, USA) packet program to determine significant differences ($P < 0.05$) between mean values, except sensory analysis results. The obtained sensory analysis results were evaluated with the aid of independent t-test by using SPSS.

RESULTS AND DISCUSSION

Change of Ascorbic Acid (AA)

The AA contents (104.86 – 0.98 mg/L) of nectar during storage were given in Fig. 1.a.. The significant decrease in AA content continued during storage until the 60. day. The initial AA content (104.86 mg/L) decreased 60% in the first 20 days of storage, while approximately all of them (~99%) degraded at the 90. day. Chemical reactions and physical conditions might be resulted with decrease in AA content of the nectar. Free radicals, hydrogen ions and hydroperoxides causing from sonolysis can also be related with the degradation of AA (Adekunte et al., 2010). Ascorbic acid present in juice is thermodynamically unstable, and it undergoes deterioration during storage. In addition to loss of nutritive value, degradation of ascorbic acid could form undesirable color in juice due to browning reactions (Bharate and Bharate, 2014). Pham et al. (2020) indicated that ascorbic acid in orange juice degraded in two phases during storage: a fast decline in the first 2 week, and a gradual decrease until the end of 8. week. These two phases of degradation were explained with the higher presence of oxygen in the medium at the first phase. AA degradation can occur simultaneously by aerobic and anaerobic mechanisms at the first phase. However, the latter pathway is slower than the former. Therefore, these two phases in AA degradation correspond to the dominance of the aerobic pathway in the presence of high headspace and/or dissolved oxygen content at the beginning of storage. The dominance of the anaerobic pathway decreases in the later phase when the oxygen level was lower (Pham et al., 2020).

Change of Hydroxymethylfurfural (HMF)

HMF contents, a thermal indicator for determining the quality of food, of nectar were between the levels of 20.4 µg/L - 494.4 µg/L (Fig. 1.b.). HMF content showed a significant increase during storage ($P < 0.01$). When the changes in the amount of ascorbic acid during storage were examined, a 95% correlation was determined between the HMF and AA contents. Kus et al. (2005) reported that HMF contents of strawberry concentrates (13-66°Bx) were between 0.4 – 4.5 mg/L. When the lower Brix values of strawberry nectar produced in this study were considered, results were found convenient. During storage of thermosonicated cloudy apple juice, HMF content (1.30 – 3.02 mg/L) increased after 7 days at 4°C and it remained unchanged during 21 days (Illera et al., 2020). Also, HMF contents of red and yellow watermelons reported between 0.108 mg/L and 0.244 mg/L after ultrasonication (26 kHz, 50% amplitude, 80 W, 4-16 min) treatment (Yıkmış, 2020). HMF content increase with storage time in fruit juices, and it was further reported that HMF formation increased with storage temperature for all fruit juices (Oral et al., 2012). This increasing can be caused from Maillard reaction or ascorbic acid degradation. The oxidized form of ascorbic acid also might have had a role as a carbonyl source in Maillard reaction. Molecules with higher acidic character might be formed from amines or sugars by Maillard reaction (Beck et al., 1990). High temperatures cause significant increase in HMF content of strawberry nectar. The relation between temperature of treatment and HMF content of strawberry nectar is linear, while HMF content is gradually increased with the increasing of ultrasound energy density (Dündar et al., 2019).

