



IMPACT OF CAROB EXTRACT SUPPLEMENTATION ON CHEMICAL AND SENSORY PROPERTIES OF YOGURT AND ICE CREAM

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ABSTRACT

In this study, yogurt and ice cream were enriched with carob extract, for giving them functional properties. Three different types of ice cream were produced with three formulations in terms of carob extract concentration. In the first type only carob extract in proportions of 8, 10 and 12% was added to the mixture. The second and third types were different from the first by their aromas: respectively lemon and chocolate. The yogurt samples were produced based on a set type of yogurt procedure. According to analytical data, considerable increments in D-pinitol content, total phenolic compounds content and antioxidant activity values were observed in both of yogurt and ice cream samples with increasing carob extract added ($P < 0.05$). The ice cream samples supplemented at a ratio of 8% had higher points in all examined sensory attributes. With respect to the results obtained from the yogurt samples, the one supplemented at a ratio of 10% was scored significantly higher ($P < 0.05$) on appearance, aroma and taste.

Keywords: Carob; D-pinitol; yogurt; ice cream

KEÇİBOYNUZU EKSTRAKTI TAKVİYESİNİN YOĞURDUN VE DONDURMANIN KİMYASAL VE DUYUSAL ÖZELLİKLERİ ÜZERİNE ETKİSİ

ÖZ

Bu çalışmada yoğurt ve dondurma, bu ürünlere fonksiyonel özellikler kazandırmak için keçiboynuzu ekstraktı ile zenginleştirilmiştir. Keçiboynuzu ekstraktı konsantrasyonu açısından üç formülasyonla

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üç farklı tipte dondurma üretilmiştir. Birinci tipte karışıma sadece %8, 10 ve 12 oranlarında keçiboynuzu ekstraktı ilave edilmiştir. İkinci ve üçüncü tipler aromalarıyla ilkinden farklı olup sırasıyla limonlu ve çikolatalıdır. Yoğurt örnekleri, set tipi yoğurt prosedürüne göre üretilmiştir. Analitik verilere göre; eklenen keçiboynuzu ekstraktındaki artışla hem yoğurt hem de dondurma örneklerinin D-pinitol miktarı, toplam fenolik madde miktarı ve antioksidan aktivite değerlerinde önemli artışlar gözlenmiştir ($P < 0.05$). Keçiboynuzu ekstraktı ile % 8 oranında takviye edilen dondurma örneklerinin incelenen tüm duyuşal özelliklerde daha yüksek puanlara sahip olduğu görülmüştür. Yoğurt örneklerinden elde edilen sonuçlara göre, % 10 oranında takviye edilenler görünüm, aroma ve tat açısından istatistiksel olarak daha yüksek ($P < 0.05$) puan almıştır.

Anahtar kelimeler: Keçiboynuzu; D-pinitol; yoğurt; dondurma

INTRODUCTION

Recent scientific researches showed that the demand for various health promoting values of food such as Carob tree (*Cerotonia siliqua* L.) also called locust bean increased. According to the Worldwide the current world production of carob extract is more than 3.000 tons per year (Bulca, 2016).

Carob fruit that grows in Mediterranean climates with high natural sugar content is rich in E and B group vitamins; calcium, magnesium, potassium, sodium, phosphorus, iron, zinc, manganese and copper minerals. Its pods represent approximately 90% of total fruit weight of Carob trees (Karkacier et al., 1995). Furthermore, its use in foods came from ancient times and has been propagated in many countries because of its high commercial value of LBG (Locust Bean Gum). Except for the richness on carbohydrates contain (45-70% of saccharose, glucose, fructose, xylose, maltose and raffinose) carob pod has a very high amount of D-pinitol, which has an important role in the regulation of various body functions taken with food diet (Oziyci et al., 2015; Turhan, 2014). Therefore, several in vivo and in vitro studies for the reduction of some diseases by the foods they contain functional compounds that there may be effective in preventing their formation was carried out. Due to the findings that these functional compounds may be nutritional supplements it develops a tendency to intake in daily diet. There as well as 5-7% D-pinitol especially since its potential influences' insulin analog can be exploited in the treatment of many diseases of insulin associated with the basic mechanisms of the human body (Bates et al., 2000).

This functional sugar alcohol begins to gradually gain importance in world trade and the importance of carob, containing high concentrations of these compounds has increased. Even the production of high-purity expensive methods such as chromatography basically the normal selling price based on these compounds is to find buyers in the more expensive fruit in that level. Thus, alternative methods and structure to incorporate this functional compound daily diet to investigate the possibility of using a source containing these compounds are naturally quite important. This aspect needs to be done when more scientific studies on the carob fruit show that it is an important agricultural resource (Tetik et al., 2011; Turhan, 2011; Nasar- Abbas et al., 2016; López-Sánchez et al., 2018; Christou et al., 2019).

