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SOME PHYSICOCHEMICAL AND SENSORY PROPERTIES OF HAZELNUT BEVERAGES ENRICHED WITH VIT-C SOURCE FRUITS AND SHELF LIFE

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ABSTRACT
In this research, drinkable hazelnut milk (HM) processed from the Tombul variety was supplemented with a ratio of 20, 40, and 60% rosehip (*Rosa canina* L.) (R) and strawberry (*Fragaria* sp.) (S) pulps as Vit-C sources and stored to determine physicochemical changes for 6 weeks at 4 and 25 ^{o}C. The influence of temperature (*T*), time (*t*), and contribution ratio (CR) factors on R and S-HM beverages was statistically significant (P <0.05). The evident changes in beverages were in total phenolics (TP) and antioxidant activity at the end of 6 weeks' storage. R1, R2, S2, and S3 formulations were the most liked for general acceptability scores, also, selected R and S purees were compatible with hazelnut milk. The optimum shelf life of R-HM and S-HM mixtures was calculated between 6-8 weeks at 15 ^{o}C using TP and Vit-C regression equations (R^2= 95.07-99.45%).
Keywords: Hazelnut milk, plant-milk beverages, physicochemical properties, functional food, modelling, stability

VIT-C KAYNAKLI MEYVELERLE ZENGİNLEŞTİRİLMİŞ FINDIK İÇECEKLERİNİN BAZI FİZİKOKİMYASAL VE DUYUSAL ÖZELLİKLERİ VE RAF ÖMRÜ

ÖZ
Bu araştırmada, Tombul çeşidinden işlenen içilebilir fındık sütüne (HM) %20, 40 ve %60 oranında kuşburnu (*Rosa canina* L.) (R) ve çilek (*Fragaria* sp.) (S) pulpları ilave edilmiş, Vit-C kaynağı olarak ve fizikokimyasal değişiklikleri belirlemek için 4 ve 25 ^{o}C'de 6 hafta süreyle depolanmıştır. Sıcaklık (*T*), süre (*t*) ve katkı oranı (CR) faktörlerinin R ve S-HM içecekleri üzerindeki etkisi istatistiksel olarak anlamlı bulunmuştur (P <0.05). İçeceklerdeki belirgin değişiklikler, 6 haftalık depolamanın sonunda toplam fenolikler (TP) ve antioksidan aktivitede olmuştur. Genel kabul edilebilirlik puanlarına göre en çok R1, R2, S2 ve S3 formülasyonları beğenilmiş, ayrıca seçilen R ve S pürelerinin fındık sütü ile uyumlu olduğu belirlenmiştir. TP ve Vit-C'ye ait regresyon denklemleri kullanılarak R-HM ve S-HM karışımlarının optimum raf ömrü, 15 ^{o}C'de 6-8 hafta arasında hesaplanmıştır (R^2= %95.07-99.45).
Anahtar kelimeler: Fındık sütü, bitkisel sütlü içecekler, fizikokimyasal özellikler, fonksiyonel gıda, modelleme, stabilite

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INTRODUCTION

Hazelnuts, a good energy source (640 kcal /100 g), contain 10-24% protein with 68-72% digestibility and about 62% oil. Also, 25 grams of hazelnuts supply 24% and 25% of the daily vitamin E and B6 needed. On the other hand, hazelnut is a rich food with complex carbohydrates called dietary fibre and phytochemicals such as phenolics and phytosterols (Köksal et al., 2006; Yorulmaz et al., 2009). Hazelnut is one of the most including Fe (3.4-5.8 mg/100 g) and Ca (209 mg/100 g) in vegetable-origin nutrients. These minerals should be consumed with vitamin C source foods (rosehip, citrus juices, kiwi, etc.) for absorption into the body (Şimşek and Aykut, 2007).

Plant-based milky products (legumes, cereals, nuts, seeds, and so-called cereal milk) are homogenized after cooking with water, supplemented with vitamins, minerals, and sweeteners, and packaged and sterilized in the same way as fresh milk (Sethi et al., 2016). On the other hand, researchers emphasize that plant-milk products are alternative food for lactose intolerant, those sensitive to cow's milk, and allergic to milk proteins. In particular, the demand for these products increases day after day because of their high digestibility, the absence of cholesterol, non-allergic proteins, unsaturated fatty acids, low in calories, Vit E, mineral substances, rich in carbohydrates and dietary fibre (Kundu et al., 2018). One of the problems faced by poor people in developing countries is not getting enough protein. To address this problem, promoting policies use of low-cost and high-quality proteins should be advanced (Albuquerque et al., 2015). Plant-based milk products may be a solution for a poor economic group of undeveloped countries, where animal milk supply is insufficient (Sethi et al., 2016).

There are studies in the literature about plant-based milk by different researchers. For example, some physicochemical parameters belonging to peanut, soybean, and coconut milk (Belewu and Belewu, 2007), almond milk (Hasan, 2012), Bambara peanut milk (Murevanhema and Jıdeanı, 2015), irradiated tiger nut milk (Okyere and Odamtten, 2014), peanut yoghurt (Isanga and Zhang, 2009), and stability of peanut milk-mango mixture (Zhang et al., 2011) had studied by different researchers. Also, Albuquerque et al. (2015) reported changes in the physical-chemical properties of peanut milk enriched with umbu and guava pulp stored for 150 days at 18 ^{o}C.

Besides, the effect of high-pressure homogenization and heat treatment on the physicochemical properties and physical stability of almond nut (Bernat et al., 2014), coconut (Tangsuphoom and Coupland, 2005), and soy milk (Cruz et al., 2007) was investigated. In another study, the optimal conditions for homogenization and sterilization in hazelnut milk, and the effects of stabilizers on the stability of hazelnut milk were defined by Lı et al. [2009]. In recent years, the optimum fermentation conditions of hazelnut milk-kefir mix (Liu and Zhou, 2012), the shelf-life (Bernat et al., 2015), and the antioxidant activity (Maleki et al., 2015) of fermented hazelnut milk with lactic acid bacteria were investigated under different storage conditions. Furthermore, Ermis et al. (2018) identified that spray-drying and freeze-drying techniques had a similar effect in obtaining high-quality hazelnut milk powder. Although many researchers have worked on hazelnut milk, there is almost no information about the milk prepared from nut and fruit extract mixtures.