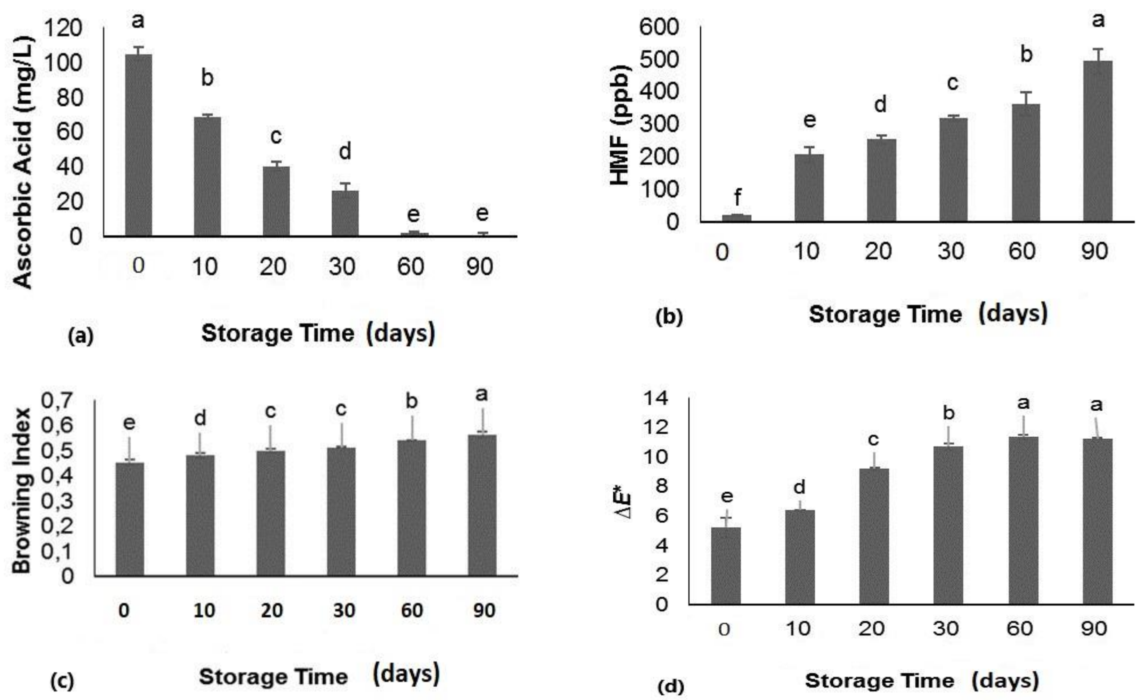

Figure 1. The ascorbic acid content (a), Hydroxymethylfurfural content (HMF) (b), browning index (c) and total color difference (ΔE^*) (d) of strawberry nectar during storage.
[a-e] The mean values with different letters are significantly different ($P < 0.05$).

Change of Browning Index (BI)

BI of nectar (0.45-0.56) increased significantly during storage ($P < 0.05$), and reached the level of 0.56 (24% increase) at the end (Fig. 2.c.). The change in BI can be related with the degradation of ascorbic acid and formation of HMF. BI had correlations with AA content (98%) and HMF level (96%). Abid et al. (2015) determined the non-enzymatic browning (NEB) values of ultrasound-treated apple juices during storage (30 days at 4°C). It was also reported that there was a significant increase in NEB values of all samples. It is possible that the change in AA can be more effective on the browning of strawberry nectar than HMF content during storage. The level of AA in nectar affect the color with browning reaction which can degrade the AA as substrate, but also decrease in AA affects the pH of medium. Consequently, change in pH can be effective on the color of nectar which contains anthocyanin compounds, which are causing pH-depended change in color. In addition, degradation of anthocyanin can have resulted with the browning of nectar. Besides AA, presence of citric acid in the medium has a stimulation effect on non-enzymatic browning in juice (Buve et al., 2021), and citric acid was added when producing nectar from strawberries as mentioned in methods of this study.