In this study, different products such as yogurt and three different types of ice cream are enriched in various proportions with carob extract. It is aimed to impart functional properties to these products consumed frequently in the daily diet. Consequently, readily carob seeds using fruits of plants of commercially valuable products with high added value, is thought to growers to obtain help strengthen economically.

MATERIALS AND METHODS

Materials

The carob extract (72 °Brix) was provided from a local factory (Yenigün Food Inc., Antalya, Turkey). The skim milk powder (Pınar Dairy, Turkey), milk cream (Pınar Dairy, Turkey), sugar (Doğuş Gıda A.Ş., Turkey) carboxymethyl cellulose (Tito, Turkey) as a stabilizer, lemon concentrate (42 °Bx, Dimes A.Ş., Turkey) and chocolate drops (bitter) (Dr. Oetker Gıda San. ve

Tic. A.Ş., Turkey) were all used for ice cream production. The pasteurized yogurt milk (16 % total solids, Sek Dairy, Turkey) and starter culture (Peyma-Chr. Hansen, Denmark) were used for yogurt production.

Production methods

Three different concentrations (8, 10, 12 %) were determined by preliminary experiments carob extracts substituted for production of ice cream and yogurt samples.

The plain ice cream mixture was formulated such as that it had non-fat total milk solid of 11 %, sugar content of 18 %, fat content of 3.5 % and stabilizer content of 0.8 %. Three different types of ice cream were produced (plain, fruit, chocolate) with three formulations in terms of carob extract concentration. In first type (PI) only carob extract in proportion of 8, 10 and 12% was added to the plain ice cream mix. The second (fruit ice cream, FI) and third types (chocolate ice cream, CI) were produced like first type (with carob extract 8, 10 and 12%) by adding lemon concentrate and chocolate drops in ratio of 10 % to the plain ice cream formulation, respectively. The ingredients were stirred until all of them completely dissolved. Then the mixture was pasteurized at a temperature of 75°C for 10 minutes. Samples were kept 24 hours at a temperature of 4°C for cooling and ripening process and after that transferred to a household ice cream maker (Tefal Gelato, China) for production. Ice cream samples have been kept in the freezer at -18°C (Figure 1).

The yogurt samples were produced on the basis of set type yogurt procedure. Because the pH of the carob extract was around 5.4, the extract (8, 10, 12%) was added to milk during the heat treatment since it did not cause the increasing of acidity of the milk. The process flow chart was shown in Figure 2 for yogurt production.

Physicochemical analyses

Descriptive analyses (total dry matter, pH and titratable acidity) were obtained according to Cemeroglu (2007).

Total phenolic content (TPC) of the samples was highlighted according to the spectrophotometric Folin-Ciocalteu's method (Folin-Ciocalteu reagent and sodium carbonate were purchased from Merck (Darmstadt, Germany)). Gallic acid (Sigma Aldrich, Steinheim, Germany) was used as a standard. Results were expressed as gallic acid equivalents (GAE) (mg GAE/L) and absorbance was measured by the help of a UV-visible spectrophotometer (Shimadzu UV-160A, Japan) at λ_{max} 765 nm (Spanos and Wrolstad, 1992).

The D-Pinitol content of samples, according to the external standard method were carry out by HPLC (Shimadzu, LC 20A Series). Mobile phase: Milli-Q water (isocratic), 0.6 mL / min, analytical and protective column: Nucleogel 87P (300x7.8 mm ID, 20x4.0 mm ID) and injection volume: 20 μ L, Column furnace temperature: 85°C, Detector: RID, cell temperature: 60°C (Tetik et al., 2011).

For the antioxidant assay, performed according to the method of Fernández-León et al. (2013); 50 μ L of diluted samples were pipetted into 0.95 mL of diphenylpicrylhydrazyl (DPPH) (Sigma-Aldrich, Steinheim, Germany) solution (60 μ M) and kept at room temperature for 30 minutes in a dark place. The absorbances of samples were read, spectrophotometrically at 515 nm. Trolox (Sigma-Aldrich, Steinheim, Germany) was used as the standard of the measurement. The antioxidant activity of samples expressed as mg Trolox Equivalent Antioxidant Capacity (TEAC) / kg were calculated using the curve obtained with trolox standards prepared at different concentrations.

The fortified products with carob extract were subjected to sensory analysis at their specific consumption forms and temperatures. The sensorial properties of products were evaluated according to a 5-point hedonic scale by trained panelists (10) from Akdeniz University, Department of Food Engineering. In this analysis, appearance-color, consistency (texture), aroma, flavor (1=dislike, 2=do not like, 3=neither like nor dislike, 4=like, 5=like very much) and purchasing preference (1= I would not buy, 3= I would buy) criteria are discussed for fortified ice cream and yogurt samples.

