



**THE THERMAL STABILITY OF PHYTOCHEMICALS AND  
PHYSICOCHEMICAL PROPERTIES OF KIRAZ AND FINDIK CHERRY  
LAUREL FRUITS (*LAUROCERASUS OFFICINALIS* L.) AND THEIR MOLLASSES**

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**ABSTRACT**

This study aimed to investigate the thermal stability of certain phytochemicals in molasses at temperatures of 50°C, 60°C, and 70°C throughout 6 to 168 hours. Additionally, the chemical makeup of Kiraz (KCLM) and Findik (FCLM) cherry laurel (*Laurocerasus officinalis* L.) fruits, as well as their molasses, was examined. The two molasses compositions were different due to the type of fruit used. The soluble dry matter (SDM) and dry matter (TDM) of the molasses ranged from 68.0-68.2% and 72.3-73.1%, respectively. The FCLM had higher values for titratable acidity (TA) (1.201%), hydroxymethylfurfural (HMF) (22.72 mg/kg), Vitamin C (66.83 mg/100 g), phenolics (TP) (5359 mg GAE/100 g), anthocyanin (ACN) (45.27 mg/kg), DPPH-RSA (80%), antioxidant capacity (AC) (33.74 µg TE/g), Hunter L\* (31.34), a\* (0.96), b\* (-0.59), and browning level (BL) (15.20) compared to KCLM. The ANOVA results showed that cultivars, temperature, and storing time significantly affected phytochemicals and physicochemical properties (P < 0.05).

**Keywords:** Phytochemicals, molasses, *Laurocerasus officinalis* L., stability

**KIRAZ VE FINDIK KARAYEMİŞ (*LAUROCERASUS OFFICINALIS* L.) MEYVE  
VE PEKMEZLERİNİN FİZİKOKİMYASAL ÖZELLİKLERİ VE  
FİTOKİMYASALLARININ ISI KARARLILIĞI**

**ÖZ**

Bu çalışma, 6 ila 168 saat boyunca 50 °C, 60 °C ve 70 °C sıcaklıklarda pekmez içindeki bazı fitokimyasalların termal stabilitesini araştırmayı amaçlamıştır. Ek olarak, Kiraz (KCLM) ve Findik (FCLM) karayemiş (*Laurocerasus officinalis* L.) meyvelerinin ve pekmezlerinin kimyasal yapısı incelendi. Kullanılan meyve türüne bağlı olarak iki pekmez bileşimi farklıydı. Pekmezlerin çözünebilir kuru maddesi (ÇKM) ve kuru maddesi (TKM) sırasıyla %68.0-68.2 ve %72.3-73.1 arasında değişmektedir. FCLM, KCLM'e göre titre edilebilir asitlik (TA) (%1.201), hidroksimetilfurfural (HMF) (22.72 mg/kg), C Vitamini (66.83 mg/100 g), fenolikler (TF) (5359 mg GAE/100 g), antosiyanin (ACN) (45.27 mg/kg), DPPH-RSA (%80), antioksidan kapasite (AK) (33.74 µg TE/g), Hunter L\* (31.34),

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a\* (0.96), b\* (-0.59) ve kahverengileşme derecesi (KD) (15.20) bakımından daha yüksek değerlere sahipti. ANOVA sonuçları çeşit, sıcaklık ve depolama süresinin fitokimyasallar ile fizikokimyasal özellikleri önemli ölçüde etkilediğini gösterdi ( $P < 0.05$ ).

**Anahtar kelimeler:** Fitokimyasallar, pekmez, *Laurocerasus officinalis* L., stabilite

## INTRODUCTION

Cherry laurel (*Laurocerasus officinalis* L.) is a popular fruit in the form of a bush or tree scattered along the Black Sea coast of Turkey and turns dark purple or black when ripe (Halilova and Ercişli, 2010). 'Oxygemmis', 'Globigemmis' and 'Angustifolia' were identified as three forms of fruits in the morphological and cytological studies on cherry laurel. The fruits of Oxygemmis are the biggest and have a bright black color when ripe, and the fruit taste is bitter and sour. It has been reported that the fruits of Globigemmis have a thinner mesocarp, are rigid and black when ripe, have a better taste and is less tart than Oxygemmis, and the fruits of this form are more preferred for fresh consumption. Angustifolia, which has a more extensive distribution than these forms, is used as an ornamental plant in Europe (Ayaz et al., 1997a; Akbulut et al., 2007).

Fruit composition varies among cultivars, likely due to ecological conditions, cultivar properties, climate, soil etc. (Sulusoglu et al., 2015). According to a study conducted by Şahan et al. (2012), the average content of cherry laurel fruits is as follows: 79.63% moisture, 0.75% ash, 0.95% protein, 11.61% sugar, and 0.16% oil. The study also found that the organic acids per 100g of fruits were: oxalic acid (1.02mg), malic acid (47.92mg), L-ascorbic acid (2.11mg), acetic acid (9.21mg), citric acid (1.28mg), succinic acid (3.84mg), and fumaric acid (5.53mg). Furthermore, the total phenolic (TP) and DPPH-radical scavenging activity (RSA) in methanol extracts of fresh fruits were 22.9mg GAE/100g and 26.70µmol trolox/g, respectively.

Akbulut et al. (2007) determined *TA* between 0.38-1.21% and *SDM* between 8.6-21.3% in 28 cherry laurel genotypes growing in the Black Sea Region. Also, Ayaz et al. (1997b) found dominant fructose, glucose, sorbitol and sucrose as sugar composition in cherry laurel cultivars. Furthermore, Alasalvar et al. (2005) reported sugars such as xylose and arabinose outside

fructose, glucose and sorbitol in fruit cultivars and their molasses. Karahalil and Şahin (2011) demonstrated that phenolics such as gallic, protocatechuic, p-OH benzoic, chlorogenic, vanillic, p-coumaric, ferulic, syringic acids and catechin and rutin were in extracts, also found high the *AC* of the fruit extracts. Ergüney et al. (2015) reported that the main anthocyanins determined in cherry laurel are cyanidin-3-arabinoside and peonidin-3-arabinoside. Furthermore, Kolaylı et al. (2003) revealed that their fruits contain significant amounts of potassium (2215 mg/kg), magnesium (179 mg/kg), calcium (153 mg/kg), sodium (55 mg/kg), manganese (24.2 mg/kg) and traces of iron ( $8.3 \pm 0.8$  mg/kg), zinc ( $1.9 \pm 0.2$  mg/kg) and copper ( $0.8 \pm 0.1$  mg/kg). Additionally, Alasalvar et al. (2006) reported that between *α*-tocopherol 0.29-0.42 mg, *γ*-tocopherol 0.55-0.69 mg and *β*-sitosterol 192.5-222 mg per 100 g of oil obtained from the seeds.

