Impact of Pistacia Terebinthus on The Antioxidant Activity and Total Phenolics of Ice Cream Depending on Roasting Conditions and Incorporation Time

Metehan ERGENEKON¹ , Çağım Akbulut ÇAKIR2*

*¹Department of Food Engineering, Faculty of Engineering, Şanlıurfa, Turkey ²Department of Food Engineering, Faculty of Engineering, Şanlıurfa, Turkey *Corresponding author: cagim@harran.edu.tr *ORCID:0000-0002-2754-0133*

Abstract

Pistacia terebinthus (terebinth) is in the same family with P. vera and contains considerable amounts of bioactive compounds with antioxidant, anti-inflammatory, hypolipidemic and neuroprotective activities. Although pistachio (P. vera) is a common ingredient used in ice cream, P. terebinthus consumption is limited to being a snack food or coffee-like drink. In this study P. terebinthus examined for its antioxidant activity under different conditions and it is added to the ice cream to increase its antioxidant activity while providing a new flavor option. P. terebinthus was added to the ice-cream after roasting at different temperatures (A: 0, B: 100, C: 125 and D: 140°C for 20 min.). Raw and roasted P. terebinthus seeds were milled, hard shells were removed and added to the ice-cream mix before pasteurization of the mix. Chemical composition, physical properties and sensory results of ice-creams (A, B, C and D) were compared to the control (K) that doesn't contain P. terebinthus. Adding P. terebinthus to ice-cream reduced its pH, lengthened its melting time, however didn't affect its viscosity as compared to K. Change in the antioxidant activity (AA) and total phenolic content (TPC) of P. terebinthus due to any possible interactions with milk proteins and sugar when heating the ice-cream mix was also examined by comparing the control P. terebinthus solution with milk solutions (9% milk powder, 10% sugar) where P. terebinthus is added before and after heating milk solutions (80°C for 1 min.). Heat treatment of milk solution together with P. terebinthus reduced its AA. Roasting increased the TPC and AA of P. terebinthus. Highest AA was observed at ice-cream C.

Keywords: Functional ice-cream, Pistacia terebinthus, Antioxidant activity, Total phenolics, Heat treatment

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INTRODUCTION

P. terebinthus L. (turpentine tree, terebinth) is in the same family with P. vera and grows in the Mediterranean region and some parts of Asia. P. terebinthus fruits possess many biological activities due to being rich in flavonoids and other phenolic compounds. Although P. vera is a common flavor used in ice cream, P. terebinthus consumption is very limited and unknown by most people. However, it drawn the interest of researchers due to its AA (Topçu et al., 2007), anti-inflammatory properties and high oil content (Matthäus and Özcan, 2006). An investigation found strong anticholinesterase activity in P. terebinthus extracts (Hacıbekiroğlu et al., 2015). Bakirel et al. (2003) showed that P. terebinthus extracts of dried fruits have hypolipidemic effect on animal tests. Orhan et al. (2012) claimed that roasted P. terebinthus could be neuroprotective due to its BChE inhibitory effects and AA. Impact of roasting on phenolic content of P. terebinthus oil was studied and an increase in TPC and AA was reported (Durmaz and Gökmen, 2011; Dalgıç et al., 2011).

Although ice cream is an important nutrient with its protein and fat content, it is not rich in antioxidants and phenolic substances alone. On the other hand, ice cream is a good carrier for many nutraceuticals, and with this feature it has been used in the production of functional foods in many studies (Goraya and Bajwa, 2015). Enriching ice cream with grape, pomegranate and sesame powder and oils (Akça and Akpınar, 2021), black carrot concentrate (Pandey et al., 2021), herbal tea (Karaman and Kayacier, 2012) blueberries (Pehlivanoğlu et al., 2024), propolis (Mehmetoğlu and Tarakçı, 2023) and pistachio shell (Ghandehari Yazdi et al., 2020) are some of these studies. Adding P. terebinthus to ice cream would produce a functional food with health benefits and would provide a new way for widespread consumption of P. terebinthus. There has been a considerable interest in the incorporation of polyphenols to dairy products to enrich their nutritional and functional properties. However, phenolic compounds can strongly interact with milk proteins, causing functional and structural changes (Jakobek, 2015); and these interactions could reduce the AA and bioavailability of the phenolics (Arts et al., 2001; Arts et al., 2002; Serafini et al., 2003; Stojadinovic et al., 2013). At what stage of the process they are added to the milk system is also important since processing conditions can influence the interactions of polyphenols. Heat treatment of the ice cream mix is one of the main processes involved in ice cream making. It was reported that AA of certain polyphenol–milk protein complexes negatively affected by heat treatment (Kılıç Bayraktar et al., 2019). Therefore, we also wanted to examine the change in the AA by the stage of adding P. terebinthus before and after heat treatment of milk solutions; and we also evaluated the masking effect of ice cream on P. terebinthus.