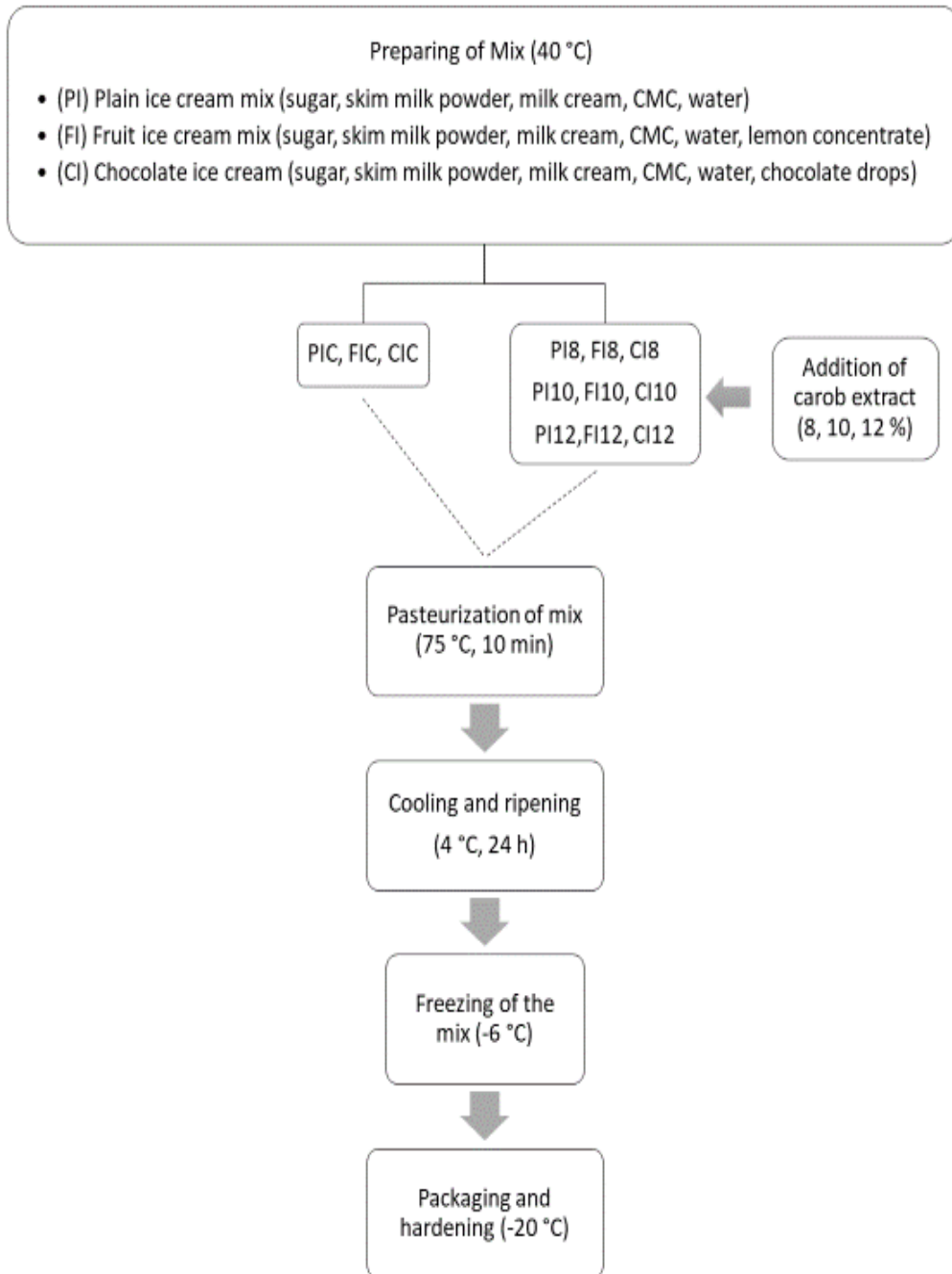


Figure 1. Flow diagram of ice cream production (control of fruit ice cream (FI), chocolate ice cream (CI) and plain ice cream (PI) were entitled as FIC, CIC and PIC, respectively)