Also, cherry laurel fruit is used as a medicinal plant to treat health issues like stomach ulcers, digestive problems, bronchitis, eczema, and hemorrhoids. The fruit also acts as a diuretic. Furthermore, they have anti-inflammatory, antinociceptive, antioxidant, neuroprotective, antidiabetic and anticarcinogenic effects (Karahalil and Şahin, 2011; Demir et al., 2017). Cherry laurel fruits are usually eaten fresh, but they can also be dried, pickled, and used to make jam, marmalade, and fruit juice, (Şahan et al., 2012). Although their fruits have been processed into molasses using traditional methods recently, their commercial production has not become widespread yet. It's important to diversify the usage of cherry laurel fruits, which is rich in phytochemicals, by turning it into different foods like molasses. This helps to increase the economic potential of this valuable fruit. Additionally, using it in different food formulations can increase the functionality of foods (Vahapoğlu et al., 2018). Molasses that are traditional foods are sweet, delicious natural products produced by

concentrating sugar-rich fruit juices by boiling them without adding any food additives or sugar, thus extending their shelf life. Molasses are produced mainly from sugar-containing fruits such as grapes, mulberries, figs, apples, plums, carob, dates, apricots, cranberry, blueberries, pomegranate, black mulberry and watermelons, as well as from yields such as andız, juniper, sugar beet or cane, and sugar millet (Şimşek and Artık, 2002; Tosun and Üstün, 2003; Turhan et al., 2007; Kalaycıoğlu, 2023). Molasses is an important food that can immediately meet the required energy needs due to quickly mixing into the blood. The 100 g of molasses contains 55-80% sugar and 0.6-0.9% nitrogenous substance, 2.2-14 µg Vitamin B1, 150 µg Vitamin B2, 1.4 mg niacin (Vitamin B3) and provides approximately 280 kcal. In addition, it has been reported that molasses is a good source of mineral substances such as K, Ca, Mg, P, Na, Fe, Zn, Cu and Mn (Şimşek and Artık, 2002; Toker and Hayoğlu, 2004; Ekin and Çelikezen, 2015).

In the literature review, although there are a limited number of studies on cherry laurel fruit and especially its molasses, there are no studies for determining the possible changes in molasses compositions at different storage temperatures. The study aimed to demonstrate the effect of model storage temperatures and times on the changes in the phytochemical compounds and physical properties of molasses produced by vacuum using two cherry laurel fruits and to obtain mathematical equations explaining the thermal stability of phytochemicals.

## MATERIAL AND METHODS

### Material

A total of 15 kg of each Kiraz (KCL) and Findık (FCL) cherry laurel cultivar (*Laurocerasus officinalis* L.) were collected on 24 July and 5 August 2017, respectively by the sampling rules from predetermined trees in Giresun province and its surroundings. The collected cherry laurels were then processed into two different types of molasses.

### Methods

#### *Production of cherry laurel molasses (FCLM, KCLM)*

To remove or reduce the dust, soil, microorganisms, and agricultural pesticide residues on the harvested and sorted KCL and FCL fruits, washing was done with tap water. Following cleaning, drying, and crushing into small pieces by hand, tap water was added to the mix at a ratio of 1:1. Wort was subjected to short-term pre-heat treatment (3 min at 80-85 °C) to inactivate enzymes and facilitate extraction and cooled to room temperature (25 ±2°C). Then, worts waited for 2 hours at 25 ±2°C for extraction, and the coarse sediment and seeds were removed using a filter cloth for clear juice (repeated twice). The obtained extracts were kept in a refrigerator (4 °C) for 12 hours and filtered through coarse filter paper to remove the fine sediment. The obtained clear filtrates were concentrated in a laboratory rotary evaporator (Heidolph Laborota 4000, Germany) at 50±2 °C, under vacuum (500-600 mmHg), at a rotational speed of 60-120 rpm (60 rpm at the beginning, 120 rpm towards the end). Evaporation terminated when the soluble dry matter (SDM) was 68% by refractometer (Hanna HI 96800, Romania) (Fig. 1).

#### *Packaging and storage of KCLM and FCLM samples*

KCLM and FCLM samples produced from FCL and KCL cultivars were placed in 50 mL glass jars, and their lids were closed. The molasses samples in the jar were stored at three different temperatures (50-60 and 70 °C) according to the experimental plan, between 6 and 168 hours, for seven storage times. The samples, whose storage period was completed, were immediately cooled with ice water and kept in a deep freezer at -24 °C until analysis.

#### *Physicochemical analyzes*

Soluble dry matter (SDM) and Total dry matter (TDM)

The SDM of fresh fruit and their molasses were determined by a digital refractometer (Hanna HI 96800, Romania). TDM was determined by calculating the weight loss caused by keeping the fresh fruit and molasses in a certain amount in glass petri dishes in an oven (Ecocell, Germany) at 70 °C until they reach a constant weight.

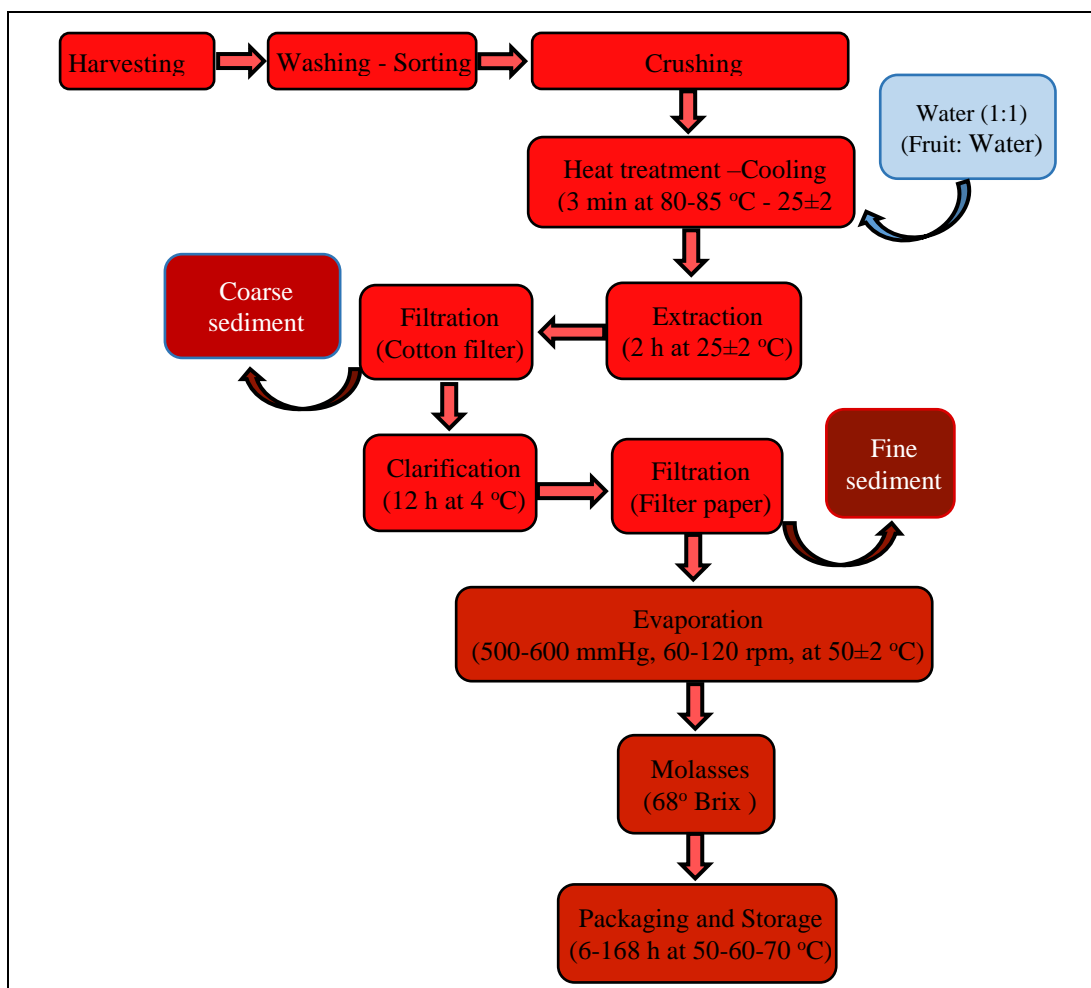


Figure 1 Production steps of KCLM and FCLM

#### pH and Titratable acidity (TA)

The pH of fruits and molasses was determined potentiometrically using a pH meter (Mettler Toledo-S210, Switzerland) calibrated with buffer solutions (pH 4.0 and pH 7.0). TA was determined by the titration of fresh fruit and molasses with 0.1 N NaOH solution using a pH meter up to pH 8.1 and expressed as g/100 g malic acid from the spent amount of NaOH.

