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Development and Optimization of the Sample Preparation Method to Determine the Antioxidants in the Yemeni Almond

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Abstract

Almonds are rich in phenolic compounds with health benefits, but their matrix complexity makes measuring antioxidants challenging, requiring a sufficient extraction technique and high method sensitivity. This study aimed to develop, optimize, and validate an extraction method to assay the total antioxidant activity (TAA), total polyphenol content (TPC), and total flavonoid content (TFC) of different parts of almonds, including whole, kernel, and skin almond samples. Conventional extraction methods were compared with ultrasound-assisted extraction efficiency. This study was performed on 34 cultivated Yemeni samples and 12 samples of imported almonds. The results showed that the combined vortex/sonication extraction method with methanol: acetone: water at a ratio of 1:1:1v/v/v was the most efficient, yielding the highest quantity of TAA, TPC, and TFC contents from the almond samples. The extraction method had high linearity and good sensitivity with a limit of detection (LOD) values of 0.019, 0.024, and 0.099 ppm for ascorbic acid, gallic acid, and quercetin respectively. The Yemeni almond had a higher antioxidant content than the imported types, with TAA, TPC, and TFC values ranging from 2.54±0.01–8.20±0.01 mg AAE/g (Ascorbic Acid Equivalent per g of almond sample), 0.75±0.01-1.91±0.03 mg GAE/g (Gallic Acid Equivalent per g of almond sample), 0.03±0.002-0.24±0.020 mg QE/g (Quercetin Equivalent per g of almond sample) respectively.

Article Info

Keywords:
Yemeni Almond, Antioxidants, Polyphenols, Flavonoids, Method Optimization.

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1. INTRODUCTION

Nutraceuticals are foods or food components that have therapeutic qualities and can be valuable for preventing diseases and promoting health. Due to their safety, nutritional value, and potential medicinal applications, these food ingredients have garnered considerable attention in recent years [1]. Owing to their therapeutic qualities, nuts are a popular choice for people seeking a balanced, nutritious diet [2]. (Prunus dulcis Mill. D.A. Webb) is a temperate nut species belonging to the genus Prunus Rosacea family [3]. Almond fruit is the most produced nut worldwide because of its exceptional nutritional value (low sugar content, high levels of proteins, unsaturated fatty acids, vitamins, and minerals) as well as health-enhancing phytochemicals [4]. The United States is the world's greatest producer of almonds, with California being the primary source of cultivation [5]. In addition, some of the Yemeni Mountains cultivate almonds, which acharacterized by their small size and delicious taste. Yemeni Almond cultivation is concentrated in Sana'a Province in three regions: Khawlan, Bani Matar, and AL-Himah, as shown in Fig.1 [6].

Almond consumption is linked to several beneficial health effects, including the control of inflammatory and immunological responses, as well as anti-cancer and anti-atherogenic properties [7]. The high polyphenol content of almond skin and other bioactive molecules

