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Effect of microwave roasting and different solvents on the extraction of bioactive compounds from date seed

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Abstract

In this study, phenolic compounds were extracted from date seeds treated with different roasting methods (microwave (MW) and traditional) with three different solvents (water, acidic water, and alcohol), and their changes were investigated. The best results for total phenolic content (TPC) and antioxidant activity were given by 80% methanol (%1 HCI) solvent. The highest amount of TPC was determined in MW roasting at 300 W (7601.71 ± 155.759 mg GAE/100 g dry weight), followed by unroasted (7333.64± 3.12 mg GAE/ 100g dry weight) samples. On the other hand, traditional roasting was less effective than MW roasting at 300 W in terms of TPC, it had the highest antioxidant activity in all samples. HPLC results showed that date seeds contained individual phenolics like caffeic acid, p-cumaric acid, gallic acid, 3,4-dihydroxybenzoic acid, and rutin, and their amounts were affected by different roasting methods. As a multivariate analysis, PCA was a successful method for the classification of roasting treatments based on the bioactive compounds and antioxidant activity. According to these results, roasting had a significant effect on the extraction of phenolic compounds from date seed.

Keywords: Antioxidant activity, Date seed, Extraction, Microwave roasting, TPC

1 Introduction

The date palm (*Phoenix dactylifera* L.) is one of the oldest plants, cultivated and known to mankind, and has more than 2000 varieties. It has been grown in a wide geography including the Middle East and North Africa. Dates have been consumed as food for about 6000 years, and have different flavors, colors, and appearances [1]. Dates are mainly consumed as dried. Dates are a good source of dietary fiber, minerals (potassium, calcium, magnesium), and B and C vitamins with low fat and protein but high phenolic content [2], [3]. There are many phytochemical compounds in date seeds as well as dates. In contrast to the fruit, date seeds have a high phenolic, protein, and fat content [4]. The seed constitutes almost 13-15% of the fruit. Date seeds are mainly used as a supplement to animal feed. However, later studies have shown that date seeds have medicinal value due to their beneficial bioactive compounds [5]. There are several animal studies reporting biological effects of date seed such as lowering blood sugar as an anti-diabetic effect [6], improving Alzheimer's, memory and learning disorders [7], reducing oxidative damage [8]. Phytochemicals, found in date seeds include flavonoids, sterols, phenolic acids, and tocopherols. Phenolic compounds predominantly found in dates are ferulic acid, proanthocyanidins, p-coumaric acid, gallic acid, quercetin, and rutin [9]. In a study, it was determined that the total phenolic content in different date seeds ranged between 3102-4430 mg GAE/100 g fresh weight, and the antioxidant content ranged between 58,000-92900 µmol Trolox equivalents/100 mg fresh weight [10]. Bouhlali et al. [11] found that date seeds contain high levels (1224-1844 mg rutin equivalents/100g dry weight (DW)) of flavonoids and determined antioxidant activity of seeds, ranging from 10 to 23 mmol trolox equivalents/100 g DW for FRAP, 4-8 mmol trolox equivalents/100 g DW for ABTS, and 0.11-0.16 g/L for IC50 of DPPH.

Roasting is heating the seeds between 170-240 ºC for a certain time. With this process, changes in the color and structure of the seeds are observed and its unique aroma is formed due to various reactions [12]. In the literature, studies examine the effect of different roasting processes on the quality characteristics and bioactive components of coffee obtained from date seed [13], [14], [15]. In a study, palm seeds were roasted at 180, 200, and 220 °C for 20 minutes and it was observed that the highest antioxidant activity was determined in the sample roasted at 220 °C for 20 minutes [16]. In a study investigating the effect of roasting conditions on the physicochemical, chemical, and sensory properties of coffee obtained from date seeds, it was reported that roasting temperature and time were highly effective on quality and the optimum roasting temperature was 199.9 °C and the optimum roasting time was 21.5 minutes [12].

In recent years, microwave roasting has been applied as an alternative to traditional roasting due to the negative effects of high roasting temperatures and long processing times on quality [17]. In studies investigating the effect of microwave roasting on the physicochemical properties and bioactive properties of peanut and sesame seeds, it was determined that microwave roasting increased the antioxidant activity and total phenolic content compared to controls [17], [18]. Phenolic compounds are naturally present in the structure of fruits and vegetables as bioactive compounds and these compounds can be obtained by extraction with different solvents[19].