#### 5-Hydroxymethylfurfural (HMF)

For HMF, 1 g of molasses samples were diluted with distilled water in appropriate proportions, made up to 50 mL with 2 mL of Carrez I and Carrez II solutions, mixed by vortex and filtered with Whatman 42 filter paper. After taking 1 mL of the filtrate and adding 2.5 mL of p-toluidine and 0.5 mL of barbutyric acid solutions,

homogenized samples' the absorbances ( $Abs$ ) were read within 1-2 min against the witness sample prepared with distilled water instead of samples at 550 nm using UV-VIS spectrophotometer (Shimadzu UV mini-1240, Japan) (Cemeroğlu, 2010).

#### Vitamin C (Vit C)

The amount of Vit C in fresh fruit and their molasses was determined with the spectrophotometric method reported by Cemeroğlu (2010). After taking 1 g sample and making it up to 25 mL with 6% metaphosphoric acid solution, it was kept in the dark for two hours, centrifuged at 727 x g (2500 rpm) for 5 min (Sigma 2-6, Germany) and filtered. The 5 mL of acetate buffer (pH 4.0), 2 mL of 2.6 dichlorofernolindophenol solution and 10 mL of

xylene were added to 5 mL of the filtrate. Then, *Abs* readings were made against the blank prepared with metaphosphoric acid after 30 min at 500 nm using a *UV-VIS* spectrophotometer (Shimadzu UV mini-1240, Japan).

#### Total Phenolics (TP)

In determining TP, the colorimetric Folin-Ciocalteu method reported by Cemeroglu (2010) was used with some modifications. Approximately 1 g of KCL and FCL were weighed and extracted using a shaker in 80% methanol (MeOH) containing 5 mL of 1% HCl for 2 hours and 10 min at 4000 rpm. Then, extracts were centrifuged and the current extraction process was repeated twice. 20  $\mu$ L of the prepared and combined MeOH extracts of fresh fruit and molasses were transferred to disposable spectrophotometer cuvettes, on added 75  $\mu$ L of Folin-Ciocalteu reagent and 750  $\mu$ L of Na<sub>2</sub>CO<sub>3</sub> (7.5%) solution and 765  $\mu$ L of distilled water. The *Abs* of mixtures kept in the dark for 90 min at room temperature was measured at 725 nm using a *UV-VIS* spectrophotometer (Shimadzu UV mini-1240, Japan). The TP was calculated over mg GAE/kg fresh fruit and molasses using the calibration curve obtained from the solutions prepared from gallic acid.

#### Total Monomeric Anthocyanin (ACN)

The total monomeric ACN was determined according to the pH differential method given by Lee et al. (2005). Accordingly, MeOH (80% MeOH + 20% H<sub>2</sub>O containing 1% HCl) extracts of fresh fruit and molasses diluted with 0.025 M KCl (pH 1.0) and 0.4 M CH<sub>3</sub>COONa\*3H<sub>2</sub>O (pH 4.5) buffer solutions at appropriate rates. The *Abs* of diluted samples hidden in the dark for 15 min were detected at 520 nm and 700 nm with a *UV-VIS* spectrophotometer (Shimadzu UV mini-1240, Japan). Results were expressed in mg/kg based on the cyanidin-3-glucoside equivalent.

#### DPPH Free Radical Scavenging Activity (DPPH-RSA) and Antioxidant Capacity (AC)

For DPPH-RSA analysis, 2.9 mL of DPPH radical solution (1 mM) was added to the 0.1 mL MeOH extracts of the samples obtained for phenolics. After keeping in a water bath at 30 °C

for 30 min, *Abs* was measured using a *UV-VIS* spectrophotometer (Shimadzu UV mini-1240, Japan) at a wavelength of 517 nm. DPPH-RSA was calculated as % inhibition according to the formula below (Equation 1). It was also expressed as Trolox equivalent (mg TE /100 g fresh fruit and molasses) using the daily prepared Trolox standard calibration curve (Cemeroglu, 2010).

$$DPPH - RSA(\%) = (1 - (Abs_s / Abs_c)) \times 100 \quad (1)$$

where *Abs<sub>s</sub>* = the *Abs* of the sample, *Abs<sub>c</sub>* = the *Abs* of the control sample

#### Viscosity

The viscosity of the molasses samples in the homogenized glass jar was measured at 20 °C at a shear rate of 100 rpm using a Brookfield viscometer and probe no s-63.

#### Browning Level (BL)

Approximately 1.5 g of fresh fruit and their molasses were weighed into centrifuge tubes and made up to 10 mL with distilled water. After adding 20 mL of ethyl alcohol and homogenizing the samples by vortex, centrifuged (Sigma 2-6, Germany) for 5 min at 727 x g (2500 rpm). 5 mL samples were taken from the supernatant part and added 5 mL of distilled water and 1 mL of K<sub>2</sub>S<sub>2</sub>O<sub>5</sub>. The *Abs* of centrifuged mixtures again at 1860 x g (4000 rpm) for 5 min and waited for 20 min were detected at 420 nm in a *UV-VIS* spectrophotometer (Shimadzu UV mini-1240, Japan). *BLs* were calculated by multiplying the read *Abs* value with the dilution factor (Cemeroglu, 2010).

#### Hunter L\*, a\* and b\* color values

The Hunter color values of fruit and molasses samples were measured by a color meter that was calibrated with the standard calibration plate of L\* = 97.79, a\* = -0.44 and b\* = +2.04 (Konica Minolta CR-410, Japan).

#### Statistical analysis

The research was set up and conducted in a Factorial Experimental Design (2 cherry laurel molasses (FCLM, KCLM) x 3 temperature (T) x 7 storage time (ST) x 2 replications, a total of 84 samples). The averages of the sources of variation found to be significant in the Analysis of Variance

(ANOVA) were compared using the Tukey Multiple Comparison Test (TMCT) (Düzgüneş et al., 1987). Also, the data of phytochemicals such as TP, ACN, and Vit C were subjected to multi-regression analysis to get mathematical models reflecting the changes with T and ST (Equation 2). The MINITAB 18 statistic program was used for data analysis.

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j \quad (2)$$

where  $\beta_0$ ,  $\beta_i$ ,  $\beta_{ii}$ , and  $\beta_{ij}$  = regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively,  $X_i$  (T) = the independent variable as temperature (°C),  $X_j$  (ST) = the independent variable as storage time (h)

## RESULTS AND DISCUSSION

### Some physicochemical properties of cherry laurel cultivars and their molasses

The physicochemical analysis results of cherry laurel cultivars and their molasses are shown in Table 1.