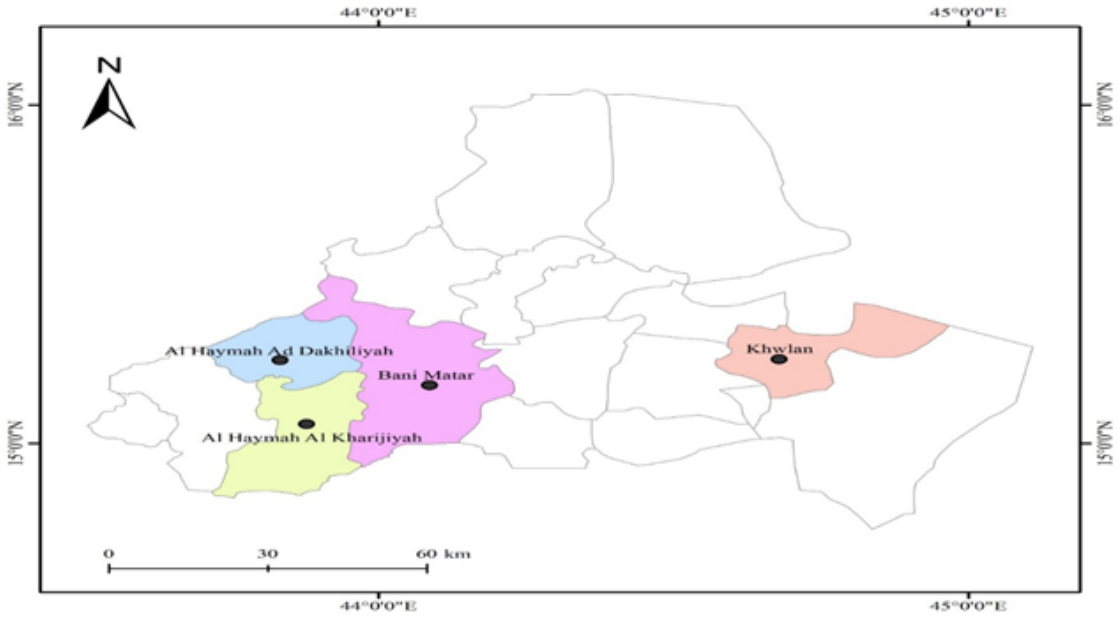

Figure 1. Map Shows the Sites of Yemeni Almonds Cultivation Regions.

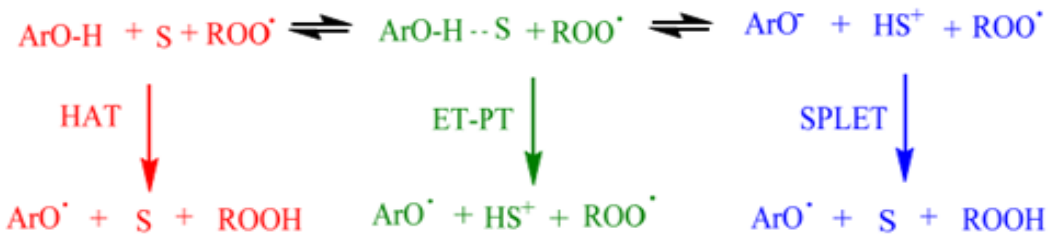

Figure 2. : Proposed mechanisms between antioxidants (ArOH) and peroxyl radicals (ROO•) comprising the hydrogen atom transfer (HAT), electron transfer–proton transfer (ET-PT), and single-proton-loss electron-transfer (SPLET), S = Solvent Adapted from reference [14].

with well-known antioxidant properties support the use of these products as sources of natural antioxidants [8]. Numerous factors, including cultivar genetics, harvest season, place of origin, climate, light, rainfall patterns, soil composition, maturity level, cultivation, processing, and storage techniques, all have a significant impact on the phytochemical content of nuts [9]. Antioxidants limit the start or spread of oxidative chain reactions, thereby preventing or delaying the oxidation of lipids or other molecules [10]. It can combat free radicals and shield the body from various degenerative illnesses [11]. The reaction of antioxidants, ArOH, with peroxyl, ROO, and radicals might occur through different mechanisms depending on the structure of the antioxidant and the solvent: one-step hydrogen atom transfer (HAT), electron transfer-proton transfer (ET-PT), proton-coupled electron transfer (PCET), or sequential proton-loss electron transfer (SPLET), as shown in Fig.2. HAT and ET-PT often occur simultaneously, but the solvent polarity, ability to form hydrogen bonds, and properties of the reactants are factors that contribute to the precise mechanism [12, 13]. Solubility plays a major role in phenolic compounds extraction. Natural phenolic compounds typically contain a large number of hydrogen acceptor sites (HAS) and hydrogen donor sites (HDS). This implies that they can form hydrogen bonds and electrostatic attractions with solvent molecules that exhibit these properties as intermolecular interactions. Various methodologies exist for approaching the theoretical understanding of dissolution mechanisms. A measure of the molecular affinity between solvents and the targeted phenolic compounds is provided by the calculated molecular parameters, such as the distribution coefficient. For example, most phenolic and polyphenolic compounds found in walnuts have low or negative log D values for their distribution coefficients. This suggests that the compounds may distribute better in water, a highly polar solvent with a higher dielectric constant, than in octanol, a nonpolar molecule with a low dielectric constant. The presence of the hydroxyl group in both water and phenolic compounds enhanced mutual solubility. In general, the water solubility of phenolic compounds increases with the number of hydroxyl groups. As a result, most phenolic glycosides are more water-soluble than the corresponding