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In this study, date seeds were subjected to roasting at different power by microwave. Phenolic compounds in unroasted (control) and roasted (microwave and hot air oven) date seeds were extracted by classical solvent extraction method with water, acidic water (containing 1% HCl), alcohol (80% methanol containing 1% HCl). The effect of roasting processes on bioactive compounds was compared by determining total phenolic content and antioxidant activity (DPPH). Phenolic compounds were determined by HPLC and those change with the roasting process was examined. As a multivariate analysis, PCA was used for the classification of roasting treatments based on the bioactive compounds and antioxidant activity.

2 Material and methods

2.1 Material

Date seeds used in this study were obtained from local markets. All date seeds were stored at +4 °C until the production process.

2.2 Methods

2.2.1 Roasting

100 g of date seeds were weighed for each roasting process. The date seeds were heated in an oven at 180 ℃ for 20 minutes in the traditional roasting method. The microwave roasting process was carried out in a household microwave oven (Samsung MS23K3515AW) at different power and times. Roasting times were determined according to the degree of roasting and applied at 300 W for 12 minutes, 450 W for 6 minutes, 600 W for 5 minutes, and 800 W for 4 minutes. Unroasted seeds were used as control. Date seeds were ground with a grinder (HC100, Lavion, Türkiye) and passed from a 250 µm sieve.

2.2.2 Extraction of bioactive compounds

All samples were extracted with water, acidic water (containing 1% HCl), and alcohol (80% methanol containing 1% HCl) by classical solvent extraction at 30 °C for 6 hours. The centrifugation was done to the extracted samples at 5000 rpm for 20 minutes and filtered.

2.2.3 Total phenolic content (TPC)

Folin- Ciocaltaeu method was applied to the samples for determining TPC. The results were represented as gallic acid equivalent (mg GAE / 100 g dry weight) [19]. Extract (0.1 ml) was added on 0.75 ml of Folin- Ciocaltaeu (%10), and after waiting for 5 minutes, 0.75 ml of Na_2CO_3 (75 g/L) was added. After vortexing, the solution was kept in the dark under room conditions for 60 minutes and absorbance was read at 725 nm.

2.2.4 Antioxidant activity

Antioxidant activity was carried out using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. The extract (0.1 ml) and 3.9 ml of 0.1 mM DPPH solution (in 80% methanol) was mixed. After vortexing, the mixing solution was kept in the dark under room conditions for 30 minutes and the absorbance was recorded at 517 nm. 0.1 ml of methanol (80%) was used instead of the sample as a control under the same conditions. Antioxidant activity was calculated by Equation (1) as inhibition (%) [19].

$$\%\ Inhibition = \left[\frac{A_c - A_s}{A_c}\right] \text{x}100 \qquad (1)$$

Ac: Absorbance of control
As: Absorbance of sample

2.2.5 Determination of phenolic compounds by HPLC

HPLC was used to determine the phenolic compounds of the samples unroasted and roasted with traditional and microwave described by Okur et al. [20] with slight modifications. Before injection, the extracts were filtered through a 0.45 µm teflon membrane filter. Then, 20 µl of the filtered extracts were injected into the HPLC (Shimadzu Corporation, Kyoto Japan) with the SIL 10AD vp automatic sampling system. The HPLC system had a pump (LC-10ADvp), degasser (DGU-14A), control oven (CTO-10Avp), and a UV-VIS detector. Polyphenols were separated using a C18 column (GL Sciences, 250x4.60 mm, 5 microns, Japan). The column temperature was 30 °C. The separation was done by applying a gradient program with a binary solvent system. The mobile phases were 5% formic acid (A) and 20% A + 80% ACN (B). The flow rate was 1.0 ml/min. Phenolic compounds were carried out at 320 nm. The gradient program for phenolic compounds was applied as follows: 0–0.01 min: A 100%; 15 min: 90% A + 10% B; 90.00 min: 60% A + 40% B; 93.00 min: 100% B; 98 min: 100% A.