Table 1. Some physicochemical compounds of cherry laurel cultivars and their molasses (n=2)<sup>a</sup>

Properties	Cherry laurel cultivars and their molasses			
	KCL	KCLM	FCL	FCLM
TDM (%)	19.78±0.74	72.60±0.43	23.70±0.64	72.79±0.31
SDM (%)	18.3±0.14	68.2±0.00	22.2±0.14	68.00±0.00
pH	4.57±0.014	4.53±0.014	4.42±0.00	4.36±0.007
TA (% as malic acid)	0.369±0.005	0.547±0.010	0.380±0.003	1.201±0.018
HMF (mg/kg)	-	9.94±1.24	-	21.07±2.33
Vit C (mg/100g)	64.01±6.67	59.98±0.52	81.52±1.07	63.17±5.17
TP (mgGAE/100g)	802.59±90.77	4287.60±20.24	1233.84±33.57	5083.20±390.25
ACN (mg/kg)	955.77±27.25	42.86±3.40	1201.22±112.20	36.03±3.08
DPPH-RSA (%)	71.39±0.00	66.50±2.15	75.11±1.95	77.94±3.66
AC (µg TE/mg sample)	29.91±0.00	27.85±0.90	31.47±0.82	32.65±1.54
Viscosity (at 100 rpm)	-	805.5±53.03	-	836.5±21.92
BL (A <sub>420</sub> /mL)	4.291±0.862	14.529±0.131	4.960±0.200	15.181±0.029
<b>Hunter Color values</b>				
L*	26.42±0.09	31.25±0.13	21.84±0.01	30.73±0.16
a*	6.52±0.04	0.94±0.04	4.10±0.10	0.74±0.03
b*	4.00±0.08	-0.14±0.02	0.83±0.01	-0.47±0.18

<sup>a</sup>: as Mean±SD (Standard deviation)

Table 1 shows that significant differences existed between compositions of both fruits in terms of Vit C, TP and ACN. Also, the composition elements like TA, HMF, Hunter L\* a\* and b\* values markedly changed during molasses production. While pH, Vit C, ACN, Hunter, a\* and b\* values of fruits generally decreased with molasses production, TA, TP, BI, and Hunter L\* values increased. On the other hand, except for pH, Hunter L\*, a\* and b\* values of FCL and FCLM, the SDM, TDM, TA, Vit C, TP, ACN, DPPH-RSA, AC, viscosity and BL values were higher than KCL and KCLM. Furthermore, the highest HMF was in KCLM compared to FCLM.

The pH values of KCL and FCL cultivars were 4.57 and 4.42, respectively, and in their molasses

were slightly lower averages of 4.53 and 4.36, respectively. Concerning pH, the TA of both fruits varied between 0.369 and 0.380% as malic acid. The average TA of FCLM (1.201%) was higher than KCLM (0.547%). According to the Turkish Food Codex, both molasses were within the pH 3.5-5.0 limits specified for sour molasses (TFC 2017).

Due to the nature of the food industry, especially the chemical composition of processed raw materials, processing conditions and techniques increase 5-hydroxymethyl-2-furfural formation in foods. In the formation of HMF, not only simple sugars but also polysaccharides, proteins (amino acids), low pH and high temperatures applied

during the process are effective (Kowalski et al., 2013). The amount of HMF of the molasses vacuum evaporated showed an average value almost two-fold higher in FCLM, which has a lower pH and high TA than KCLM. HMF findings were consistent with between 0.15-166 mg/kg values that were reported for different molasses (Şimşek and Artık, 2002; Tosun and Üstün, 2003; Toker and Hayaloğlu, 2004; Turhan et al., 2007).

Compared to KCL, FCL contained more Vit C at 81.52 mg/100g. However, Vit C decreased to 59-63 mg in both molasses during the vacuum evaporating process. After all, FCLM had a higher Vit C degradation rate at 23%. Consistent with our results, Kuşçu and Bulantekin (2016) reported a gradual decrease of ascorbic acid in apple pekmez by open-pan evaporating (56%) and vacuum evaporating (23%).

In our samples, the TP of FCL was about 53% higher than KCL, and the TP of cultivars increased 5.34 fold in KCLM and 4.12 fold in FCLM due to concentration during molasses production. As can be seen, cherry laurel fruits are a good source of antioxidants such as Vit C, TP and ACN. In a previous study, TP in KCL, FCL and molasses samples was determined by Alasalvar et al. (2005) as 454, 651, and 1444 mg GAE/100 g, respectively. Celep et al. (2012) reported the average TP in dry extracts of cherry laurel fruits as  $23.64 \pm 0.84$  mg GAE/g. Ayaz et al. (1997a), in the phenolic profile of cherry laurel fruits and the wild form, determined vanillic acid as the predominant phenolic acid besides cinnamic (p-coumaric and caffeic acids), benzoic (p-hydroxybenzoic, protocatechuic, and vanillic acids) acids. According to the literature, our data shows approximately 2-fold higher from fruit and 3-4-fold higher from molasses, except for Celep et al. (2012), where the dry extract was used. Furthermore, Tunç et al. (2021) reported that the total phenolic contents of the grape pekmez samples produced by ohmic heating-assisted vacuum evaporation varied between 1.36 and 1.67 mg GAE/g sample. As a result, it turned out that cherry laurel fruits are a good source of TP. Cherry laurel fruits and molasses' TP differences

may be due to ecological conditions and harvest time. During the extraction, crushing and heat treatment stages of the fruits, bioactive compounds with antioxidant effects such as amygdalin, tocopherols and sterols pass through the crushed seeds and may have increased the AC in both molasses. As a matter of fact, in the previous study, Elmastas et al. (2013) determined that the ACs of amygdalin, prunasin and  $\beta$ -sitosterol isolated from cherry laurel seeds were higher than BHA and lower than BHT and  $\alpha$ -tocopherol.

Alasalvar et al. (2005) reported a higher ACN in KCL, FCL variety and their molasses than our results to be 123, 174 and 9.3 mg/100 g, respectively. Although washing, sorting and pre-heating to remove contaminants for fruits and evaporating at low temperatures (vacuum) to prevent the color loss for molasses were made, discolorations might have been due to the effects of moderate light intensity and heat rather than endogenous and microbial enzymes (glycosides, peroxidases and polyphenol oxidases). Additionally, anthocyanin monomers might have polymerized into brownish oligomers, known as polymeric color. Also, researchers reported that the color losses catalyzed by high temperature, time, oxygen and metal ions were even more accelerated by ascorbic acid, glucose and fructose and their degradation products (Stintzing and Carle, 2003). In this study, increasing concentration may have affected the decrease of monomeric anthocyanin. Similarly, Kırca et al. (2006) reported that the degradation of monomeric anthocyanins in black carrots increased with increasing solid content during heating and decreased during storage. Furthermore, it is important to note that both molasses used in the study have a pH level above 4. This means that four different types of anthocyanins, namely flavylium cation, anhydrous quinoidal base, colorless carbinol base, and pale yellow chalcone, will likely coexist in KCLM and FCLM. These various forms of anthocyanins significantly impact the process of thermal degradation (Jiang et al., 2019). Additionally, it has been found that the stability of anthocyanins is affected by whether they are acylated or

unacylated. Studies have shown that colorants from red sweet potato and purple carrot, which are rich in acylated anthocyanins, exhibit higher stability than colorants from purple corn and red grape, which are rich in non-acylated anthocyanins, under different pH, temperature, and light conditions (Cevallos-Casals and Cisneros-Zevallos, 2004).

DPPH-RSA varies between 71.39% and 76.49% among fruits. FCL exhibited a higher mean DPPH-RSA ( $75.11 \pm 1.95\%$ ), due to its higher TP and Vit C content DPPH-RSA increased in FCLM, reaching 80.53%, but decreased to 66.50% in KCLM. AC of FCL varied between 30.89 and 32.05  $\mu\text{g}/\text{mg}$  over TE, higher than KCL ( $29.91 \mu\text{g}/\text{TE mg}$  sample). Furthermore, AC ( $27.85 \pm 0.90$ ) decreased in KCLM but increased in FCL ( $32.65 \pm 1.54$ ). Additionally, Liyana-Pathirana et al. (2006) found AC to be higher in cherry laurel molasses on a fresh weight basis and hydrogen peroxide and DPPH-RSA in fruit on a dry weight basis. This situation explained by the authors that it was due to the moisture content of both samples and the possible destruction of antioxidant compounds during molasses production. They calculated the inhibition values of their fruits as 23.4, 20.7 and 14.0% at concentrations of 400, 200 and 100 mg/kg, respectively. On the other hand, many phenolics in nature are shown AC due to their reducing, singlet oxygen-scavenging and metal-chelating properties (Robards et al., 1999). Additionally, AC has reflected several phytochemicals substantial in the fruit and their synergistic effects. Also, studies have shown a direct relationship between the TP and AC of many fruits and vegetables (Jacobo-Velázquez and Cisneros-Zevallos, 2009; Matthes and Schmitz-Eiberger, 2009). Indeed, regarding the issue, Kolaylı et al. (2003) found that the cherry laurel fruits' AC of TP were higher than the reference ascorbic acid.