This work aimed to study the main characteristics of hazelnut milk enriched by rosehip and strawberry puree (rich in Vit-C) and revealed by mathematical equations the shelf-life of enriched hazelnut milk at 4 and 25 ^{o}C for 6 weeks.

MATERIALS AND METHODS

Materials

Tombul hazelnut cultivar and strawberry fruit (Albion (*Fragaria* sp.) in the 2017 harvest period were collected in the Ordu province. Strawberry fruits were converted to puree by pulper (Moulinex Masterchef 750 duotronic). Rosehip fruit (*Rosa canina* L.) purees as a commercial sample were obtained from Turkish producers (Öncüler Food-Nesil Food Industry and Trade

Ltd. Co., Merzifon) in a 2.5 kg plastic aseptically filled package that does not contain any additives.

Preparation of hazelnut beverage formulations

Hazelnut milk (HM) was converted from hazelnuts with a combination of production techniques: soybean, almond, peanut milk, etc. (Wallace and Khaleque, 1971; Rubico et al., 1987; Maghsoudlou et al., 2016). Hazelnuts between 11-13 mm were roasted at 155 ^{o}C for 45 minutes, cooled, and removed from their pellicle. After adding tap water (water: hazelnut, 2:1), the mixture was cooked at 100 ^{o}C for 20 minutes and passed through a grinder, and then made up to 5lt water. The rosehip, strawberry pups and sugar based on the weight of hazelnut milk were added respectively as 20% (R1, S1), 40% (R2, S2), 60% (R3, S3) and 6%. Then, the mixtures were transformed into drinkable hazelnut beverages with a homogenizer within 5 minutes with a gradual increase rate from 6500 rpm to 24000 rpm, placed in glass jars (200 ml), pasteurized at 85 °C for 15 minutes and stored for 6 weeks at 4 and 25 °C after cooling.

Methods

Physicochemical analysis

The dry matter (DM) was determined with kept at 70 ºC (Ecocell, Germany) for 4 hours and calculated as % with weight difference. Water-soluble dry matter (SDM) was measured by a refractometer (HANNA) at 20 ºC. The pH values of the samples were detected by a pH meter (Mettler Toledo seven compact S210). Total acidity (TA) in R-HM and S-HM were specified using 0.1N NaOH and expressed as malic and citric acid in terms of g/100g, respectively (Cemeroğlu, 2010). The absorbance of the red colour of hydroxymethylfurfural (HMF) with p-toluidine and barbutyric acid was determined by a spectrophotometer (Shimadzu UV mini-1240) at 550 nm wavelength (Cemeroğlu, 2010). Vit-C was identified by spectrophotometric measurement of the colour of the ascorbic acid reaction with the 2,6-dichloro-phenol indophenol solution (Cemeroğlu, 2010). The total monomeric anthocyanins (AC) concentration was calculated by the pH differential method using a spectrophotometer such as mg cyanidin-3-glycoside/L (Cemeroğlu, 2010). The antioxidant activity (AA) of the samples was determined by the DPPH-RSA (DPPH-radical scavenging activity) method based on measuring the strength of a purple-coloured compound, 2,2-diphenyl-1-picrylhydrazase (DPPH) radical. The results were expressed as mol TE/g by using the Trolox standard curve (Cemeroğlu, 2010). Total phenolics (TP) were assigned by spectrophotometric measurement of the colour of phenolic compounds with the Folin-Ciocalteu solution in the alkaline medium as gallic acid equivalent (mg GAE/100 g sample) (Sıngleton and Rossi, 1965). The viscosity (V) was measured in cP (centipoise) using a viscometer in a 35 ml sample cup at 20 ^{o}C (And SV-10 SineWave Vibro Digital Viscometer). Hunter L*, a*, and b* values of products were measured by a color meter (Konica Minolta Cr-410). Stability (serum separation rate, SSR) was calculated by the % ratio of the volume of serum separated during storage periods (0, 2, 4, and 6 weeks) to the total volume (Bernat et al., 2015). The sugar profile of the samples was determined by HPLC (Thermo Scientific Dionex Ultimate 3000) after filtration and precipitation with Carrez 1 ($K_4Fe(CN)_6 . 3H_2O$) and Carrez 2 ($ZnSO_4 . 7H_2O$) solutions.

HPLC Conditions

Column: Thermo Scientific Gold Amino 4.6x250mm (5µ)

Mobile Phase: %80:20 v/v, ACN (acetonitrile): H_2O (water)

Flow Rate: 1.3 ml /min

Temperature: 30 ℃

Injection Volume: 20 µl

Detector: ERC Refractomax 521RID Detector

Pump System: Thermo Scientific SR 3000

Degasser: MPMINIPURE SUPER UP M

Column oven: Thermo Scientific TCC-3000

Program: Chromeleon

Sensory analysis

Pasteurized and cooled R and S-HM formulations were matured for 24 hours to balance the color, aroma, texture (mouth feeling), structure-consistency (test with a spoon), and flavor. Sensory parameters were scored by 10 panellists trained in Food Engineering using a hedonic scale (Worst rating: 1, Best rating: 5).

Statistical analysis and modelling
Experimental design was established in 96 samples with 2 fruit purees x 3 contribution ratio (CR) x 2 storage temperature (*T*) x 4 storage time (*t*) x 2 repetitions. MINITAB 18 program was used for the statistical analysis (ANOVA and Tukey's multi-comparison test) and modelling.