2.2.6 Multivariate analysis

Principal component analysis (PCA) was used to differentiate unroasted and roasted samples, and the results were presented as a score plot. PCA analysis was performed with the Minitab 17 (State College, PA, USA) program. The TPC and antioxidant activity of the samples were chosen as variables [19].

2.2.7 Statistical analysis

The Minitab (17.1.0.0, State College, PA, USA) was applied for the statistical analysis of the results. One-way ANOVA (analysis of variance) and Tukey's multiple range test were employed to determine the statistical differences.

3 Results and discussions

Roasted and unroasted samples were subjected to classical solvent extraction with different solvents. The TPC and antioxidant activity were determined in the extracted samples. The total phenolic content of unroasted and roasted samples extracted with different solvents was shown in Table 1.

According to Table 1, the highest TPC was determined in the sample subjected to microwave roasting at 300 W for 12 minutes ($p < 0.05$). The amount of TPC determined in the sample subjected to traditional roasting was lower than in the unroasted sample ($p < 0.05$).

Table 1. The TPC of unroasted and roasted samples extracted with different solvents

Treatment	TPC (mg GAE/100 g dry weight)		
	80% methanol (1% HCI)	Acidic water (1% HCI)	Water
Unroasted	7333.64± 3.12^{Aab}	542.10 ± 34.99^{Ca}	1331.32 ± 23.91^{Bb}
Traditional roasting	7139.43 ± 24.45^{Abc}	448.13 ± 12.85^{Ca}	1749.88 ± 21.85^{Ba}
MW roasting-300 W	7601.71 ± 155.75^{Aa}	231.98 ± 59.24^{Cb}	984.09 ± 85.73^{Cc}
MW roasting-450 W	6757.23 ± 153.99^{Ad}	484.77 ± 84.09^{Ca}	886.37 ± 51.49^{Cc}
MW roasting-600 W	6865.31 ± 223.55^{Acd}	428.36 ± 9.42^{Ca}	1335.71 ± 3.07^{Cb}
MW roasting-800 W	6789.30 ± 32.03^{Acd}	202.86 ± 48.76^{Cb}	1374.50 ± 105.87^{Cb}

Different uppercase letters (A, B, C) in the same line indicated a significant difference between the extraction solvents; Different lowercase letters (a, b, c) in the same column indicated a significant difference between the treatments

Table 2. The antioxidant activity of unroasted and roasted samples extracted with different solvents

Treatment	% inhibition		
	80% methanol (1% HCI)	Acidic water (1% HCI)	Water
Unroasted	49.18 ± 0.72^{Abc}	14.71 ± 1.75^{Ca}	18.93 ± 1.95^{Ba}
Traditional roasting	54.79 ± 0.15^{Aa}	14.25 ± 0.87^{Ba}	10.66 ± 0.93^{Cc}
MW roasting-300 W	46.81 ± 0.51^{Ac}	8.53 ± 0.61^{Bb}	9.88 ± 1.02^{Bc}
MW roasting-450 W	51.29 ± 1.18^{Aab}	7.45± 0.46^{Cb}	14.09 ± 0.30^{Bb}
MW roasting-600 W	48.92 ± 1.29^{Abc}	9.93 ± 0.26^{Bab}	10.25 ± 0.46^{Bc}
MW roasting-800 W	50.87 ± 2.11^{Aabc}	8.39± 1.08^{Cb}	18.82 ± 2.37^{Ba}

Different uppercase letters (A, B, C) in the same line indicated a significant difference between the extraction solvents; Different lowercase letters (a, b, c) in the same column indicated a significant difference between the treatments

The highest TPC in classical solvent extraction was determined with methanol in terms of solvents used. Similarly, methanol was found to be more effective than water for the extraction of phenolic compounds from date because the extraction of phenolic contents has been influenced by the polarity of extracting solvents [21] The antioxidant activity of unroasted and roasted samples extracted with different solvents was shown in Table 2.

Contrary to the TPC of the samples, the highest antioxidant activity was determined in the sample subjected to traditional roasting ($p < 0.05$). The antioxidant activity of the samples, treated with traditional roasting, was statistically higher than that of the samples, treated with microwave roasting. In the literature similar results reported that date seeds roasted with MW had higher antioxidant activity than those roasted with hot air [22] Similarly, it was determined that extraction with 50% methanol showed the highest total antioxidant activity compared to all other solvents [21]. Jdaini et al. (2023) [23]determined that binary extracts water-acetone and water-methanol revealed the highest antioxidant activity in date fruit. The sample, treated with MW roasting at 300 W showed the lowest antioxidant activity and this was significantly different from the results of the samples roasted with MW. The samples treated at 450 W, 600 W, and 800 W have antioxidant activity that are statistically similar to each other, but still different from the samples of unroasted and traditional roasting.