The BL of the fruit extracts was partially higher in FCL than in KCL as Abs/mL at 420 nm. BL value increased in KCLM and FCLM and reached the values of 14.621 and 15.201, respectively. The most significant change in color values was that

the Hunter  $a^*$  value, which is an index of the red color in fruits, decreased, and the positive Hunter  $b^*$  value increased in a negative trend ( $-b^*$ ) with molasses production. That is, while the red color tone decreased in molasses, the violet-purple color tone became dominant.

#### **Thermal changes in some physicochemical properties and phytochemical compounds of molasses during storage at different temperatures and times**

Total dry matter (TDM) and Soluble dry matter (SDM)

ANOVA results showed that only the amount of TDM and SDM was affected by C at the  $P < 0.01$  level. T and ST with CxT, CxST, TxST, and CxTxST interactions did not affect TDM and SDM. When comparing TDM averages of molasses using the TMCT, the FCLM had the highest average value of 72.85%, while the KCLM had the lowest average of 72.42%. According to the TMCT results of SDM for C, SDM had significantly different values in KCLM (67.83%) and FCLM (67.68%).

#### *pH and Titratable acidity (TA)*

ANOVA showed that variations in C, T and ST sources, besides CxT, TxST, CxST and CxTxST, had a statistically significant impact on the pH value and TA of all molasses at  $P < 0.01$ . Comparing the pH and TA averages based on TMCT, KCLM molasses experienced a reduction in pH (from 4.53 to 4.41) and an increase in TA (from 0.515 to 0.714%) as the T and ST increased. Additionally, similar changes in FCLM occurred in the form of a decrease from 4.36 to 4.18 for the pH and an increase from 1.12 to 1.48% for TA. Similarly, Buckow et al. (2010), when pasteurized blueberry juice was stored at 4, 25, and 40 °C for approximately 6, 2, and 0.5 weeks, respectively, the pH decreased from 3.0 to 2.85, and the latter remained steady. The authors suggested that this reduction may be due to the rise in phenolic acids and ACN degradation products. In addition, increasing T and ST may cause the release of galacturonic acid from the breakdown of pectin, leading to an increase in TA and a decrease in pH value (Anthon and Barrett, 2012).



*Vit C and HMF*

ANOVA analysis indicated that Vit C was affected significantly by C and ST factors. Additionally, all sources of variation and interaction effects on the HMF level were statistically significant at  $P < 0.01$ . When comparing the averages of Vit C with TMCT for C and ST, Vit C in KCLM had a higher value of 55.01 mg/100 g than FCLM's 51.09 mg/100 g. Also, the Vit C averages of both molasses samples

decreased with ST compared to the control samples, with a range of 37.1-43.7 mg/100 g (30-41%) (Fig. 2). Similarly, Kuşçu and Bulantekin (2016) determined that after 4 months of storage, the loss in ascorbic acid was 69.54% in vacuum evaporation for apple pekmez. Beşe and Polatoğlu (2017) reported that oxygen and light in the environment are the most significant factors in the loss of Vit C at the rate of 51.1% after sun drying the cranberry fruit.

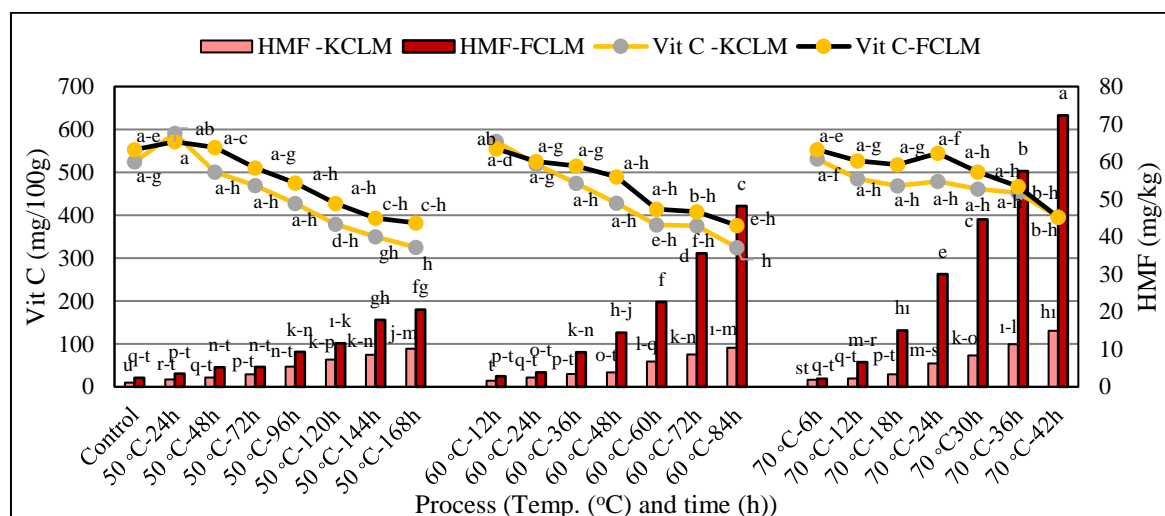


Figure 2 Thermal change of Vit C and HMF

Means shown with the same capital letter (a-h for Vit C, a-u for HMF) in the bar chart are not statistically different from each other ( $P < 0.05$ , Tukey's test).

HMF is a cyclic aldehyde produced by ascorbic acid and sugar deterioration. It can also form through the Maillard reaction, which occurs during food processing or long-term food storage (Torribio and Lozano, 1984; Cemeroglu, 2013; Shapla et al., 2018). While the HMF content of FCLM was approximately 2-fold (21.07 mg/kg) at the start of storage compared to KCLM, the difference reached up to 5-fold with increasing T and ST (Fig. 3). Babsky et al. (1986) explained the increase of HMF during 111 days of storage of concentrated apple juice at 37 °C, the first period including an induction time of about two weeks, a rapid increase in HMF within 50 days, the second period, and the decrease in the rate of HMF formation in 3 periods as the third period. A study conducted by Burdurlu and Karadeniz (2003) discovered that two apple juice concentrates kept at 5 °C and 20 °C did not

experience a significant increase in HMF levels. However, when stored at 37 °C, the HMF levels at the end of storage had reached 963 and 190 mg/kg in Golden Delicious and Amasya apple juice concentrates, respectively. Similarly, a study by Simsek et al. (2006) determined HMF levels in two grape juice concentrates after thermal storage. Results showed that HMF amounts were affected by grape type, concentration, temperature, and duration of storage. Most researchers reported that the increase of HMF is related to heat processing, temperature and storage, and the presence of simple sugars (like glucose and fructose), acids (with low pH), aw (water activity), protein, and minerals (such as Ca, K, Mg, Na<sup>+</sup>, Fe<sup>2+</sup>, and Fe<sup>3+</sup>) (Torribio and Lozano, 1984; Burdurlu and Karadeniz, 2003; Şimşek et al., 2007; Cemeroglu, 2013; Karataş and Şengül, 2018; Shapla et al., 2018). These results are

similar to the research findings and the highlighted results on the subject.