aglycone [15]. Many techniques have been published for the extraction of antioxidants from nut samples, including maceration [16, 17], soxhlet [18, 19], and stirring [20, 21]. Despite the efficiency of these methods for the extraction of antioxidants, they have several drawbacks, including the consumption of large amounts of solvents, which increases the cost. Elevate the exposure of the analyst to toxic solvents and maximize the impact on the environment, in addition to the long extraction time, which decreases the method throughput [22]. The various methods for evaluating total antioxidant activity fall into three distinct categories: spectrometry [23]. Electrochemical [24, 25]. and chromatography assays [26]. Many studies have described spectrophotometric methods for determining the reducing capacity of antioxidants using Fe(III)-bipyridyl reducing capacity (FBRC) reagent [27–29]. The method involves the reduction of Fe (III)-bipyridyl reagent to a stable orange-red color. Fe (II)-bipyridyl chelate by antioxidants in a buffered medium. This stable orange-red Fe (II)-bipyridyl complex can be measured spectrophotometrically; therefore, this study aimed to develop, optimize, and validate a new simple, fast, efficient, and environmentally friendly sample preparation method for the extraction of total antioxidants and to verify and validate analytical methods for the quantification of total antioxidant activity (TAA), total polyphenol content (TPC), and total flavonoid content (TFC) of almond samples cultivated in Yemen and compared with those of some imported types.

2. MATERIALS AND METHODS

2.1. Chemicals and solvents

All reagents used were pure analytical grade, ascorbic acid 99.7%, from BDH, Gallic acid 99.8% from ACS, Quercetin 99.9% from Pspark Scientific, Methanol 99%, acetone 99.5%, ethanol 96%, acetonitrile 99.97%, and ethyl acetate 99.5% from Scharlau (Spain), and purified using a Direct-Q3 water purification system (Millipore, Bedford, MA, USA).

2.2. Almond Sample Collection and Homogenization

Different types of Yemeni almond samples were collected from three regions in Sana'a province: Khawlan, Bani-Mater, and Al-Haimah, whereas imported samples were purchased from local markets in Sana'a City. All the samples were transferred to the laboratory. Subsequently, the sample was ground using an electrical grinder, homogenized using a mortar and pastel, and then kept in a cold dry place at room temperature until use.

2.3. Selection of the Solvent for the Recovery of TAA from Almonds

Experimental studies were performed to determine the optimal extraction solvent for the recovery of total antioxidants from the almonds. Therefore, the initial step in the preparation of extracts was to select an appropriate extraction solvent for the total antioxidant compounds present in the almonds. Nineteen different solvent systems have been selected based on their wide use in the extraction of phenolic and flavonoid compounds from different types of nuts or fruits [30, 31]. Extraction of antioxidant compounds was carried out by the proposed magnetic stirrer method with some modifications; 1 g of almond sample was mixed with 20 ml of different tested solvents for one hour at room temperature. Subsequently, the extracts were centrifuged at 3500 rpm for 10 min and the supernatant was collected and used for the TAA assay [32].