Phenolic compounds and their amounts determined by HPLC in roasted and unroasted samples were shown in Table 3. HPLC chromatogram of roasted and unroasted samples of date seed was shown in Figure 1.

Table 3. Phenolic compounds and their amounts in roasted and unroasted samples

	Gallic acid (mg/kg)	3,4-Dihydroxybenzoic acid (mg/kg)	Caffeic acid (mg/kg)	*p*-coumaric acid (mg/kg)	Rutin (mg/kg)
Unroasted	ND	ND	73.61 ± 1.04	18.14 ± 0.56^{a}	790.48±36.92^{a}
MW roasting-300 W	2936.91±136.31^{b}	690.42 ± 30.03^{b}	ND	15.57± 0.35^{b}	229.85 ±8.44^{b}
Traditional roasting	4907.45±108.34^{a}	1477.83 ± 63.60^{a}	ND	14.97± 1.04^{b}	97.34±1.50^{c}

ND: Not detected
Different letters (a, b, c) in the same column indicated a significant difference between the treatments

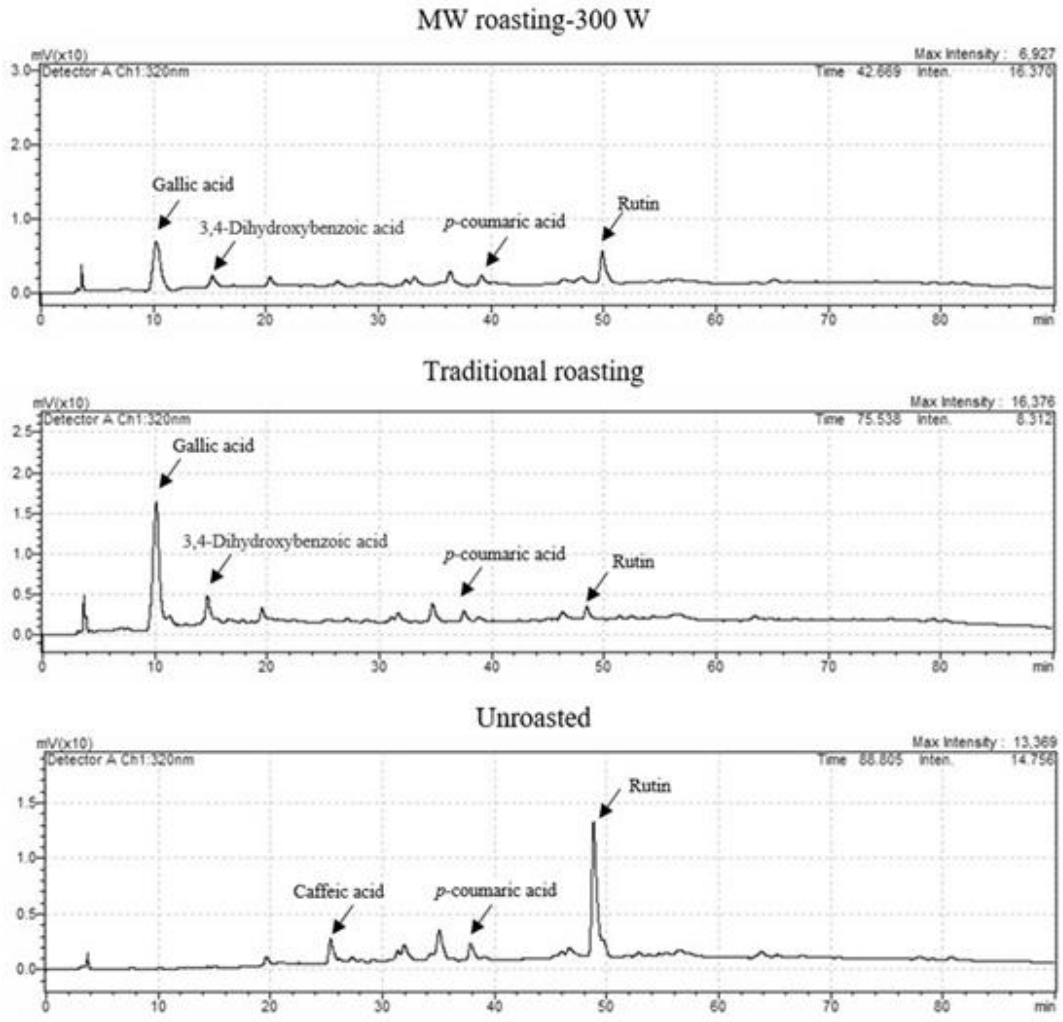

Figure 1. HPLC chromatogram of roasted and unroasted samples of date seed at 320 nm.

According to Table 3, the phenolic compounds detected in unroasted date seeds were caffeic acid, p-coumaric acid, and rutin, while gallic acid and 3,4-dihydroxybenzoic acid were found differently in roasted date seeds. Similarly determined that the dominant phenolic components of the roasting and unroasting date seed were (+)-catechin, 1,2-dihydroxybenzene, 3,4- dihydroxybenzoic acid, quercetin, syringic acid, and gallic acid [24] The effect of roasting on phenolic components in date seed showed significant changes ($p < 0.05$). The roasting process enables the release of phenolic compounds in the structure. Another research also reported roasting enables the release of polyphenols incorporated with various cellular components [22]. On the other hand, roasting caused a decrease in the amount of p-cumaric acid and rutin compounds. The phenolic compound determined in the highest amount in roasted samples was gallic acid. MW roasting was less effective than traditional roasting in terms of the release of bound phenolic compounds in the structure.