*Total Phenolics (TP) and DPPH-RSA*

According to the ANOVA results, the effects of T, ST and CxT on TP and C, T, ST, CxT and CxST on DPPH-RSA were found significant ( $P < 0.05$ ). After comparing the statistically significant CxT interaction averages with the TMCT, it is found that the TPs of KCLM and Insert Fig. 3 here

FCLM decreased due to an increase in T and ST in all thermal processes. The TP averages reached during the last storage period at 50 and 60 °C were statistically similar in both molasses, but the TP of both molasses differed at the end of the storage period at 70 °C. After all, it has been found that FCLM TP's stored for 6-42 h at 70 °C are better preserved than other samples. Furthermore, it showed that the ST had a higher effect on the TP of both FCLM and KCLM than the T (Fig. 3).

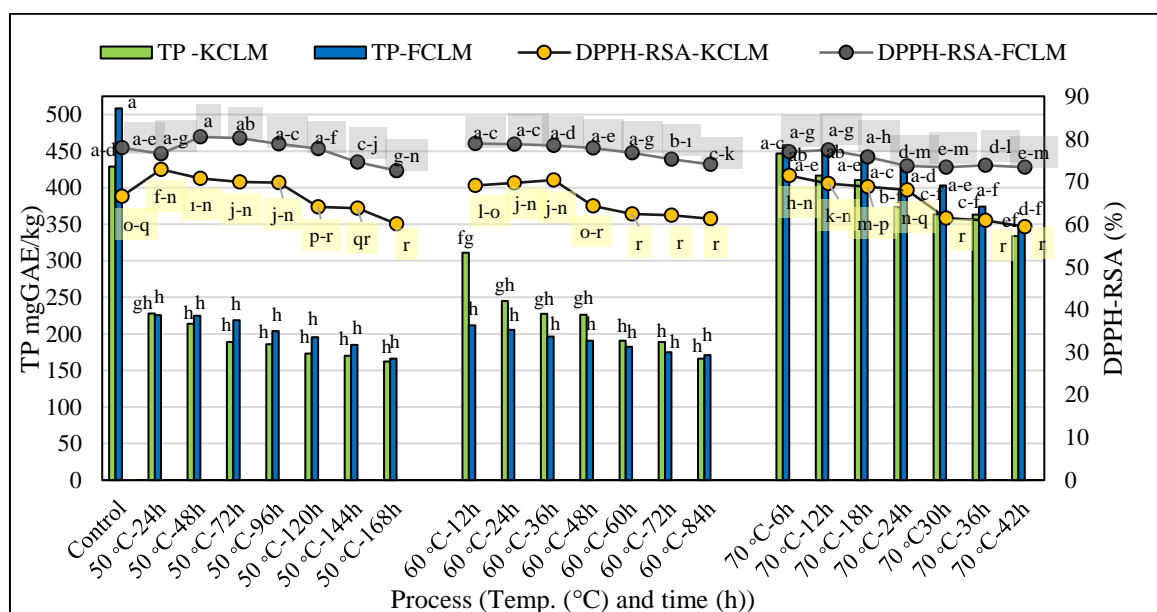


Figure 3 Thermal change of TP and DPPH-RSA with process

Means shown with the same capital letter (a-h for TP, a-r for DPPH-RSA) in the bar chart are not statistically different from each other ( $P < 0.05$ , Tukey's test).

Kuşçu and Bulantekin (2016) reported that the highest TP in apple molasses produced by two different methods (boiler and vacuum) was in molasses produced by vacuum. However, the amount of TP as epicatechin (114-138 to 92-112 mg/kg) and catechin (94-115 to 83-97 mg/kg) decreased during storage compared to the initial value. Moldovan et al. (2016) discovered that extracts' TP decreased slightly (between 3.1% and 22.6%) after being stored at 2 °C in the dark for 10-60 days. However, the TP of stored extracts at room temperature (22 °C) decreased significantly after 60 days (25.4%). Furthermore, TP deterioration was 8 and 16 times higher at 55 °C and 75 °C, respectively, compared to 2 °C. The

researchers explained that high temperatures cause the polyphenolic content of the extracts to decrease due to an increase in the oxidation rate of bioactive components. Similar decreases in TP (16.31-9.31 µg GAE/mg sample) and AC (21.29-17.38%) were observed by Karataş and Şengül (2018) in mulberry molasses stored at  $20 \pm 2$  °C for 6 months. The findings of this study coincide with the literature findings reported above.

When comparing the DPPH-RSA mean values of CxT interaction to TMCT, the highest and lowest values were observed at 50 and 70 °C, respectively. Additionally, the mean value of FCLM is greater than that of KCLM. Comparing

CxST averages with TMCT, DPPH-RSA of both molasses reduced as the storage process progressed. However, the DPPH-RSA value of FCLM was higher than KCLM throughout the same storage period (Fig. 3).

*Total monomeric anthocyanin (ACN) and Antioxidant capacity (AC)*

As a result of ANOVA, the effect of CxTxST interaction on the amount of ACN and AC was significant at the  $P < 0.01$  level. The ACN amount of the control samples was higher (42.86 mg/kg)

in KCLM than in FCLM. When the averages of CxTxST ACNs data are compared according to TMCT, they decreased with increasing time of 50, 60 and 70 °C. While these decreases were between 71.60-80.91% at the end of 50 °C-168 h in both molasses and 85% in KCLM at the end of 60 °C-84 h, it completely disintegrated in FCLM at the end of 60 °C-60 h. At the end of the highest temperature of storage, at 70 °C-42 h, the amount of ACN decreased in KCLM to 3.73 mg/kg with a loss of 91%, while ACN completely degraded in FCLM at the end of 70 °C- 24 h (Fig. 4).

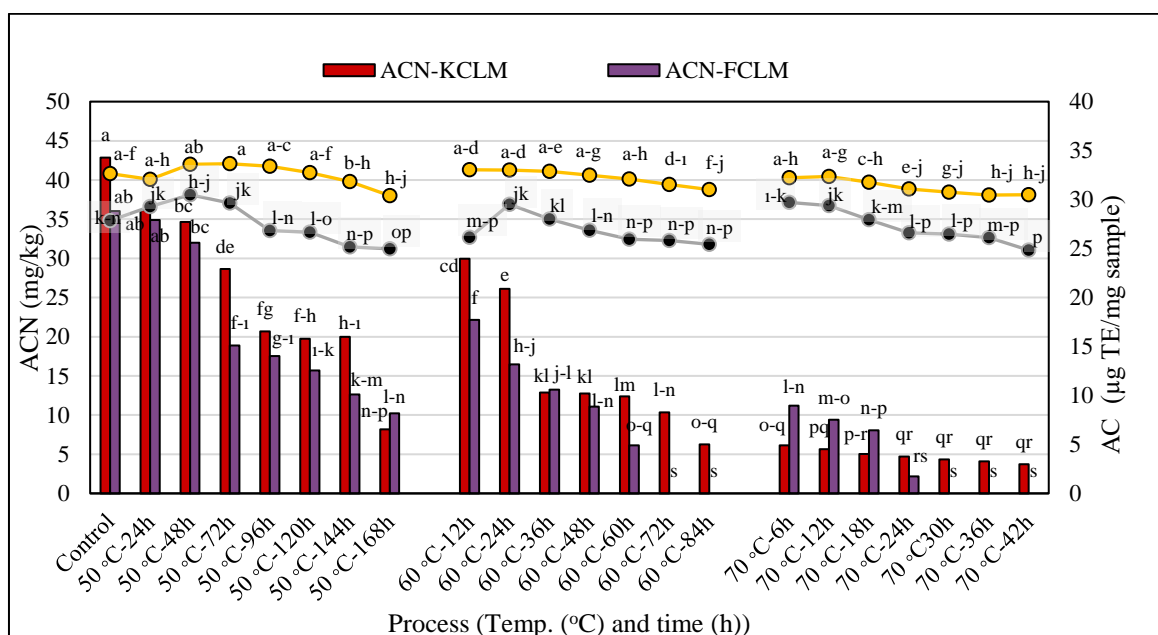


Figure 4 Thermal change of ACN and AC with process

Means shown with the same capital letter (a-s for ACN, a-p for AC) in the bar chart are not statistically different from each other ( $P < 0.05$ , Tukey's test).