Change of Total Color Difference (*ΔE)**

The Δ*E** values of nectar changing between 5.21-11.36 were given in Fig. 2.d.. The color of nectar changed during storage, especially during the first month. Δ*E** value showed 23% and 116% increase in the first 10 days and at the end of storage, respectively. A high correlation between AA and Δ*E** values was determined (R^2=0.955). Buglione and Lozano (2002) reported that the Δ*E** values of grape juices stored at 10, 20 and 30°C for 21 weeks increased during storage. Similarly, Abid et al. (2015) reported that there was a significant increase in the Δ*E** value of ultrasonicated apple juices during storage ($P < 0.05$). It has been stated that the color change may have occurred due to the acceleration of carotenoid isomerization caused from physical conditions such as cavitation, storage temperature and high temperature. An increase in the Δ*E** value of ultrasonicated kiwi juice during 7 days of storage was also reported by Tomadoni et al. (2016), and this increase was faster in the first 2 days. Cavitation occurs during thermosonication may accelerate the chemical reactions, increase diffusion rate, inactivate some enzymes, and therefore it can also affect color change (Adekunte et al., 2010). It has been reported that a small change in pH value is also effective in increasing the sensitivity of the color to temperature application as well as the color of strawberry juice (Wang et al, 2015). Change in pH value during storage may also have caused color change in nectars.

Kinetic Parameters

The kinetic parameters rate constant (*k*), half-time ($t_{1/2}$) or doubling time (t_2) as well as the R^2, adjusted-R^2 and RMSE of the zero, half, first and second-order models through a least square fitting procedure of the main compounds and some important quality parameters were given in Table 1. The content of each compound that was investigated, and the kinetic model with highest correlation coefficient is accepted to explain the order of changing.

Table 1. The correlation coefficients of different degradation/formation orders and kinetic performance outputs

		AA	HMF	Δ*E**	Browning Index
R^2	*Zero*	0.7725	0.8492	0.6784	0.9422
	Half	0.9136	0.6825	0.6578	0.9338
	First	0.9755	0.4958	0.6362	0.9249
	Second	0.8989	0.2989	0.591	0.9056
R^2_{adj}	*Zero*	0.7156	0.8115	0.5979	0.9277
	Half	0.892	0.6031	0.5723	0.9173
	First	0.9694	0.3698	0.5453	0.9062
	Second	0.8736	0.2989	0.4888	0.8820
RMSE	*Zero*	21.53	69.38	1.670	0.01075
	Half	1.205	3.836	0.3023	0.008068
	First	0.3353	0.9155	0.2216	0.02415
	Second	1.455×10^{-1}	1.743×10^{-2}	3.084×10^{-2}	5.366×10^{-2}
Reaction Rate constant*(k)		5.58×10^{-2} 1/day	4.34 mg/L.day	6.40×10^{-2} mg/L.day	1.14×10^{-2} mg/L.day
ConfInt		2.46×10^{-2}	5.08	1.22×10^{-1}	7.87×10^{-4}
$t_{1/2}$ or t_2 (days)		12	2	41	20

AA: Ascorbic acid, HMF: Hydroxymethylfurfural, Δ*E**: total color difference

$t_{1/2}$: half-life, t_2: doubling time.

RMSE: root mean square error, ConfInt: confidence interval was calculated with 95% of probability.

*The reaction rate constants and half-lives were determined according to best fitted kinetic model for each compound/quality parameter.

The properties directly related browning which are HMF content, BI and ΔE* value of strawberry nectar followed the zero-order kinetic during storage, while AA degradation was best fitted to the first-order kinetic model. The half-lives or doubling times of AA, HMF, ΔE* and BI were 12, 2, 41 and 20 days, respectively. The half-time of ascorbic acid in blood orange juice during storage at 4°C was also 12 days (Remini et al., 2015), and daily AA intake should be 60 mg/day (Lukaski, 2004). The obtained results showed that the consumption of optimally thermosonicated strawberry nectar at the level of 100 mL can meet the need of daily AA until the 31. day of storage. Dhakal et al. (2018) showed that AA degradation followed the first order kinetic during thermal treatment only or a thermal treatment combined with pressure in pineapple juice. In literature, ascorbic acid degradation has been described by first order kinetics (Nisha et al., 2004, Polydera et al., 2003, Tola and Ramaswamy, 2015, Vieira et al., 2016). On the other hand, similar to the results of this study, Wibowo et al. (2015) indicated that HMF formation during storage of pasteurized orange juice could be best fitted to zero-order model (R^2_{adj} = 0.96). Burdurlu et al. (2016) were also reported zero-order model as the best fitted to the change of HMF content in orange juice concentrate. When the relationships between the quality properties and ΔE* value of nectar were evaluated, the strongest relationship was found with AA (R^2=0.955) and followed by BI (R^2=0.859).