MATERIAL and METHOD

Materials

Fresh fruits of P. terebinthus were purchased at local markets in Şanlıurfa, Turkey. Non-fat milk powder (96% total solids), cream (35% fat) (Pınar Dairy, Turkey), lecithin (Sosa Ingredients, S.L. Ctra de Granera, Spain) as emulsifier and a blend of stabilizers that contain equal amounts of Karragenan (E 407), Guar gum (E 412), Sodium alginat (E 401), Xanthan gum (E 415) and dextrose (KATPA, Katkı Maddeleri Gıda Sanayii ve Ticaret Ltd. Şti., Türkiye) was used for ice-cream making.

Preparation and heat treatment of Pistacia terebinthus extracts

Damaged fruits and foreign matters were discarded and layered in aluminum trays as a single layer for heat treatment at temperatures selected by preliminary trials. One group kept untreated (A), and other groups were roasted in oven that is set to 100 (B), 125 (C) and 140°C (D) for 40 min. After cooling down to room temperature, they were milled using a laboratory mill in such a size that the shells do not pass under the sieve. Obtained P. terebinthus paste was sieved by adding water (1:6) to be able to remove the hard-shell pieces. These P. terebinthus extracts were prepared the day before ice-cream production and kept at 4° C.

Ice cream manufacture

Mix (11% non-fat solids (NFS), 5% fat, 18% sugar, 0.8% emulsifier and 0.2% stabilizer) was prepared and 5% P. terebinthus roasted at 0° C (A), 100° C (B), 125° C (C) and 140°C (D) was added. One batch from each production was set as control (K) and no extract was added. All ice cream mixes were heat treated at 80°C for 1 min, stirred well with a blender while still hot and aged for 24h at 4°C. A vertical freezing machine with 6 kg capacity (Uğur, Nazilli, Turkey) was used for freezing the mix and the ice cream was packed in 200 mL cups and stored at −18˚C. All trials were duplicated, and the analyses were carried out at least in duplicate.

Determining the impact of P. terebinthus incorporation stage

AA and TPC were compared between control (5% P. terebinthus solution) and milk solutions (9% milk powder, 10% sugar) where 5% P. terebinthus is added before and after pasteurizing the milk solutions at 80°C for 1 min. Raw and roasted (125°C) P. terebinthus extracts were used. Sample codes for controls; A0: Raw P. terebinthus, C0: Roasted (125°C 40 min) P. terebinthus, for milk solutions; A1: raw P. terebinthus added before pasteurizing, A2: raw P. terebinthus added after pasteurizing, C1: roasted P. terebinthus added before pasteurizing, C2: roasted P. terebinthus added after pasteurizing.

Masking of total antioxidant activity

Masking of total AA calculated as percent difference between sum of AA of P. terebinthus $+ K$ and AA of ice creams with P. terebinthus (Stojadinovic et al., 2013).

Proximate analysis of ice cream samples

Fat (IDF, 1991), total solids, ash, pH and titratable acidity (Bradley et al., 1992) were determined 1 week after the production.

ABTS assay

ABTS·+ radical scavenging activity was measured at 1 week. Briefly, 7 mM ABTS stock solution was reacted with 2.45 mM $K_2S_2O_8$ and kept in the dark at room temperature for 12–16 h. Then it was diluted to an absorbance of 0.70 ± 0.03 at 734 nm by adding 5 mM phosphate buffered saline (pH 7.4). For the photometric assay, 2.980 ml of the ABTS·+ solution was added to ethanol extracts of samples and after waiting 6 min, their absorbance measured at 734 nm (Re et al., 1999). Calibration was done with trolox stock solution. Inhibition of $ABTS + was calculated by the following equation:$

ABTS⁺⁺ Inhibition $(\%) = [(Ac-As)/Ac \times 100]$ Ac; Absorbance of the blank, As; Absorbance of the sample

DPPH assay

DPPH radical scavenging activity was analyzed at 1 week. Briefly, 2.9 ml of 0.1 mM DPPH was added to ethanol extracts of samples and absorbance was detected at 517nm after 30 min. DPPH inhibition capacity of the samples were calculated by the following equation (Blois, 1958):