The AC of FCLM and KCLM samples were increased slightly at baseline relative to their controls but decreased with increasing time at 50, 60 and 70 °C. Additionally, FCLM's AC decrease was higher than KCLM's. The fact that the compounds such as Vit C, TFM and ACN, which have AC in both molasses, decreased with increasing T and ST probably may have contributed to the slight decrease in AC with extension ST. However, the slight increases seen in the first process applications may be related to the HMF increase, which is known to have AC, as well as phloroglucinaldehyde and protocatechic

acid, which have higher antioxidant activity revealed by ACN degradation (Sadilova et al., 2007). According to Karataş and Şengül (2018), mulberry molasses stored at  $20 \pm 2$  °C for 6 months showed a decrease in AC (from 21.29% to 17.38%) and TP (from 16.31 µg GAE/mg sample to 9.31 µg GAE/mg sample).

*Hunter L\*, a\*, b\* values and Browning Levels (BL)*

As a result of the ANOVA, the effect of CxT interaction on Hunter L\* and BL, CxTxST interaction on Hunter a\* value and CxT, CxST and TxST interactions on Hunter b\* value were

found significantly at the  $P < 0.05$  level. When the Hunter  $L^*$  value averages belonging to C were compared with TMCT, it was determined that KCLM had a lighter color tone than FCLM. Hunter  $L^*$  value of both molasses cultivars was affected the most during storage at 50 and 70 °C, Insert Fig. 5 here

and this effect was partially high in KCLM. Presumably, the differences in the composition elements of the cultivars (Vit C, ACN concentration, HMF, amount of mineral matter, etc.) caused the differences in the Hunter  $L^*$  value of molasses (Fig. 5).

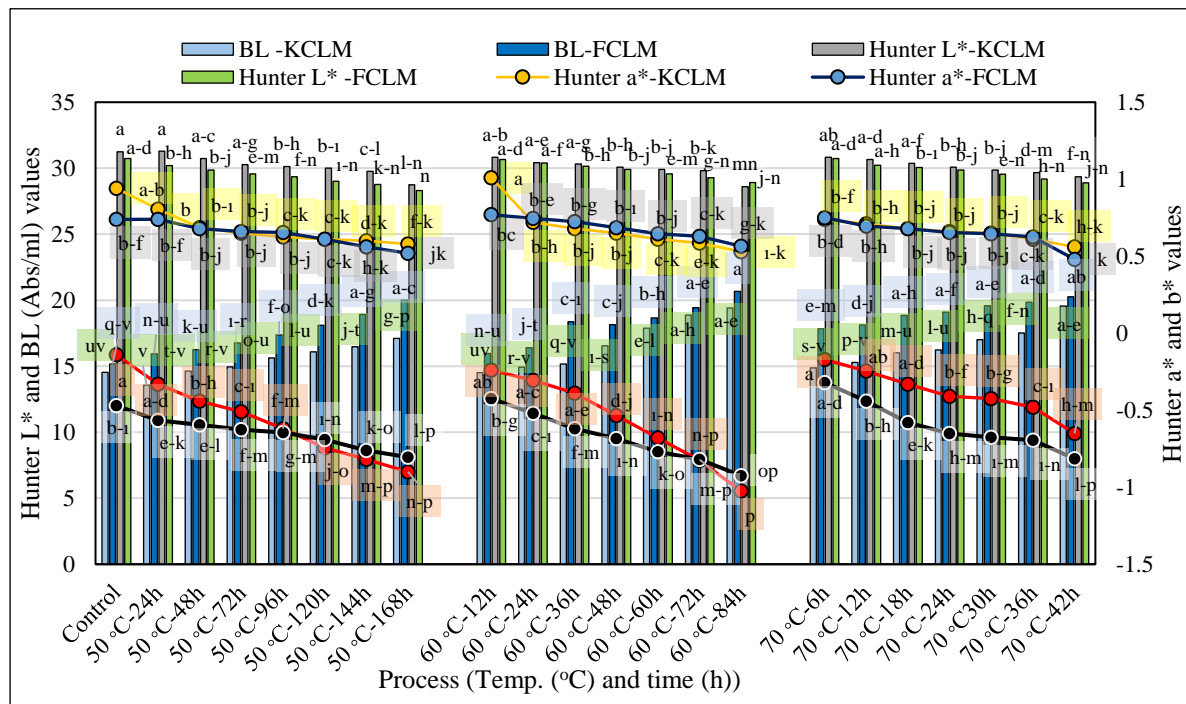


Figure 5 Thermal change of Hunter  $L^*$ ,  $a^*$ ,  $b^*$  values and BI

Means shown with the same capital letter (a-n for Hunter  $L^*$ , a-k for Hunter  $a^*$ , a-p for Hunter  $b^*$ , a-v for BI) in the bar chart are not statistically different from each other ( $P < 0.05$ , Tukey's test).

TMCT results showed a reduction in cyanidin derivatives that provide the red color (Hunter  $a^*$  value) due to increased T and ST. The read Hunter  $a^*$  value after 168 hours of storage at 50 °C varied between 0.520 and 0.580, and the effect of 72-84 hours at 60 °C and 42 hours at 70 °C on Hunter  $a^*$  values were similar for both molasses (Fig. 5).

Additionally, according to the statistically significant CxT interaction, the difference between increasing T and Hunter  $b^*$  value was highest in KCLM (-0.3871). Also, CxST co-interaction decreased the Hunter  $b^*$  value of molasses cultivars, and the blue color tone turned into purple-violet (increase in delphinidin

derivative) tones and reached the highest average values in the last storage period. Hunter  $b^*$  values of both molasses were statistically indifferent ( $P < 0.05$ ) at the end of the ST. According to the TMCT compared results of SxDS Hunter  $b^*$  means for both molasses, the highest increase was at 60 °C in the negative direction, followed by increases at 50 °C and 70 °C, respectively. However, there was no difference between these increases at the statistical significance level ( $P < 0.05$ ).

According to the TMCT results of the CxT interaction found to be important in the BL data, BL increased significantly with increasing T based on C. The highest BL values were determined at

70 °C (19.08) for FCLM, at 60 °C (16.76) and 70 °C (16.64) for KCLM, but there was no statistical difference between both T.

*Viscosity*

According to the ANOVA analysis, factors T and ST had a significant effect, along with the interactions CxT, CxST, and TxST, on the viscosity of KCLM and FCLM at a statistical level of  $P < 0.05$ . However, the C and CxTxST did not

have any effect on viscosity. According to the TMCT results of viscosity averages of CxT, while the measured viscosity of KCLM at a 100 rpm shear was 718 at 50 °C, it reduced with the increased T compared to the control to 653 and 522 at 60 and 70 °C, respectively. FCLM also showed similar decreases, but the reductions at 50 and 70 °C were no different from each other statistically ( $P < 0.05$ ) (Fig. 6).