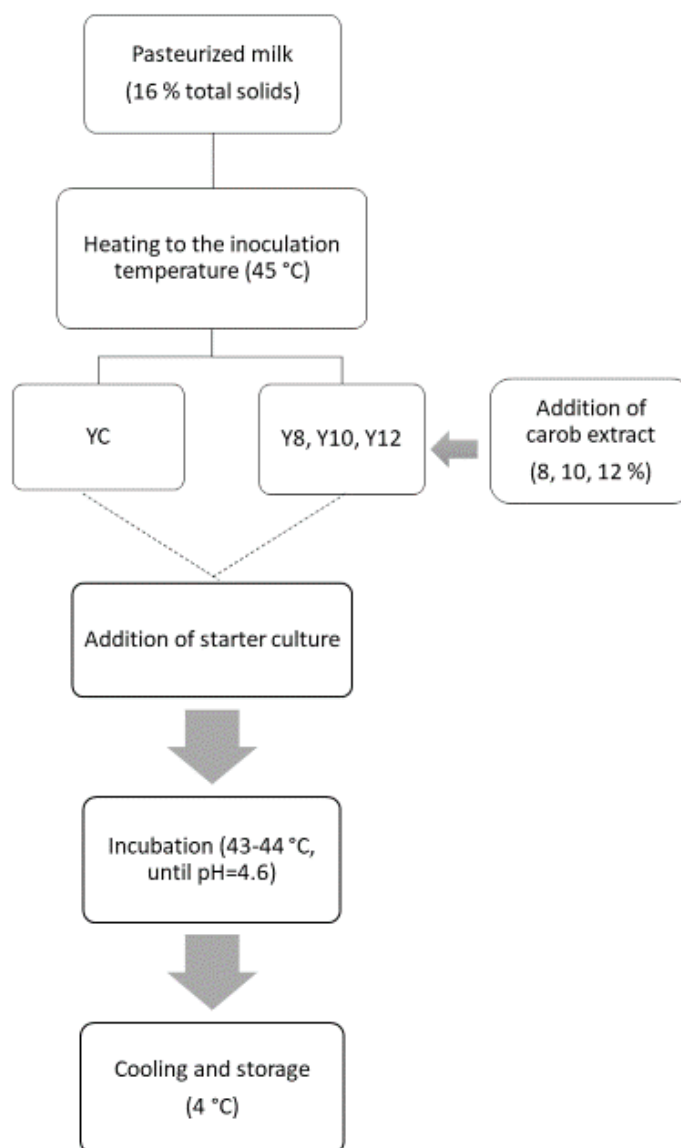


Figure 2. Flow diagram of yogurt production (control of yogurt were entitled as YC)

Statistical analysis

The obtained data were analyzed statistically by using SAS software, Version 7 (SAS Institute Inc., Cary, NC). All experiments were conducted in duplicate. The values of all parameters ($n = 4$) were presented as mean \pm standard deviation and the analysis of variance (ANOVA) test was used to evaluate the means of different treatments at a significance level of 0.05.

RESULTS AND DISCUSSION

Physicochemical properties of yogurt and ice cream samples

It was observed that, total dry matter content of yogurt samples increased significantly ($P < 0.05$), with increasing amount of carob extract added. Considering the fermentation process was terminated at the same pH value (4.6) in all samples, there was no statistical difference among yogurt samples in terms of titratable acidity

content and pH value. In the study of Karaca et al. (2011), yogurt samples produced by adding various concentrations (6%, 10%, 14%) of grape, mulberry and carob molasses had pH values ranged between 4.23 and 4.50. In another study conducted by Atasoy (2009), yogurt was produced by adding carob juice concentrate in different proportions (2.5%, 5%, 7.5%, 10%). It has been stated that the pH value of yogurts varies between 4.54 and 4.92. Nasser (2020) produced yogurt by adding 0%, 5%, 10% and 20% carob extract. He determined that the pH values of the yogurts

produced varied between 4.32-4.54 after 7 days of storage. He also reported that their total acidity varied between 1.16% and 1.43%.

As a result of the analyzes performed in the yogurt samples, on the HPLC device: D-pinitol was not detected in yogurt controls since the carob was not added. At the moment of 8%, 10% and 12% of carob extract was added in the yogurt, the amount of D-pinitol increased, significantly ($P < 0.05$) (Table 1).

Table 1. Chemical properties of yogurt samples produced with different levels of carob extract.

Level of carob extract (%) (w/w)	Total solids (%)	pH	Titrateable acidity (%)	D-pinitol (g/kg)	TPC (mg GAE/kg)	Antioxidant activity (mg TEAC/kg)
0 (YC)	13.13 ± 0.04 ^D	4.64 ± 0.05 ^A	0.78 ± 0.04 ^A	ND*	643.46 ± 1.22 ^D	30.56 ± 3.56 ^D
8 (Y8)	18.22 ± 0.03 ^C	4.64 ± 0.05 ^A	0.77 ± 0.04 ^A	7.76 ± 0.19 ^A	1057.02 ± 25.54 ^C	1428.95 ± 65.79 ^C
10 (Y10)	19.63 ± 0.01 ^B	4.50 ± 0.02 ^A	0.78 ± 0.02 ^A	8.42 ± 0.14 ^B	1216.37 ± 0.01 ^B	1830.27 ± 19.74 ^B
12 (Y12)	20.715 ± 0.01 ^A	4.57 ± 0.02 ^A	0.75 ± 0.02 ^A	10.09 ± 0.29 ^C	1246.78 ± 8.51 ^A	2576.98 ± 16.44 ^A

Means with different superscripts letters in the same column differ significantly ($P < 0.05$).

*ND: Not detected.