> DPPH Inhibition $% = [(Ac-As)/Ac \times 100]$ Ac; Absorbance of the blank, As; Absorbance of the sample

Determination of Total Phenolic Contents

The amount of phenolic compounds in ethanol extracts of samples were determined by the Folin–Ciocalteu colorimetric method described by Slinkard and Singleton (1977) with some modifications. Sample extract (0.03 mL) was mixed with 2.37 mL distilled water and 0.15 mL Folin–Ciocalteu's reagent. After waiting for 8 min. 0.45 mL Na₂CO₃ was added and kept 30 min. at room temperature for reading. The absorbance was measured at 750 nm and total phenolic concentrations (mg/kg) were estimated using gallic acid standard curve.

Physical analysis of ice cream samples

The apparent viscosity of the samples were determined according to Dervisoglu et al. (2004) using ice cream samples equilibrated to 4°C for 12h prior to the test. A Brookfield Viscometer (Model DV-II; Bookfield Engineering Laboratories, Inc., Stoughton, MA, USA) with spindle no 5 was used.

The overrun of the ice cream samples was estimated using the following equation (Jimenez-Florez et al., 1993).

Overrun = (Weight of unit mix - weight of equal volume of ice cream) / (Weight of equal volume of ice cream) * 100

Melting behavior expressed as the first dripping time and melting rate, was evaluated by weighing 80 ± 5 g ice cream sample on a 0.2 cm wire mesh screen that was left to melt at room temperature $(24 \pm 2^{\circ}\text{C})$ (Abd El-Rahman et al., 1997; Cotrell et al., 1979). The time it takes to see the first drop of ice cream was measured as first dripping time. The melted ice cream weight was recorded at 30th, 45th, 60th and the 90th min. Time of complete melting was also recorded.

Sensory analysis

Sensory analysis was conducted by ten untrained panelists involving the staff from the Harran University Department of Food Engineering. Panelists were familiar with dairy products and were checked based on sensory perception and reliability. A 10-point hedonic scale was used to examine color and appearance, firmness, smoothness, gumminess, meltdown, iciness, flavor and taste and general acceptability (1=strongly unacceptable, $10 =$ very good) as described by Aime et al. (2001).

Each panelist received 5 samples of ice cream to taste and to evaluate at each serving. Panelists were also requested to drink water between the samples in order to maintain discretion. All physical, chemical and sensory analyses were carried out 1 week after the production.

Statistical analysis

Statistical analysis was performed by SPSS version 16 (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) was done to establish statistical differences between the chemical and physical properties of the samples. Statistically different groups were determined by the Duncan multiple comparison test $(p<0.05)$.

RESULTS and DISCUSSION

Composition and physical properties of ice creams

Control ice cream (K) had $36.2 \pm 0.72\%$ total solids, $3.55 \pm 0.21\%$ fat, $1.1 \pm 0.03\%$ ash, 0.25 \pm 0.03% titration acidity and a pH value of 6.9 \pm 0.03. No significant difference (p>0.05) was observed between ice cream samples with 5% P. terebinthus, having 38.69±0.19% total solids, $5.18\pm0.13\%$ fat, $1.19\pm0.00\%$ ash, 6.75 ± 0.01 pH and $0.31\pm0.00\%$ acidity in average. Adding P. terebinthus increased the total solids, fat and ash content as expected, and due to their higher acidity, they increased the acidity of the ice creams as well.

Measurement		K		B		
Viscosity (cP)		12021 ± 218 ^a	$12258 \pm 7^{\text{a}}$	$11376 \pm 0^{\circ}$	12862 ± 891 ^a	$12795 \pm 1050^{\text{a}}$
Overrun $(\%)$		29.37 \pm 2.95 $^{\rm a}$	28.30 ± 1.77 ^{ab}	24.82 ± 0.11 ^{bc}	28.70 ± 0.60 ^{ab}	22.18 ± 0.52 ^c
First dripping time (s)		709 ± 0.00 ^a	690 ± 4.24 ^c	683 ± 0.00 d	698 ± 1.41 ^b	710 ± 0.71 ^a
Melting rate	$15th$ min			1.69 ± 2.38		
(%)	$30th$ min	17.64 ± 7.17 ^a	0.82 ± 1.15 ^c	11.04 ± 3.24^{b}	1.72 ± 1.83 ^c	2.47 ± 2.09 ^c
	45^{th} min	70.22 ± 7.98 ^a	23.79 ± 7.80 ^d	67.55 ± 7.34 ^a	46.85 \pm 7.02 ^b	$32.30\pm0.16^{\circ}$
	$60th$ min	90.31 \pm 0.13 ^a	$80.03 \pm 7.45^{\rm b}$	83.21 \pm 850 ^{ab}	77.64 \pm 5.76 $^{\circ}$	86.05 ± 5.68 ^{ab}