RESULTS AND DISCUSSION

The physicochemical properties of hazelnut beverage formulations
Table 1 shows some physicochemical properties of drinkable hazelnut beverages enriched with R and S puree at different concentrations.

Table 1 Some physicochemical properties of hazelnut milk enriched with R and S purees (n= 2)[α]

Properties	Formulations					
	R1	R2	R3	S1	S2	S3
DM (%)	22.08 ± 0.22	21.06 ± 0.58	19.94 ± 0.03	23.56 ± 1.08	22.42 ± 1.57	21.85 ± 2.46
SDM (°Briks)	10.35 ± 0.07	11.70 ± 0.00	12.65 ± 0.07	9.95 ± 0.21	11.45 ± 0.21	12.05 ± 0.07
pH	5.02 ± 0.01	4.40 ± 0.02	4.12 ± 0.04	5.52 ± 0.12	4.67 ± 0.01	4.32 ± 0.01
TA (%)	0.141 ± 0.006	0.263 ± 0.011	0.400 ± 0.012	0.199 ± 0.040	0.308 ± 0.028	0.520 ± 0.002
Sugar Composition (g/100g)						
Glucose	0.356±0.021	0.803±0.018	0.93±0.041	0.833±0.047	1.739±0.033	2.024±0.084
Fructose	0.448±0.028	0.944±0.003	1.076±0.020	0.915±0.024	1.129±0.023	1.914±0.006
Sucrose	6.574±0.004	6.140±0.036	6.276±0.027	6.158±0.021	6.156±0.004	6.334±0.001
Total sugar	7.378±0.053	7.887±0.050	8.283±0.089	7.906±0.091	9.024±0.059	10.271±0.092
HMF (mg/kg)	2.06 ± 0.29	1.87 ± 0.02	2.08 ± 0.28	4.10 ± 0.01	4.86 ± 0.87	4.83 ± 0.66
Vit-C (mg/100g)	17.66 ± 0.46	21.80 ± 0.51	24.96 ± 1.31	11.31 ± 0.34	17.19 ± 0.41	17.37 ± 0.47
AC (mg/L)	-	-	-	7.16 ± 0.60	11.43 ± 0.34	16.75 ± 1.06
TP (GAE mg/ 100g)	183.48 ± 4.81	364.42 ± 6.95	556.78 ± 21.36	38.99 ± 2.46	60.57 ± 1.93	66.15 ± 0.60
DPPH-RSA (%)	63.37 ± 0.49	67.24 ± 0.02	73.96 ± 1.18	62.89 ± 0.30	64.98 ± 1.39	65.16 ± 1.37
AA (µg TE/mg sample)	21.97 ± 0.18	23.41 ± 0.07	25.92 ± 0.44	28.25 ± 0.14	29.20 ± 0.63	29.29 ± 0.62
V (Cp)	11.10 ± 0.42	21.60 ± 0.28	34.20 ± 0.85	28.55 ± 0.64	87.45 ± 0.64	206.50 ± 3.54
Hunter color values						
L	51.78 ± 0.01	49.37 ± 0.82	43.17 ± 0.32	52.79 ± 0.92	51.21 ± 1.15	46.53 ± 0.15
a	6.74 ± 0.03	7.94 ± 1.26	11.11 ± 0.04	5.72 ± 0.03	7.46 ± 0.08	10.50 ± 0.12
b	12.98 ± 0.01	14.51 ± 0.57	14.11 ± 0.22	8.10 ± 0.01	8.19 0.11	7.66± 0.11

[α] Mean+Standard deviation (SD)

It is clear from the table that the addition of all two purees caused a significant change in the physicochemical properties of the samples. An increase in puree concentration in the HM samples caused a decrease in the DM and pH of the final products. On the other hand, an increasing trend was observed for SDM, TA, glucose, fructose, sucrose, total sugar, Vit-C, TP, DPPH-RSA, AA, V, Hunter L*, and a* values of the samples. Hunter b* and HMF values showed oscillation in similar limits. Moreover, as seen in Table 1, the amount of AC tends to increase according to the rise of S ratios (20, 40, and 60%).

Sensory evaluation of hazelnut beverage formulations
Sensory evaluation results are shown in Fig. 1. The R2 sample was the most admired by panellists for color, flavor, and acceptability among R-added hazelnut beverages. The rosehip aroma has suppressed the hazelnut aroma in the R2 and R3 examples. For this reason, the R1 product aroma is thought to be more balanced and appreciated. As a result of the evaluation, the rate of fruit puree in the R-added products increased, the feeling (texture) in the mouth was not affected negatively, the flow rate from the spoon decreased, and the consistency increased. The highest score was given to the R3 sample by panellists. The additive rates in the R1 and R2 formulations were the most appropriate according to the general acceptability scores. S-added hazelnut beverages were more liked with their increasing fruit puree ratio. It was an expected result as the increased fruit ratio lowered the product pH and caused the anthocyanin pigment to give a more pronounced

red color under acidic conditions (Cemeroğlu, 2004). The S3 was more liked for color and aroma among S-added groups. However, S3 was found to be more viscous by the panellists and evaluated with the lowest for structure-consistency and texture (sensation in the mouth). Also, the S2 was assessed with the highest texture and structure consistency score by the thickening effect of pectin and sugar in the strawberry structure. Also, as the total sugar content of S was higher than R, it increased the taste ratio, and therefore, the S2 flavor was the most liked. The general acceptability scores of the S-added groups show that the contribution rate in S2 and S3 formulations were most appropriate. Besides, the panellists stated that R and S puree selected as vitamin C sources are very compatible with hazelnut milk and that the product obtained is a cold drink that can be consumed with pleasure.