2.4. Optimization of the Extraction of Antioxidants

2.4.1. Optimization of the Soxhlet, Maceration, and Vortex Methods

In this study, several extraction methods were examined to select the most suitable and efficient extraction method. A 5 g homogenized paste of almond sample was extracted with 100 mL ethanol 96% v/v using the soxhlet method for 2 and 6 h [19]. In addition, according to a previously reported method [33], with some modifications, 1 g of homogenized paste of almond sample was extracted with 20 mL methanol: acetone: water in a ratio of 1:1:1v/v/v using the maceration method for different times (1, 12, and 24 h). Similarly, 1 g/20 mL solvent (methanol: acetone: water at a ratio of 1:1:1 v/v/v) was extracted using a vortex (Velp Scientific, 60 Hz, W 45, made in Europe) for (10-40 min), at room temperature. The mixture was then centrifuged at 3500 rpm for 10 min. Subsequently, the supernatants were collected separately for each extraction method and used for TAA-assayed [34].

2.4.2. Optimization of the Magnetic Stirring Method

The parameters affecting the extraction efficiency of total antioxidants from almonds were optimized using a magnetic stirrer extraction method (Gallenhamp hotplate 400, volts 220/40, 50 Hz, made in England) [32]. Extraction was performed in triplicate by weighing 1 g of homogenized paste of almond sample in 20 mL (methanol: acetone: water ratio 1:1:1v/v/v). Different extraction parameters such as stirring speed (50-300 rpm, at 25OC), stirring time (5-180 min, at 25 oC, 200 rpm), different sample weights (0.1-2g/20 mL solvent for 60 min, at 200 rpm and 25 oC), different temperatures (25 and 50 oC), and different ionic strength (0.117-1.638 M sodium

chloride) were used. After magnetic stirring, the mixture was centrifuged at 3500 rpm for 10 min. Subsequently, the supernatants were collected and used for the TAA assays.

2.4.3. Optimization of the Sonication Method

The parameters affecting the extraction efficiency of total antioxidants from almonds were optimized using the sonication extraction method (Bransonic Ultrasonic Cleaner, Model B-5200 E4, 450 W, 50-60 Hz) [35]. With some modifications, 0.05 g of almond sample was mixed with 1 mL (methanol: acetone: water in ratios 1:1:1v/v/v), different extraction parameters such as the effect of different sonication times (5-60 min, at 40 OC), different sample weight/solvent ratios (0.05/1 mL, 0.25/5 mL, 0.5/10 mL, and 1/20 mL), and different ionic strengths using sodium chloride concentrations (0.17-3.42 M) were studied. After sonication processes were performed, the supernatant obtained after centrifugation at 3500 rpm for 10 min was used for the TAA assay.

2.4.4. Combination of Vortex/Sonication Extraction Method

An integration of different extraction methods might be an adequate approach towards extracting such total antioxidant content at a higher extraction efficiency, so in an Eppendorf, 0.05 g of homogenized paste of almond sample was added to 1 mL (methanol: acetone: water 1:1:1 v/v/v), contained 0.85M sodium chloride salt. The mixture was vortexed for 1 min and extracted in an ultrasonic bath for 20 min. The supernatant obtained after centrifugation at 3500 rpm for 10 min was used for the TAA assay.

2.5. Determination of Antioxidants in Almond Samples

2.5.1. Determination of Total Antioxidant Activity TAA

The Total antioxidant activity assay was performed according to the methods described by Othman et al., Evelyn et al., and Sacchi et al., [27–29] with some modifications using ferric (III), 2.2-Bipyridyl Reducing Capacity (FBRC). 100 muL of almond extract (0.05g/mL) was added to a 10 mL measuring flask. Then, 2mL of acetate buffer solution (pH 4), 1.5 mL $FeCl_3$ (0.01M), and 1.5mL 2.2-dipyridyl solution (1000ppm) was added, and the solution was made up to 10 mL with deionized water, mixed well, and placed in a water bath at 50°C for 30 min. After cooling, the absorbance was measured at 520 nm using a reagent as a blank. The concentration of TAA in the samples was correlated with the calibration curve of ascorbic acid. The results were expressed as mg (AAE/g).