Sensorial Evaluation

The sensory evaluation scores of strawberry nectar changed between 6.39 and 7.70 (Fig. 2).

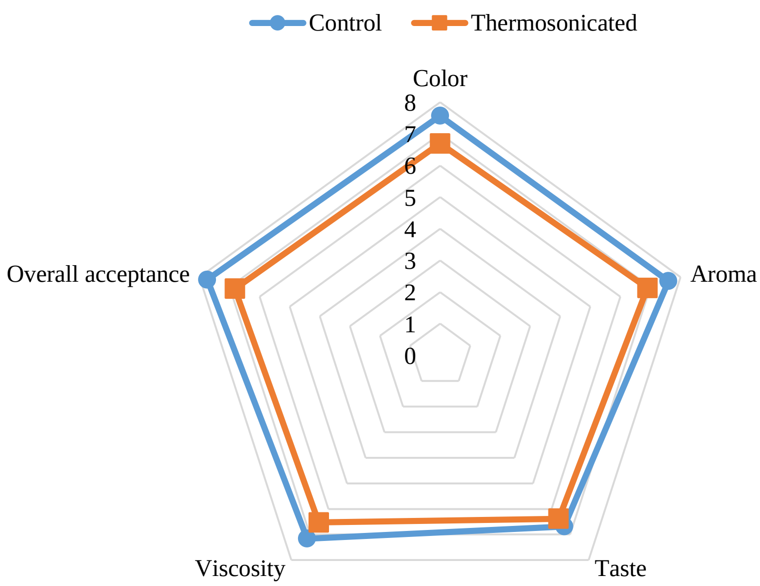

Figure 2. Spider plot for the sensory score based on the viscosity, taste, aroma, color and overall acceptance of control and thermosonicated strawberry nectars.

Although thermosonication caused decrease in all of the rated sensory attributes, the difference between control and the thermosonicated nectar was not significant. Therefore, it can be said that the optimum thermosonication conditions have no significant adverse effect on the sensorial properties of strawberry nectar. It has been reported in the literature that thermosonication may have positive effects as well as negative effects on sensory properties of fruit juice (Walkling-Ribeiro et al., 2009). Conditions caused by thermosonication such as removal of the gas in the nectar, lightening of the nectar color, decreased enzyme activity, and increased prevention of ascorbic acid may have caused no significant difference between the control and processed nectars in terms of sensory properties.

CONCLUSION

Thermosonication is considered as an alternative way to conventional thermal treatment in fruit juices. In this study, the effect of storage (three months at 4°C) on ascorbic acid, HMF, browning index, ΔE^* and the changing kinetics of optimally thermosonicated strawberry nectar were determined. Ascorbic acid degradation fitted the first order model. AA was the most sensitive parameter among them with the half-life of 12 days. The highest correlation with the change of total color was observed in AA (R^2=0.955) and followed by BI (R^2=0.859). Therefore, the greatest effect on color might be caused by the AA degradation among the studied quality properties. The knowledge gained from this study could be useful for further shelf-life studies of strawberry nectar after thermosonication, evaluating the effect storage on quality parameters and relations between them. The obtained degradation kinetics and half-lives could be suitable for the juice processing industry and consumers.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

AUTHOR CONTRIBUTIONS

Asiye Akyıldız conceptualized and designed the study. Material preparation, data collection and analyses were performed by Burcu Dündar Kirit. The first draft of the manuscript was written by Burcu Dündar Kirit. Asiye Akyıldız edited the manuscript. All authors approved the final manuscript.

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