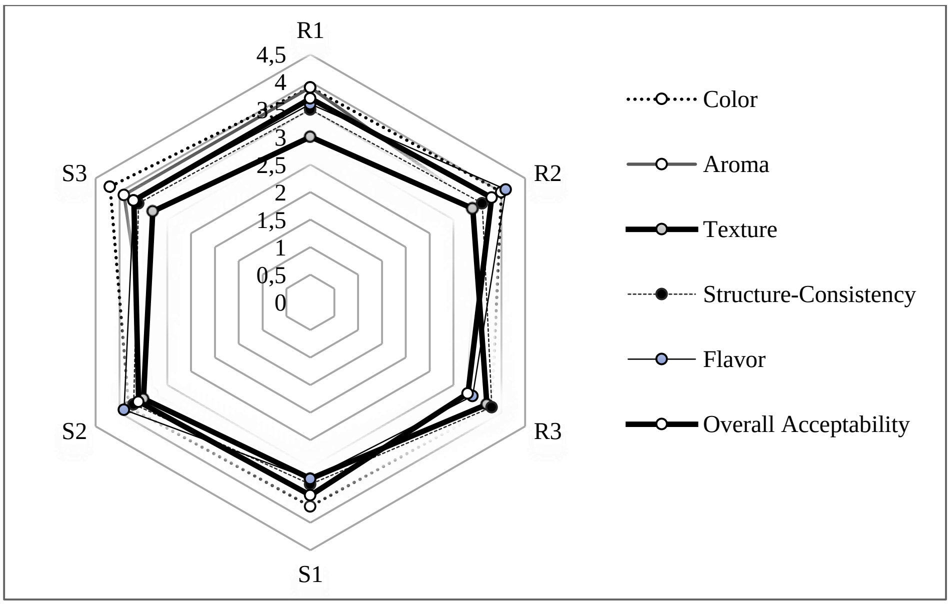

Figure 1. Sensory evaluations of hazelnut milk supplemented with fruit puree

Change of some physicochemical properties of hazelnut beverages during storage

In the second part of the study, some physicochemical properties of the HM-fruit puree mixtures stored at 4 and 25°C for 6 weeks were analyzed statistically and obtained mathematical equations that reflected the changes. As a result of ANOVA (Variance analysis), the effect of CR, *T* and *t* factors on the composition of R-HM and S-HM mixtures was found significant ($P < 0.05$). Tukey multicomparison test results belonging to means of physicochemical parameters that were found significant are discussed below.

DM and SDM showed a slight increase in both puree-added hazelnut milk. The pH values decreased from 5.53 to 4.42 in R-HM and from 5.69 to 4.45 in S-HM as CR increased. On the other hand, T and t factors and pH values increased until the end of the 4th week (4.51-4.95) in R-HM samples, then decreased. Differently, S-HM's pH values increased until the end of the 6th week (4.83-5.16). Also, the pH increases at 25 C in both types of hazelnut milk was higher than those stored at 4°C. Moreover, the change in titration acidity was consistent with pH (Fig. 2a).

Perhaps the reduction in acidity may have been efficient in the damage of Vit-C. The increase in titration acidity (reduction in pH) after 4 weeks may be due to free fatty acids and amino acids released by the breakdown of proteins and fats with organic acids (Albuquerque et al., 2015). On the other hand, CRx*t* and *T*x*t* interactions increased the HMF content of all R-HM and S-HM samples (Fig. 2b).