Table 1. Physical properties of the ice cream samples

a,b,c Values with different superscript letter in the same column are significantly different $(P<0.05)$

*K: Control with no *P. terebinthus*, A: Raw *P. terebinthus,* B: 100°C roasted *P. terebinthus,* C: 125°C roasted *P.terebinthus,* D: 140°C roasted *P. terebinthus*

Physical properties of the ice creams are given in Table 1. No significant difference was observed between the apparent viscosities of the ice cream samples $(p>0.05)$. Slight differences were observed between overrun values, with K having a little higher overrun, which could be due to the hindrance of the overrun by P. terebinthus. Most of the studies that involve adding fruits or nuts reported a decrease in overrun (Ghandehari et al., 2020, El-Samahy et al., 2009, Hwang et al., 2009). While first dripping time was longer than the others for ice cream K, its melting rate was faster. Previous studies showed that adding ingredients such as polysaccharides and fibers with high water holding capacity would reduce the melting rate of the ice cream (Hwang et al, 2009, Karaman et al., 2014; Erkaya et al. 2012). Fiber content of the P. terebinthus could have reduced the melting rate. It appears that roasting the P. terebinthus influenced the melting rates.

While raw P. terebinthus reduced the melting dramatically, the fastest melt had occurred at milder temperatures within the roasted P. terebinthus. This could be due to a decrease in the water binding capacity of the components in P. terebinthus caused by heat treatment.

Antioxidant activity and total phenolics content of ice creams

Changes in the antioxidant capacity and TPC of the ice cream samples are given in Table 2. Roasting the P. terebinthus significantly influenced the AA and TPC of ice creams (P<0.05). Ice creams with raw P. terebinthus had the lowest TPC and AA. There was a proportional increase in both AA and TPC with the increase in roasting temperature until 125°C. Above that temperature it was either stable or a decrease was observed (ABTS inhibition). It is previously reported that, roasting induce several changes to phenolic compounds of nuts and seeds. While some phenolics break down, some other new phenolic compounds are formed with heat treatment (Durmaz and Gökmen, 2011). Certain Maillard reaction products formed by heat treatment also have AA (Lertittikul, et al., 2007). In a previous study, roasting increased the TPC, AA and oxidative stability of P. terebinthus oil, while the level of tocopherols, lutein and β-carotene was decreased (Durmaz and Gökmen, 2011).Dalgıç et al. (2011) also reported an increase in TPC and AA of P. terebinthus oil together with an increase in total carotenoids and most tocopherols.

Table 2. Changes in antioxidant capacity and total phenolic content of the ice cream samples

DPPH $(\%)$	$1.72 \pm 1.49^{\mathrm{a}}$	53.89 \pm 0.35 ^b	$55.71 \pm 0.35^{\rm bc}$	58.49 ± 0.96 ^d	56.46 ± 0.97 ^{cd}
ABTS(%)	$17.00 \pm 1.63^{\circ}$	$50.59 \pm 0.93^{\circ}$	53.02 \pm 0.77 $^{\circ}$	57.93 ± 0.19^e	$55.72 \pm 0.19^{\circ}$
Total Phenolics (mg/kg)	$21.28 \pm 0.15^{\circ}$	$80.71 \pm 8.58^{\circ}$	$85.83 \pm 1.66^{\circ}$	96.48 ± 1.35 ^c	$95.52 \pm 5.42^{\circ}$

Values with different superscript letter in the same column are significantly different (P<0.05) *K: Control with no *P. terebinthus*, A: Raw *P. terebinthus,* B: 100°C roasted *P. terebinthus,* C: 125°C roasted *P. terebinthus,* D: 140°C roasted *P. terebinthus*

Impact of P. terebinthus incorporation stage and masking of total antioxidant activity