Principal component analysis (PCA) was used as a multivariate analysis and categorized the samples that were unroasted and roasted according to different methods based on the bioactive compounds (Figure 2).

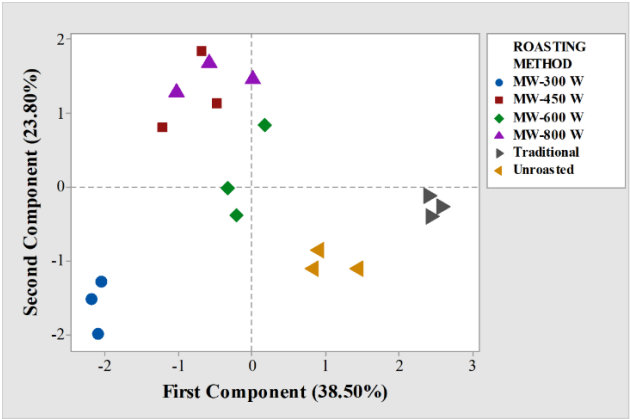

Figure 2. Score plot of two components for the effect of different roasting methods on the extraction of date seeds.

As shown in the PCA score plot, two principal components explained 62.20% of the total variance whereas the total variance was 38.50 % for PC1 and 23.80 % for PC2. The samples roasted by MW were mostly differentiated from the samples that were unroasted and roasted with the traditional method according to PC1. However, the samples

unroasted, roasted with MW at 300W, and traditional roasting were differentiated from those roasted with MW at 450, 600, and 800 W according to PC2. Similarly, PCA results revealed a positive relationship between antioxidant activity and phenolic compounds of date seed extracts roasted at 160 and 180 °C for 45 minutes [25].

4 Conclusions

According to our research, date seeds had high phenolic compounds and antioxidant activity. Among the solvents, 80% methanol (1% HCl) was selected as the best extraction solvent. Moreover, roasting had a significant effect on the extraction of phenolic compounds, the best results were obtained for TPC with MW roasting at 300 W and for antioxidant activity with traditional roasting. These results were in accordance with the PCA results. HPLC results showed that the roasting process caused the release of bound phenolic compounds in the structure like gallic acid and 3,4-dihydroxybenzoic acid. In further studies, the effects of new extraction techniques such as microwave and ultrasound on the extraction of phenolic compounds from roasted and unroasted date seeds can be investigated.

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Conflict of interest

The authors declare that there is no conflict of interest.

Similarity rate (iThenticate): 19%

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