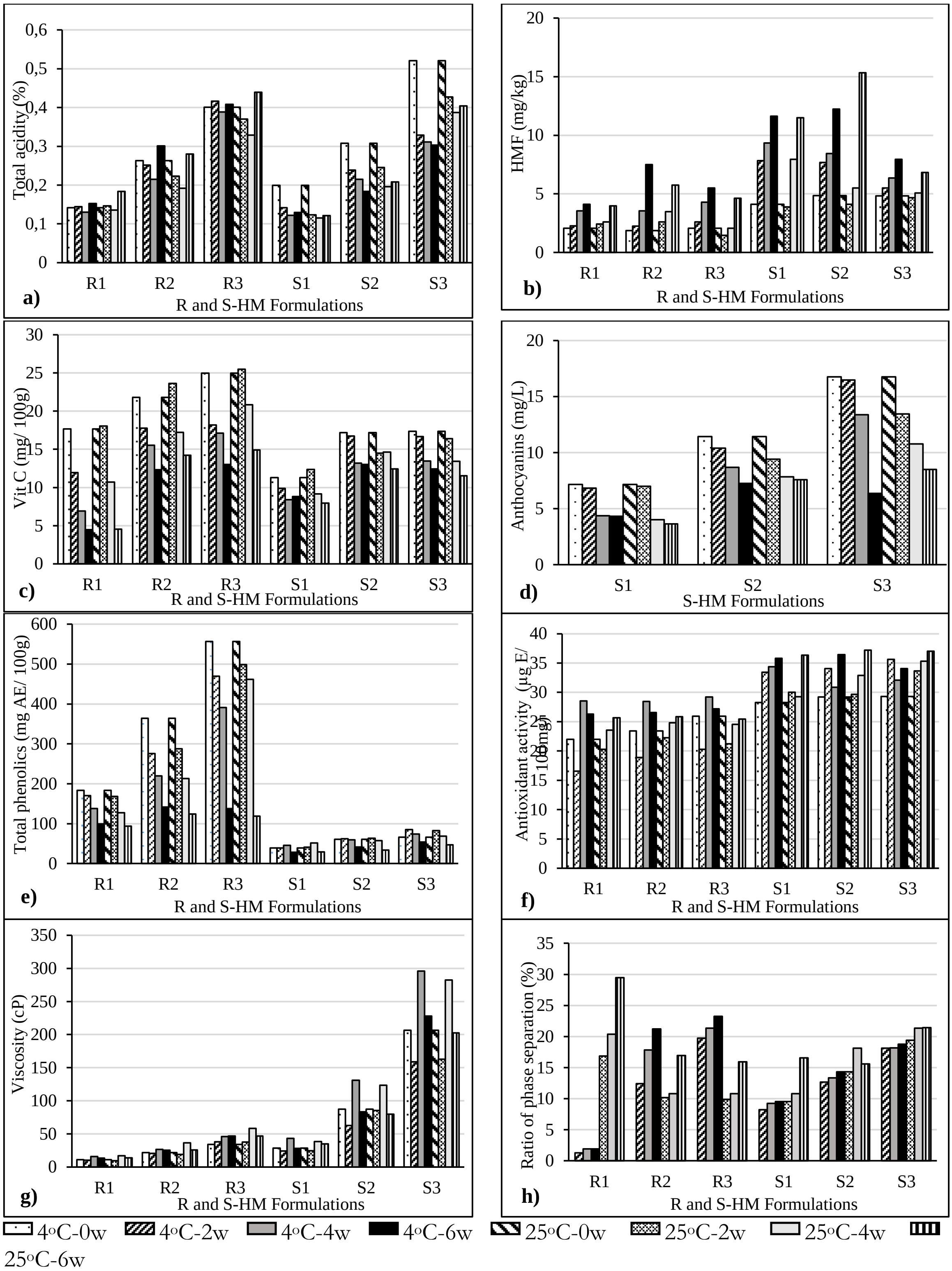

Figure 2. Changes in the physiochemical properties of R and S-HM mixtures during storage at 4 and 25 °C for 6 weeks (n=2).

HMF is also one of the decomposition products of ascorbic acid and combines with amino acids to form brown pigments (Cemeroğlu, 2004). As a matter of fact, in our study, ascorbic acid decreased in increasing storage processes and increased HMF. Moreover, according to the contribution ratio (R3 and S3), the acid environment and glucose amount increase the rate of formation of the reaction in the formation of levulinic acid and acetic acid with the decomposition of HMF (Chang et al., 2006). Indeed, the high total acidity in R3 and S3 confirms this approach.

Vit-C increased with increased CR, while it decreased with increased *t*. Vit-C losses in the last week of storage were 47-53% in R-HM and 25-32% in S-HM. There was no statistical difference between storage at 4 and 25 °C (Fig. 2c). A study on rosehip puree determined that storage at different temperatures and time causes a decrease in ascorbic acid due to increasing temperature and time (Kadakal et al., 2017). Another study reported that the amount of ascorbic acid in strawberries at 5, 10, and 25 ºC, 15-day storage was decreased between 53.2-57.3% (Derossi et al., 2010). A similar study found that the ascorbic acid amount of pasteurized strawberry juice decreased by 63.7% after 3 months of storage at refrigerator temperature 4-15 ºC (Ayub et al., 2010). Also, ascorbic acid degradation depends on many factors such as storage time and temperature, oxygen, light, pH, and mineral substances (Cemeroğlu, 2004).

In the S-HM beverages, anthocyanin (AC) was affected by CRx*T*x*t* at the end of 6 weeks' storage at 4 and 25 °C. As CR, *T*, and *t* increased, the amount of AC decreased (Fig. 2d). The decomposition of anthocyanins depends on many factors such as storage temperature and duration, pH, oxygen, heat, light, anthocyanin concentration and chemical structure, and the concentration of phenolic, enzyme, protein, and minerals (Patras et al., 2010). Similarly, Zavala et al. (2004) examined that the amount of anthocyanin in strawberries decreased at 0 and 5 ºC during the first 5 days of storage. In another study, Oszmianski and Wojdylo (2009) reported that the total anthocyanin content of 3 different strawberry purees was decreased after storage for 4 months at 4 and 20 ºC.

The total phenolics (TP) belonging to R and S-HM increased with CR but decreased with *t* and no significant differences were found for storage at 4 and 25 °C. Also, the TP of the R groups was higher than the S groups (Fig. 2e). Similarly, Zavala et al. (2004) affirmed that the effect of different storage temperatures (0, 5, and 10 ºC) on the TP composition of strawberries was significant. In a similar study on strawberries, the TP was affected by temperature, humidity, and storage time, but the change was not regular (Shin et al. 2007). Reductions in the amount of TP can be explained by the oxidation of phenolic compounds or polymerization with proteins (Cao et al., 2011). However, the decrease in the TP could also result from the degradation of phenolic structures due to an organic acid increase (Maleki et al., 2015).

DPPH-RSA and AA values in our samples showed oscillation during the first 4 weeks of storage by CRx*T*, CRx*t*, and *T*x*t* interactions ($P > 0.05$). Then, a slight increase in R-HM beverages and a decrease in S-HM beverages were detected in the 6th week of storage (Fig. 2f). In addition, AA was expected to be higher in R group beverages with higher TP and Vit-C, but they were higher from S groups. The changes in DPPH-RSA and AA may have been influenced by the broken-down of antioxidant compounds such as Vit-C, TP and AC (for S-HM) during storage. On the other hand, the fact that AA is higher in S group samples than in R group samples may be caused by AC, phenolic compounds and derivatives (flavonol, ellagic acid, p-coumaric acids, ellagitannins and glycosylated derivatives of ellagic acid) it contains. In addition, the difference in the amount of Maillard reaction products with known AA properties according to S and R pulps may also have been affected (HMF is higher in S).