Otherwise, the amount of phenolic substances and antioxidants was very low in the yogurt samples which were not added to the carob extract; as the proportion of carob extract in the yogurt increases, the amount of phenolic compounds (TPC) and antioxidant activity increased, significantly ($P < 0.05$). Likewise, Açıkgözoglu (2008) reported that the TPCs of yogurts prepared with addition of 15% (w/w) pomegranate juice concentrate and 15% (w/w) cherry juice concentrate were 70.51 mg GAE/150 g and 63.35 mg GAE/150 g, respectively. In another study, the effects of olive leaf extract (0, 0.1, 0.2 and 0.4%, v/w) on low-fat apricot yogurt were investigated. According to the results, the added olive leaf extract has influenced significantly ($P < 0.05$) total dry matter and pH value of low-fat apricot yogurt. The lowest phenolic content and antioxidant activity were found in control sample (Peker and Arslan, 2017). Furthermore, in their study Cervenka et al. (2019), prepared muffins by replacing wheat flour by carob powder (5, 10, 15 and 20% (w/w)) and

determined some physicochemical properties. TPC of fortified muffins significantly increased from 348.1 to 829.1 µg GAE/g DM with the increase of amount of carob powder from 5% to 20% (w/w) ($P < 0.05$) while in control they found 193.2 µg GAE/g DM. Additionally, researchers observed that control muffin sample did not exhibit antioxidant capacity in terms of DPPH assay. TEAC values of fortified muffin samples with %5, 15% and 20% (w/w) were determined as 222.9, 1099.5 and 1228.3 µg Trolox/g DM, respectively. Babiker et al. (2020) obtained cookies enriched with carob flour. They reported that the antioxidant activity values of the cookie samples varied between 2.32% (0.0% carob) and 76.75% (50% carob). It was also stated that the total phenolic substance content varied between 36.59 mg GAE/100 g (0.0% carob) and 123.61 mgGAE/100 g (100% carob). Srouf et al. (2016) obtained a carob-based milk beverage using 6 different carob powders (roasted and unroasted). It was stated that the total phenolic contents of the beverages produced with roasted and

unroasted carob powder varied between 8-16 g GAE/kg and 5-14 g GAE/kg, respectively. In addition, antioxidant activities were reported to vary between 6.9-10.8 mmol Trolox equivalent/kg and 3.5-12.2 mmol Trolox equivalent/kg, respectively.

Total solids content, pH value, titratable acidity content, D-pinitol content, TPC and antioxidant activity of ice cream samples supplemented with carob extract were shown, in Table 2.

Table 2. Chemical properties of ice cream samples produced with different levels of carob extract.

Sample	Level of carob extract (%) (w/w)	Total solids (%)	pH	Titratable acidity (%)	D-pinitol (g/kg)	TPC (mg GAE/kg)	Antioxidant activity (mg TEAC/kg)
Plain ice cream	0 (PIC)	29.815 ± 0.01 ^D	6.78 ± 0.04 ^A	0.09 ± 0.00 ^C	ND*	641.03 ± 13.38 ^D	15.37 ± 0.00 ^D
	8 (PI8)	31.47 ± 0.01 ^C	6.57 ± 0.07 ^B	0.18 ± 0.02 ^B	8.78 ± 0.18 ^A	868.49 ± 19.46 ^C	1550.65 ± 23.03 ^C
	10 (PI10)	32.11 ± 0.04 ^B	6.48 ± 0.04 ^B	0.19 ± 0.01 ^B	9.54 ± 0.28 ^B	1318.54 ± 14.60 ^B	1708.55 ± 49.34 ^B
	12 (PI12)	33.44 ± 0.07 ^A	6.47 ± 0.01 ^B	0.23 ± 0.01 ^A	10.87 ± 0.11 ^C	1864.69 ± 8.51 ^A	1932.24 ± 29.61 ^A
Lemon ice cream	0 (FI0)	33.12 ± 0.01 ^C	3.59 ± 0.01 ^C	1.61 ± 0.01 ^B	ND	645.89 ± 8.51 ^D	111.87 ± 4.34 ^D
	8 (FI8)	32.75 ± 0.04 ^D	3.70 ± 0.02 ^B	1.65 ± 0.01 ^B	8.25 ± 0.01 ^A	1077.7 ± 0.01 ^C	1682.24 ± 9.87 ^C
	10 (FI10)	34.41 ± 0.04 ^B	3.75 ± 0.04 ^B	1.72 ± 0.02 ^A	9.15 ± 0.25 ^B	1201.77 ± 2.43 ^B	1981.58 ± 13.16 ^B
	12 (FI12)	35.31 ± 0.05 ^A	3.90 ± 0.02 ^A	1.62 ± 0.03 ^B	10.31 ± 0.27 ^C	1526.54 ± 37.71 ^A	2123.03 ± 3.29 ^A
Chocolate ice cream	0 (CI0)	35.70 ± 0.03 ^A	7.24 ± 0.00 ^A	0.08 ± 0.01 ^C	ND	583.86 ± 19.46 ^D	783.58 ± 16.84 ^D
	8 (CI8)	34.12 ± 0.06 ^A	6.93 ± 0.01 ^B	0.15 ± 0.01 ^B	8.16 ± 0.19 ^A	863.62 ± 7.30 ^C	1353.29 ± 9.87 ^C
	10 (CI10)	35.53 ± 0.6 ^A	6.75 ± 0.00 ^C	0.24 ± 0.01 ^A	9.19 ± 0.20 ^B	1001.07 ± 3.65 ^B	1889.47 ± 6.58 ^B
	12 (CI12)	36.54 ± 2.45 ^A	6.75 ± 0.02 ^C	0.22 ± 0.00 ^A	10.11 ± 0.29 ^C	1125.14 ± 6.08 ^A	2567.11 ± 26.32 ^A