2.5.2. Determination of Total Polyphenol Content TPC

TPC was determined using the method published by Singleton et al., with some modifications [36]. By Diluting 100 μL of almond extract (0.05g/mL) in a 10 mL measuring flask, 3.6mL of deionized water and 0.4mL of Folin–Ciocalteau reagent were added, the mixture was shaken and left for 5 min, 4mL of 7% sodium carbonate solution was added. The solution was made up to 10 mL with deionized water and incubated in a water bath at 50oC for 20 min. TPC was measured at λmax 750 nm against a reagent blank. The TPC concentration was correlated with the calibration curve of gallic acid. The results were expressed as mg (GAE/g).

2.5.3. Determination of Total Flavonoid Content TFC

The TFC was determined using the method described by Madjitoloum et al. [18]. By diluting 3mL of almond extract (0.5g/10 mL) in a 10 mL measuring flask with 2 mL of 2% w/v of aluminum chloride. The volume was made up to 10mL with a solvent (ethanol: water) (2:1). After 10 min, absorbance was measured at 415 nm against a reagent blank. The analysis was performed in triplicate, and the results were expressed as mg (QE/g).

2.6. Methods Validation

The applicability of the methods for the quantitative determination of TAA, TPC, and TFC in almond samples were demonstrated by performing analytical validation by the analysis series of spiked almond samples with ascorbic acid, gallic acid, and quercetin standard solutions in triplicate, and the absorbance were used to plot a standard calibration curves. The Linearity regression equation, correlation coefficient (R2), precision as relative standard deviation (%RSD), Limit of Detection (LOD), Limit of Quantification (LOQ), and relative recovery (R%) were calculated from the calibration curve according to the AOAC and perivous published papers [37–42].

3. RESULTS AND DISCUSSION

3.1. Solvents/ Solvent Mixture Screening

The results obtained when quantifying total antioxidant activity in terms of the solvents used are presented in Fig. 3. The observed data revealed that methanol: acetone: water in ratios 1:1:1v/v/v was the most suitable solvent to extract the highest quantity of total antioxidant with a value of 2.14±0.01 mg AAE/g, followed by methanol, and (methanol: ethanol: water) mixture solvent 1:1:1v/v/v ratios) with 1.81±0.01 and 1.78 ±0.01 mg AAE/g, respectively. Also, water and (methanol: acetonitrile: water 1:1:1v/v/v ratios) and ethanol gave moderate values of TAA with 1.56± 0.01, 1.54±0.01 and 1.53±0.02