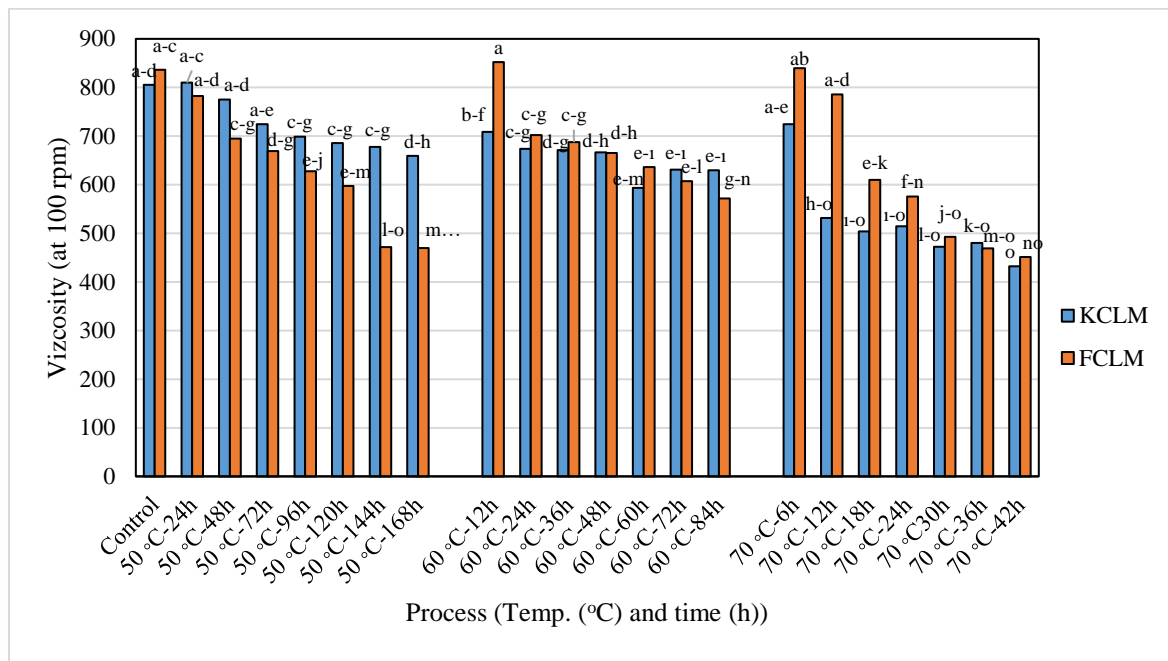


Figure 6 Thermal change of viscosity

Means shown with the same capital letter (a-o) in the bar chart are not statistically different from each other ( $P < 0.05$ , Tukey's test).

The viscosity averages of two different molasses were significantly affected by the interaction of CxST, as revealed by TMCT. The increase in ST led to a decrease in viscosity, and this decrease varied depending on the type of molasses. The KCLM and FCLM's viscosities decreased by 29% and 40%, respectively, compared to the control sample. Differences in viscosity between the molasses are likely due to the characteristics of each cultivar. However, while pectin remaining in molasses initially provided a specific consistency to the samples with the presence of sugar, increased T may have caused a decrease in viscosity by disrupting the structure of pectin (decomposition into galacturonic acid) and

sucrose content. Studies indicate that low pH values and high temperatures can cause the breakdown of glycosidic bonds and the deterioration of pectin structure (Sundar Raj et al., 2012). The interaction between SxDS had an impact effect on the change in molasses viscosity. When comparing the averages based on TMCT, the highest viscosity value was observed after 42 h of storage at 70 °C compared to the control sample, followed by 168 h at 50 °C and 84 h at 60 °C, respectively. However, there was no statistical difference found between the last two applications.

### Mathematical equations reflecting the thermal change of some phytochemicals

One of the critical factors to consider in food processing is nutrient loss. Therefore, kinetic studies are needed to minimize undesirable variation and optimize the quality of certain foods. Kinetic models are frequently used to ensure safe food production is objective, fast and economical. Kinetic models are also used to predict and examine the impact of the application and process on critical quality parameters (Patras et al., 2009). We took the average values of Y (Vit C, TP, and ACN) from two separate experiments and used them to create a quadratic polynomial model. As a result of the multi-regression analysis, it determined that the relationship between Vit C, TP and ACN phytochemicals and the T and ST could be disclosed by a three-dimensional polynomial or paraboloid regression equation that had a high R<sup>2</sup> value. The R<sup>2</sup> value (74-94.7 %) of calculated regression equality for the average values of molasses phytochemicals using regression analysis was less than the equations formed according to the KCLM (76-96.4 %) and FCLM (84-97.2 %). This model predicts the amount of phytochemicals in mg/100 g, mg GAE/100 g, and mg/kg, respectively.

$$\text{Vit C} = 148.6 - 2.31 T + 0.121 ST + 0.016 T^2 - 0.00049 ST^2 - 0.0806 TST$$

(R<sup>2</sup> = 74.55%)

$$\text{TP} = 21798 - 749 T + 49.2 ST + 7.192 T^2 - 0.0020 ST^2 - 1.056 TST$$

(R<sup>2</sup> = 94.73%)

$$\text{ACN} = 192 - 3.86 T - 0.310 ST + 0.0185 T^2 + 0.00076 ST^2 - 0.00047 TST$$

(R<sup>2</sup> = 90.40%)

However, TP and ACN equations with high regression coefficients (R<sup>2</sup>), which vary within the same limits as the regression equations created according to data belonging to cultivars, can be used to determine optimum conditions in kinetic calculations belonging to thermal stability. In the models of phytochemicals such as Vit C, TP and ACN of molasses, besides the linear (primary) effect of T and ST, the quadratic (secondary or T<sup>2</sup>, ST<sup>2</sup>) and interaction (TxST) effects were also found to be significant (P < 0.001).

As observed from equalities, the thermal change of phytochemical compounds such as Vit C, TP and ACN in different applications (including the T and ST) didn't show compliance with the first-degree reaction kinetics. Consistently, Kanner et al. (1982) reported that the fragmentation course did not comply with the first-order reaction kinetic can be used in temperatures of 25 °C and below because ascorbic acid decomposed at 36 °C, but in the decomposition of ascorbic acid during the storage of orange juice concentrate. Similarly, Moldovan and David (2014) explained the degradation of ascorbic acid in the same conditions with the Arrhenius equation. In a previous study, the reaction kinetics reflecting the thermal decomposition of cranberry fruit polyphenols during storage has shown compliance with the Arrhenius equation (Moldovan et al., 2016). Furthermore, Buckow et al. (2010) demonstrated the relationship between ACN degradation and pressure, T and application time (ST) factors in blueberry juice using a nonlinear regression equation.

### CONCLUSIONS

Cherry laurel (*Laurocerasus officinalis* L.) is a fruit whose consumption is limited in quantities due to its astringent taste, processed into pickles, jams, dried fruit, juices, molasses and brine products with traditional methods in the Blacksea region of Turkey. Considering the nutrients it contains, such as anthocyanins, phenolic substances and Vit C, and its relations with health, this fruit should be delivered to most consumers as converted into molasses with appropriate technology in modern factories and evaluated in different foods as an additive. As a result, a significant reduction of nutrient losses is possible by determining thermal changes in some physicochemical properties and phytochemical compounds during molasses production and storage under different T and ST conditions. The results of this study demonstrated that phytochemicals such as Vit C, TP and ACN were quality parameters in KCLM and FCLM, and the equations reflected the change with T and ST of Vit C, TP, and ACN may be used to calculate the phytochemicals thermal stability.

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**AUTHORS' CONTRIBUTION**

Vesile Başar: Investigation, formal analysis, original draft. Atilla Şimşek: Project administration, supervision, conceptualization, methodology, formal analysis, data curation, validation, writing - review and editing. Emre Turan: Supervision, formal analysis, resources, validation, writing, original draft.

**DECLARATION OF CONFLICTING INTERESTS**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**DATA AVAILABILITY STATEMENT**

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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