Means with different superscripts letters in the same column differ significantly ($P < 0.05$).

*ND: Not detected.

The total amount of dry matter increased by 8%, 10% and 12% in ice cream samples generally. Since the pH value of the carob extract is higher than the pH value of the lemon concentrate, only the pH value of FI samples are increased by increasing amount of carob extract. While in the samples CI and PI, increase in acidity and decrease in pH value were observed as the amount of carob extract increased. In a similar study, Guler-Akin (2016) investigated some physicochemical properties of probiotic yogurt ice cream supplemented with carob extract and whey powder. It was observed that in samples of carob extract and whey powder supplemented, the pH decreased, and the acidity increased while the dry matter content (%) increased slightly as the carob extract and whey powder was added. Ice creams containing carob molasses were produced by Badem (2006) using different stabilizers. It was

stated that the pH values of the ice creams produced varied between 6.30-6.37. Ice cream containing different proportions of carob pod powder was produced by El-Kholy (2015). It has been reported that the pH values of ice creams varied between 6.22-6.46.

D-pinitol could not be detected in the control samples of FI, CI and PI, whereas the amount of D-pinitol were significantly higher than in control for all kinds of fortified ice creams without regard of carob concentrate proportion. A considerable increment in D-pinitol content was observed in samples with increasing carob extract added ($P < 0.05$).

The TPC and antioxidant activity value were found to be the lowest in PIC. Due to the presence of cacao; CIC had the highest

antioxidants among all the control samples. As can be seen from the results, enrichment with increasing amount of carob extract, increased the amount of phenolic substances and antioxidants, significantly ($P < 0.05$) for each kind of ice cream examined in this study. In the same spirit, Sanguigni et al. (2017) produced an ice cream based on a mixture of dark cocoa powder with hazelnut and green tea extract, naturally very rich in antioxidants. The TPC in the latter was 1817 mg GAE/L compared to only 96 mg GAE/L in control ice cream.

Sensory analysis of yogurt and ice cream samples

According to the results of sensory evaluation yogurt sample supplemented in ratio of 10% (Y10) was scored higher in sensorial attributes such as appearance-color, aroma and flavor except for consistency. The increment of carob extract in yogurt showed a negative influence on consistency. As can be seen from the Figure 3, that the consistency (texture) of Y8 was more preferred than others. However, Y10 and Y12 were found to be close to each other in terms of appearance and color.

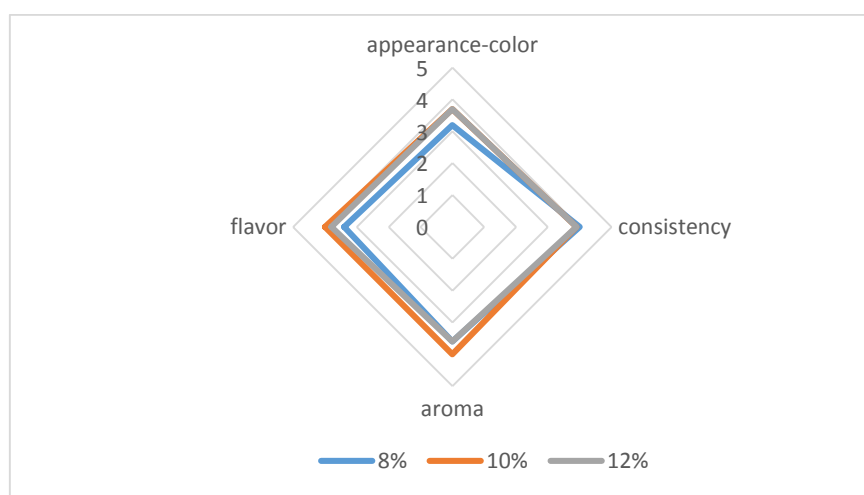


Figure 3. Results of sensory analysis of yogurt samples added by 8%, 10% and 12% of carob extract.