As the fruit pulp ratio increased, the increase in viscosity showed a more significant increase in the S group beverages compared to the R group, yet, the change course of viscosity of samples stored at 4 and 25 °C was found to be similar. Consistent with the pH change, the viscosity (V) of the samples increased until the 4th week and decreased at the end of the 6th week due to the increase in CR, *T* and *t* (Fig. 2g). The difference in the composition of the pulp, the amount of sugar, organic acid, polysaccharide and protein it contains, and the type of sugar is likely to affect the viscosity. In addition, the degree of pasteurization applied under these conditions may have affected the formation of conjugates, namely glycolization, differently. Xi et al. (2020) determined that conjugates formed from the interaction (glycolization) of proteins and sugars in alkaline conditions are emulsifiers and viscosity increases. The sugar type is effective, for example, while glucose exhibits a higher glycolization, dextran was more effective than glucose for emulsion formation and stability. In addition, emulsion stability decreased as the pH increased, which was explained by the formation of disulphide bonds and aggregation. Semenova et al. (2002) summarized the gelation mechanism of globular proteins in the presence of sugars in 3 ways. The first is to increase the protein gelation temperature when sugar is present, the second is to reduce the frequency of protein-protein encounters with the continuous sugar phase is a slower gelling rate increase in viscosity, and the third is to heat at a temperature above the protein denaturation heat and then cool. The third process causes the formation of hard protein gels, but when heated at a temperature below protein denaturation heat or possibly for a short period gives weaker gels. Bernat et al. (2015) reported that the denaturation of protein with the effect of the homogenization (62, 103, 172 MPa) and heat treatment (85 ºC-30 min or 121 ºC-15 min) process increases viscosity. Cruz et al. (2007) explained that the ultrahigh-pressure homogenization process applied to soy milk increases the viscosity compared to the UHT method, with the growth of particle surface and internal friction. Yıldız and Alpaslan (2012) determined that the viscosity values of rosehip puree and marmalades were affected by pH, pectin and sugar content and the degree of heat treatment applied for production, also reported that peanut drinks with higher processing temperatures were more viscous. This situation is probably due to changes in protein and carbohydrate structures that were reported by Rubico et al. (1987). Similarly, Wallace and Khaleque (1971) have explained the increase in viscosity in concentrated soymilk during sterilization by the progressive unfolding and aggregation of proteins.

One of the most important problems encountered in the presentation of plant-based milk is the serum separation from the emulsion. In R-HM and S-HM beverages, the serum separation rate (SSR) increased with an increase in the puree rate (CR), temperature (*T*) and storage time (*t*) except for R-HM stored at 25 °C. Also, the rise in SSR of R1 for 6 weeks (45 days) at 25 °C was more than other R and S-HM beverages. The SSR in R-HM beverages at 4 °C increased with increasing the CR and *t* factors and decreased at 25 °C with an increase in the CR. But, R1 was stable for up to 6 weeks at 4 °C among all formulations (Fig. 2h). S-HM beverages stored at 4 and 25 °C for 6 weeks showed a linear increase between the increase in S puree ratio, *T* and *t*, and SSR. SSR varied between 1.91% and 29.5% in R-HM and 9.53-21.4% in S-HM over 45 days. In addition, the increase in SSR was more in S-HM stored at 25 °C and in R-HM stored at 4 °C. Factors such as the composition of the fruit (sugar, TA, protein, pectin, dietary fibre), the amount of added sugar, and the gelation that occurs as a result of thermal degradation during pasteurization can be affected the SSR. As can be seen from Table 1, increasing the pulp ratio decreased the pH, and increased the amount of TA and sugar, so the viscosity increased, the probable cause was gelatination or protein aggregation with the role of sugars (glycosylation). Also, the destabilization in the samples may have been caused by reaching the isoelectric point (pI) in hazelnut milk with the added low-pH pH purees and the precipitation of the proteins. Similarly, Semenova et al. (2002) declared that at pH<pI (protein isoelectric point), weakens as the hydrogen bond between sugars and more protonated protein molecules, sugars become more effective than proteins in binding water molecules, leading to protein dehydration and

subsequently extensive protein aggregation. Bernat et al. (2015) reported that fermented hazelnut milk (pH=3.70-4.80, acidity= 0.100-0.338) had serum separation of 25% after 28 days of storage at 4 °C. They stated that this was due to the low protein content in plant-based milk, which acts as an emulsifier in oil-water emulsions.

In R and S-HM beverages stored at 4 and 25 °C, only water was separated as a phase at the end of the 2nd week, while 3 distinct phase separations were observed after the 2nd week. Phase separation was listed from the bottom to the top as sediment of hazelnut flour, fruit puree-nut milk, and water phase. The homogenization process applied to the hazelnut milk may cause a change in protein structure. Also, the pasteurization temperature may have increased the sedimentation rate by causing both protein denaturation and fat droplets-protein flock or fruit-fat-protein flock and an increase in particle size (Bernat et al., 2015). For this purpose, Li et al. (2009), for optimal stabilization of hazelnut milk, suggested 0.08% carrageenan, 0.08% guar gum, 0.1% sucrose fatty acid ester and 0.15% compound stabilizer. Also, in the same study, the optimum condition of homogenization and sterilization was respectively determined to be 35 MPa at 70 °C and 20 min at 121 °C. Maghsoudlou et al. (2016) reported that the application of ultrasound-assisted stabilization to almond milk to which modified starch, agar, and lecithin were added prevented phase separation due to time and thus preserved the stabilization. Hasan (2012) determined that the sediment layer in unprocessed almond milk stored at 4 and 25 °C for 7 days is higher than in pasteurized almond milk and lecithin-added almond milk. This situation was explained by the fact that homogenization applied before pasteurization and heat treatment caused a more stable distribution. On the other hand, lecithin added to almond milk increased the physical stability and balanced the particle distribution. In another similar study, it was determined that the two-stage homogenization (4-40 MPa) and heat application (1 hour at 50-60-70-80-90 °C) are effective in maintaining emulsion stability in coconut milk (Tangsuphoom and Coupland, 2005).