A0: Control with 5% raw P. terebinthus, A1: Milk solution with 5% raw P. terebinthus added before pasteurization, A2: Milk solution with 5% raw P. terebinthus added after pasteurization, C0: Control with 5% roasted (125°C 40 min) P. terebinthus, C1: Milk solution with 5% roasted (125°C 40 min) P. terebinthus added before pasteurization, C2: Milk solution with 5% roasted (125°C 40 min) P. terebinthus added after pasteurization. a,b,c Bars with different superscript letter are significantly different (P<0.05)

Several studies showed the polyphenol-protein interactions and impact of those interactions on their AA (Arts et al., 2001; Xiao et al., 2011; Gallo et al., 2013). Milk proteins have an affinity to bind phenolic compounds which can mask the AA of those compounds and can reduce their bioavailability (Arts et al., 2001; Arts et al., 2002; Serafini et al., 2003; Stojadinovic et al., 2013). It has also been showed that AA of certain polyphenol–milk protein complexes affected by heat treatment (Kılıç Bayraktar et al., 2019). Therefore, we evaluated change in the AA by the stage of adding P. terebinthus before and after heat treatment of milk solutions; and we also evaluated the masking effect of ice cream on P. terebinthus. According to ABTS assay P. terebinthus adding stage didn't influence the AA; however, we observed differences between samples at DPPH assay (Fig. 1). Heat treatment (80°C 1 min) of milk solution together with P. terebinthus reduced its antioxidant capacity. Samples with P. terebinthus added after heat treatment had similar DPPH inhibition level with control. TPC of milk solutions were higher than the control (Fig. 2). Addition stage of roasted P. terebinthus didn't influence the TPC. Raw P. terebinthus on the other hand, had higher TPC when added to the milk solution before heat treatment.

A0: Control with 5% raw P. terebinthus, A1: Milk solution with 5% raw P. terebinthus added before pasteurization, A2: Milk solution with 5% raw P. terebinthus added after pasteurization, C0: Control with 5% roasted (125°C 40 min) P. terebinthus, C1: Milk solution with 5% roasted (125°C 40 min) P. terebinthus added before pasteurization, C2: Milk solution with 5% roasted (125°C 40 min) P. terebinthus added after pasteurization. ^{a,b,c} Bars with different superscript letter are significantly different (P<0.05)

Figure 2. Change in the total phenolics content depending on incorporation stage.

AA of both raw and roasted P. terebinthus were masked similarly in ice cream (Fig. 3). However different masking ratios were obtained from different measurement methods. Masking of AA by ice cream was much higher according to ABTS assay (30%) as compared to DPPH assay (9%). Differences between different test methods have been reported in previous studies as well (Skrede et al., 2004), therefore it is necessary to use more than one method when evaluating antioxidant capacity.

Figure 3. Masking of total antioxidant capacity of A0: 5% raw P. terebinthus and C0: 5% roasted (125°C 40 min) P. terebinthus when added in ice cream.

Sensory Analysis Results

Sensory analysis results are given in Table 3. No significant difference was observed between smoothness, gumminess, meltdown, iciness and taste and flavor of ice cream samples ($p>0.05$). Ice creams with P. terebinthus were found to be firmer than the K. It appears from the sensory evaluation that, the dark color of P. terebinthus ice creams was not liked, and received lower scores than the K. It was indicated in comments by most panelists that, although the taste and flavor of the P. terebinthus ice creams was good, the amount used was too high and therefore flavor was too intense. Reducing the P. terebinthus amount to an acceptable level would produce an ice cream with better color and flavor. General acceptability of roasted ice creams found better with C being slightly higher than the other P. terebinthus ice creams, while D and A received lower scores than K.

^{a,b,c} Values with different superscript letter in the same column are significantly different (P<0.05)

*K: Control with no *P. terebinthus*, A: Raw *P. terebinthus,* B: 100°C roasted *P. terebinthus,* C: 125°C roasted *P.terebinthus,* D: 140°C roasted *P. terebinthus*

CONCLUSION

Significant differences were observed between AA and TPC of ice creams with P. terebinthus roasted at different temperatures. Ice creams with raw P. terebinthus had the lowest TPC and AA; and there was a proportional increase in both AA and TPC with the increase in heating temperature up to 125°C. Highest AA and better sensory properties were obtained by P. terebinthus roasted at 125°C for 40 min.

Increase in AA by roasting was due to the increase in TPC as well as the contribution of possible Maillard reaction products. Adding P. terebinthus to ice cream mix before heat treatment could reduce its AA according to our tests with milk solutions. About 30% of the AA of P. terebinthus was masked in the ice cream. The masking of the AA was presumably due to interactions between milk proteins and phenolics.

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