For the flavor analysis the Y10 was more popular because it was close to fruity yogurt flavor. Although, 8%, 10% and 12% of the carob extract was added to the yogurt samples, the aroma of the yogurt sample with 10% of carob extract was more appreciated contrary to 8% and 12%. Atasoy (2009) obtained yogurt using different proportions of carob juice concentrate. He reported that, in general, yogurts containing 7.5% -10% carob juice concentrate were mostly preferred by panelists. He also stated that yogurts containing 2.5% -5% carob juice concentrate were short of sweetness. Nasser (2020) reported that yogurts containing carob extract in different proportions (0%, 5%, 10% and 20%) generally have high acceptability in terms of sensory properties in all storage period.

Results of sensorial assessment of ice cream samples are shown in Figure 4. As understood from Figure 4a, in terms of appearance-color FI8 was found more appreciated ($P < 0.05$) whereas the FI10 and FI12 had not too much differences. However, the aroma and the consistency of FI8 and FI10 were more popular ($P < 0.05$).

As shown in Figure 4b, CI8 was better than CI10 and CI12 in terms of appearance-color and consistency ($P < 0.05$). Otherwise, the aroma characteristic of the chocolate ice cream sample with low carob extract was more preferred ($P < 0.05$). Considering the evaluation made on flavor, it was found that there was a significant difference between CI8 and C10 to CI12 ($P < 0.05$). The increase in amount of carob extracts added, negatively affects the flavor of chocolate ice cream.

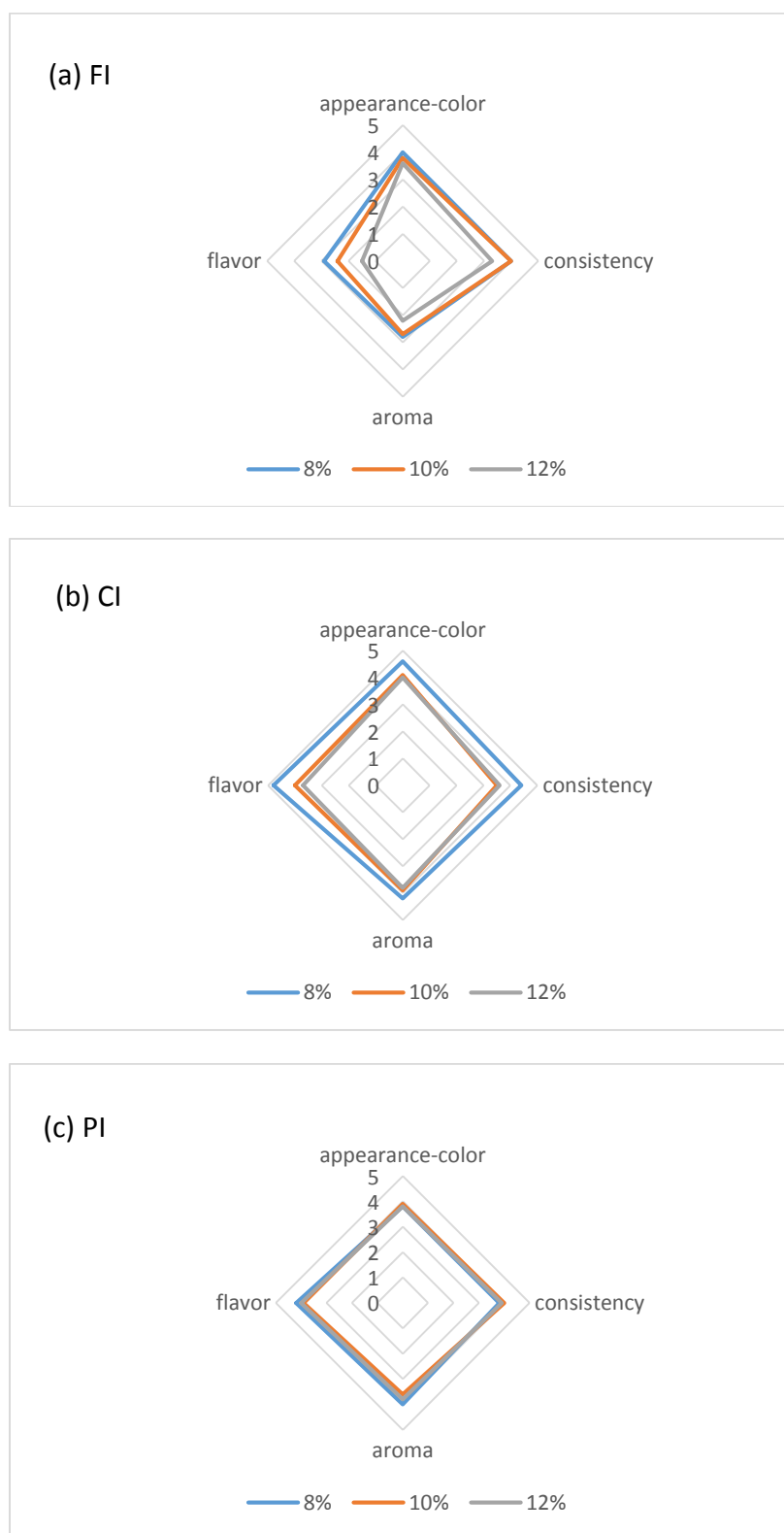


Figure 4. Results of sensory analysis of ice cream samples added by 8 %, 10 % and 12 % of carob extract; (a) fruit ice cream (FI), (b) chocolate ice cream (CI), (c) plain ice cream (PI).