Hunter L*, a*, and b* colour values of R-HM and S-HM were determined to be affected by CR, *T*, and *t* factors ($p < 0.05$, ANOVA). Hunter L* value decreased with increasing CR in R and S-HM beverages and decreased in both formulation groups during storage. While the decreases at 4 and 25 °C were statistically similar in R1 and S1, the reduction became more pronounced as the pulp ratio increased. Hunter a* value increased with the increase in CR in R and S-HM beverages and was more in the R groups. While the Hunter a* value did not change in the R group samples at both temperatures during storage, it decreased in the S2 and S3 beverages and at 25 °C were more than 4 degrees. Hunter b* values were increased similarly to Hunter a* with increasing CR, and increases were more in R group beverages than in S groups. Although there were slight fluctuations at both temperature degrees during storage, there was a slight decrease in R2 and R3 in all samples, and these decreases were found to be statistically insignificant ($P < 0.05$). The decrease in L*, a* and b* values during storage was probably related to the decomposition of carotene (R groups) and anthocyanins (S groups) under the influence of pasteurization and storage temperature and the pH value of the environment. In the previous study, Wang et al. (2015) reported that the colour stability of strawberry juice is preserved at pH 3.0 or below during production and storage, too small changes in pH changed significantly the colour stability of strawberry juice due to the pH-dependent characteristics and molecular structures of anthocyanins. Similarly, Buve et al. (2018) reported that ascorbic acid content and Hunter a* values decreased with increasing temperature and time as a result of storage (32 weeks) of pasteurized strawberry juice at different temperatures (20, 28, 35 and 42 °C).

Mathematical Equations for Kinetic Calculations

The mathematical equations of TP, Vit-C, Hunter L*, a* and b* values, V, HMF and AC (for S-HM) belonging to R-HM and S-HM beverages related to *T* and *t* are given respectively in Table 2.

Table 2. Mathematical equations showing the effects of *T* and *t* on some quality parameters of R-HM and S-HM ($P < 0.001$)

Pulp Mix	CR (%)	Parameter	Mathematical equations	R^2(%)
R-HM	20	TP=	$0.0 + 69.87 T + 2.7 t - 2.469 T^2 - 7.20 t^2 + 0.377 T*t$	95.07
	20	Vit-C=	$0.0 + 4.422 T - 0.987 t - 0.1496 T^2 - 0.1538 t^2 + 0.0102 T*t$	98.32
	20	L*=	$0.0 + 15.039 T - 0.853 t - 0.51788 T^2 + 0.1297 t^2 - 0.01025 T*t$	99.96
	20	a* =	$0.0 + 1.9303 T + 0.1761 t - 0.06662 T^2 - 0.0184 t^2 + 0.00070 T*t$	99.84
	20	b* =	$0.0 + 3.8304 T - 0.019 t - 0.13268 T^2 - 0.0014 t^2 + 0.00140 T*t$	99.87
	20	V=	$0.0 + 1.993 T + 3.63 t - 0.0728 T^2 - 0.429 t^2 + 0.0517 T*t$	97.77
	20	HMF =	$0.0 + 0.4932 T - 0.008 t - 0.01680 T^2 + 0.1126 t^2 - 0.01004 T*t$	96.59
	40	TP=	$78.8 + 69.87 T + 2.7 t - 2.469 T^2 - 7.20 t^2 + 0.377 T*t$	95.07
	40	Vit-C=	$6.076 + 4.422 T - 0.987 t - 0.1496 T^2 - 0.1538 t^2 + 0.0102 T*t$	98.32
	40	L*=	$-2.966 + 15.039 T - 0.853 t - 0.51788 T^2 + 0.1297 t^2 - 0.01025 T*t$	99.96
	40	a* =	$1.622 + 1.9303 T + 0.1761 t - 0.06662 T^2 - 0.0184 t^2 + 0.00070 T*t$	99.84
	40	b* =	$0.902 + 3.8304 T - 0.019 t - 0.13268 T^2 - 0.0014 t^2 + 0.00140 T*t$	99.87
	40	V=	$10.34 + 1.993 T + 3.63 t - 0.0728 T^2 - 0.429 t^2 + 0.0517 T*t$	97.77
	40	HMF =	$0.798 + 0.4932 T - 0.008 t - 0.01680 T^2 + 0.1126 t^2 - 0.01004 T*t$	96.59
	60	TP=	$253.4 + 69.87 T + 2.7 t - 2.469 T^2 - 7.20 t^2 + 0.377 T*t$	95.07
	60	Vit-C=	$8.436 + 4.422 T - 0.987 t - 0.1496 T^2 - 0.1538 t^2 + 0.0102 T*t$	98.32
	60	L*=	$-8.017 + 15.039 T - 0.853 t - 0.51788 T^2 + 0.1297 t^2 - 0.01025 T*t$	99.96
	60	a* =	$4.035 + 1.9303 T + 0.1761 t - 0.06662 T^2 - 0.0184 t^2 + 0.00070 T*t$	99.84
	60	b* =	$1.231 + 3.8304 T - 0.019 t - 0.13268 T^2 - 0.0014 t^2 + 0.00140 T*t$	99.87
	60	V=	$30.05 + 1.993 T + 3.63 t - 0.0728 T^2 - 0.429 t^2 + 0.0517 T*t$	97.77
	60	HMF =	$0.207 + 0.4932 T - 0.008 t - 0.01680 T^2 + 0.1126 t^2 - 0.01004 T*t$	96.59
S-HM	20	TP=	$0.0 + 11.420 T + 8.33 t - 0.3920 T^2 - 1.728 t^2 - 0.0366 T*t$	99.12
	20	Vit-C=	$0.0 + 3.484 T - 0.644 t - 0.11977 T^2 - 0.0094 t^2 - 0.00392 T*t$	99.45
	20	L*=	$0.0 + 15.3871 T - 0.099 t - 0.53107 T^2 + 0.0280 t^2 - 0.00942 T*t$	99.98
	20	a* =	$0.0 + 1.938 T - 0.576 t - 0.06704 T^2 + 0.0527 t^2 + 0.00021 T*t$	97.12
	20	b* =	$0.0 + 2.3235 T + 0.1898 t - 0.07984 T^2 - 0.02646 t^2 + 0.00115 T*t$	99.92
	20	V=	$0.0 + 3.73 T + 13.22 t - 0.122 T^2 - 1.37 t^2 - 0.095 T*t$	95.96
	20	HMF =	$0.0 + 1.718 T - 0.117 t - 0.06156 T^2 + 0.1741 t^2 + 0.0065 T*t$	95.94
	20	AC=	$0.0 + 2.499 T - 0.739 t - 0.08743 T^2 - 0.0448 t^2 + 0.00427 T*t$	98.10
	40	TP=	$15.77 + 11.420 T + 8.33 t - 0.3920 T^2 - 1.728 t^2 - 0.0366 T*t$	99.12
	40	Vit-C=	$4.957 + 3.484 T - 0.644 t - 0.11977 T^2 - 0.0094 t^2 - 0.00392 T*t$	99.45
	40	L*=	$-1.777 + 15.3871 T - 0.099 t - 0.53107 T^2 + 0.0280 t^2 - 0.00942 T*t$	99.98
	40	a* =	$1.258 + 1.938 T - 0.576 t - 0.06704 T^2 + 0.0527 t^2 + 0.00021 T*t$	97.12
	40	b* =	$0.0125 + 2.3235 T + 0.1898 t - 0.07984 T^2 - 0.02646 t^2 + 0.00115 T*t$	99.92
	40	V=	$61.2 + 3.73 T + 13.22 t - 0.122 T^2 - 1.37 t^2 - 0.095 T*t$	95.96
	40	HMF =	$0.333 + 1.718 T - 0.117 t - 0.06156 T^2 + 0.1741 t^2 + 0.0065 T*t$	95.94
	40	AC=	$3.691 + 2.499 T - 0.739 t - 0.08743 T^2 - 0.0448 t^2 + 0.00427 T*t$	98.10
	60	TP=	$28.82 + 11.420 T + 8.33 t - 0.3920 T^2 - 1.728 t^2 - 0.0366 T*t$	99.12
	60	Vit-C=	$4.937 + 3.484 T - 0.644 t - 0.11977 T^2 - 0.0094 t^2 - 0.00392 T*t$	99.45
	60	L*=	$-6.786 + 15.3871 T - 0.099 t - 0.53107 T^2 + 0.0280 t^2 - 0.00942 T*t$	99.98
	60	a* =	$2.839 + 1.938 T - 0.576 t - 0.06704 T^2 + 0.0527 t^2 + 0.00021 T*t$	97.12
	60	b* =	$-0.3106 + 2.3235 T + 0.1898 t - 0.07984 T^2 - 0.02646 t^2 + 0.00115 T*t$	99.92
	60	V=	$186.6 + 3.73 T + 13.22 t - 0.122 T^2 - 1.37 t^2 - 0.095 T*t$	95.96
	60	HMF =	$-1.790 + 1.718 T - 0.117 t - 0.06156 T^2 + 0.1741 t^2 + 0.0065 T*t$	95.94
	60	AC=	$7.243 + 2.499 T - 0.739 t - 0.08743 T^2 - 0.0448 t^2 + 0.00427 T*t$	98.10