The sensory analysis made on plain ice cream samples (Figure 4c), observed that there was not much differences in appearance-color and consistency between all samples. The flavor taste rankings of plain ice cream samples are PI8 PI12 and PI10. But samples which have 8% and 10% carob extract were much more appreciated. Considering the aroma evaluations, the aroma of PI8 was better ($P < 0.05$). Ice creams containing carob molasses were produced by Badem (2006) using different stabilizers. Sensory properties (color-appearance, texture-consistency and taste-

odor) of ice creams were evaluated over 7 points. It was reported that the color-appearance score varied between 5.50-6.50, the texture-consistency score varied between 4.33-6.66, and the taste-odor score varied between 4.83-6.33. Ice cream containing carob powder was produced by Sabatini et al. (2011). It has been stated that the ice cream produced has an acceptability index of over 87% in terms of all sensory properties. Thus, it has been reported that ice cream can be put on the market.

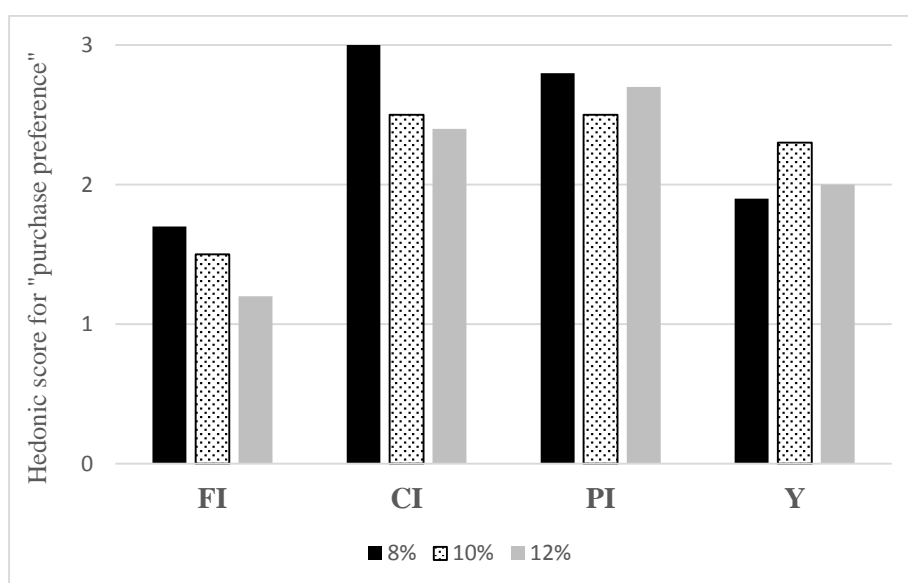


Figure 5. Hedonic scores (1: I would not buy, 2: Undecided, 3: I would buy) for preference of purchasing of fruit ice cream (FI), chocolate ice cream (CI), plain ice cream (PI) and yogurt (Y) samples fortified with carob extract.

According to the results of hedonic scores in our study, when all the analysis evaluations are taken into consideration, PI8 and PI12 are close together purchasing preference mark, while PI10 is less preferred (Figure 5). It was concluded that FI8 and CI8 can be more purchased. So, the decrease in purchasing preference was found out as the proportion of carob extract increased among fruit and chocolate ice cream samples. Considering the purchasing preferences of panelists in accordance with other criteria of yogurt, it was observed that Y10 can be the most saleable. This can be explained by the fact that the 10% carob extract yogurt is preferred by panelists

who consume both sugar-free and sugary product.

CONCLUSION

The supplementation of carob extract in yogurt and ice cream impact their physicochemical and sensory properties. When the proportion of carob extract added to the yogurt and ice cream samples increased, D-pinitol contents, total dry matter contents, amount of phenolic compounds and antioxidants values also increased. Furthermore, when we take into consideration all sensory analysis' evaluations, samples added with 8% of carob extract either yogurt or ice-cream got better results. To this effect, the use of carob to impart

functional compounds in daily diet products can be thorough, considering that: in yogurt and ice cream as the proportion of carob extract increases, the amount of D-pinitol, antioxidant value and phenolic compounds increases. Furthermore, tastes and purchasing preference was found proportionately opposed to the increase of carob extracts in the products.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

MK designed the research. MA, ŞK, DY, NK, and AAK performed research and carried out statistical analyzes. UTZA, AAK, AH, MK wrote the paper. All authors contributed to the article and approved the submitted version.

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