CR: Ratio of contribution, *T*: temperature (°C), *t*: time (week)

Temperature, (*T*) time (*t*) and fruit puree ratio (CR) were effective on some quality parameters of R and S-HM beverages. As a result of the regression analysis of the data belonging to the used quality parameters in determining the shelf life, paraboloid regression was found very important (P <0.001). Also, R^2 values belonging to mathematics equal for both puree-HM beverages changed between 95.07-99.98% (Tables 2). *T* and *t* linear (or primary), quadratic (or secondary) (T^2, t^2), and interaction (Txt) effects on all parameters of R and S-HM were observed (P <0.001). Another significant result, the primary (direct) or linear effects of *T* and *t* were higher than the secondary effects in all parameters of both R and S puree mixtures according to coefficients in the regression equation.

The primary effect of *T* was positive in the TP and Vit-C equations quality parameters for all mixtures of both purees. Besides, the secondary effect of T (T^2) belonging to TP and Vit-C was negative in all R and S-HM mixtures ratios. On the other hand, while the secondary effect of *t* was negative in both quality parameters, the primary effect of *t* was negative in Vit-C and positive in TP. The interaction (Txt) effect was negative on the TP and Vit-C equations of the S-HM mixtures. It also showed a positive effect on the same quality parameters of R-HM. Optimum shelf life can be calculated from both equations, assuming that Vit-C and TP are reduced by at most half during the storage period in R and S-HM beverages. As a result, the optimum shelf life of 3 different mixtures of R-HM and S-HM was calculated respectively as 6 and 8 weeks at 15 ^{o}C, using the TP equation. On the other hand, the optimum shelf life of R-HM and S-HM was calculated as 7.5 and 8 weeks at 15 °C, using the Vit-C equations.

CONCLUSION

The addition of R and S fortified the rich composition of hazelnut milk with Vit-C, phenolic compounds, sugars, organic acids, colourants (anthocyanins, xanthophylls etc.), and pectins. Hence, the obtained beverages might be considered a new functional food with potential health benefits, such as being suitable for obese groups (decreased amount of oil due to dilution), vegetarians, lactose-intolerant, or people allergic to animal proteins. In this study, the addition of R and S fruit pulps as a source of Vit-C, to hazelnut milk, although it enriched the composition of the hazelnut milk and improved its drinking properties, negatively affected the stabilization during the storage process. As it is understood, it does not seem possible to eliminate the stabilization problem in such foods by homogenization only. Also, stabilizers should use as an alternative solution. On the other hand, based on the half-lives of the quality parameters, the shelf life of the R and S-HM can be calculated using the mathematical equations formed between temperature and time.

DECLARATION OF COMPETING INTEREST

The authors have declared no conflict of interest.

AUTHORS' CONTRIBUTIONS

Sakine Kübra Çelik: Conceptualization, methodology, investigation, formal analysis, writing - original draft. Emre Turan: Conceptualization, methodology, investigation, formal analysis, writing - original draft. Atilla Şimşek: Project administration, supervision, conceptualization, methodology, writing - review and editing. All authors read and approved the final manuscript.

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