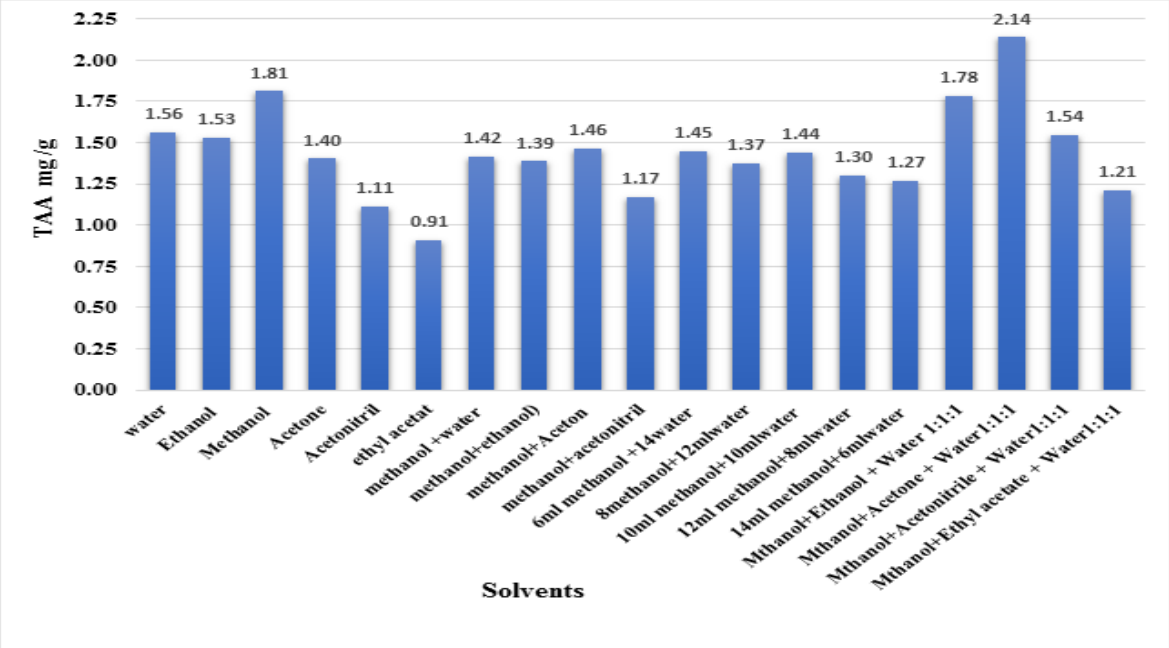

Figure 3. Capacity of Different Extraction Solvents on the Extraction of TAA from Almond Sample.

mg AAE/g respectively. In contrast, the ethyl acetate extract showed the lowest amount of TAA with only 0.91± 0.01 mg AAE/g.

3.2. Maceration, Soxhlet, and Vortex Extraction Methods at Optimal Conditions

From the obtained data for the maceration extraction method, the obtained results have shown that the extraction time for 24 hours gives more extraction efficiency than one and 12 hours with1.74±0.02 mg AAE/g, while of soxhlet extraction method, showed that, the higher value of TAA was achieved after 6 hour with value 1.01 ±0.01 mg AAE/g. In addition, the results showed that the optimal time for vortex extraction was 30 min. with a value of 1.66±0.002 mg AAE/g, on the other hand; the results showed that the total antioxidant content was increased with increasing the sample weight of almond sample.

3.3. Optimization of the Magnetic Stirring Method

The obtained results showed that the extraction at a speed of 200 rpm gives more extraction efficiency of total antioxidants with a value of 1.71±0.01 mg AAE/g. In addition, the optimum stirring time for TAA extraction from the almond sample was 60 min, with a value of 2.21±0.01 mg AAE/g. and the analytical signals remained constant at longer periods, that is, above 60 min. Also, the total antioxidant content was increased by increasing the sample weights, while the temperature did not affect the extraction efficiency of the total antioxidant over the temperature studied. Also, the extraction efficiency was increased with increasing Sodium chloride concentration up to 0.68M, with a value of 2.54 ±0.01 mg AAE/g, and remained constant at higher concentrations of sodium chloride.

3.4. Optimization of the Sonication Extraction Method

The results showed that the extraction efficiency increased with time up to 20 min with a value of 2.17±0.03 mg AAE/g, after that, the efficiency was constant over the period. Also, the results showed that the extraction efficiency increases with increasing Sodium chloride concentration up to 0.85M, with a value of 3.02±0.01 mg AAE/g; while at higher concentrations of sodium chloride, the extraction efficiency remained constant. The addition of salt may improve the partition of the analyte to the organic phase or diminish the solubility of the organic solvent in the water phase due to the salting-out effect.

3.5. Combined Vortex/Sonication Extraction Method Efficiency

The obtained results in Table 1 showed that the total antioxidant extracted by the combined extraction method gave the highest total antioxidant value with 3.52±0.02 mg AAE/g followed by the sonication method with 3.02 ± 0.01 mg AAE/g, while the lowest value was 1.01 ±0.01 mg AAE/g by Soxhlet extraction method. Therefore, we used the combined extraction method in subsequent experiments.

Table 1: Comparison Results of Optimized Extraction Techniques in the Extraction of TAA from Almond Sample.

No	Extraction Method	Average TAA mg AAE/g (n=3)
1.	Soxhlet	1.01 ±0.01
2.	Vortex	1.66±0.002
3.	Maceration	1.74 ±0.02
4.	Magnetic Stirrer	2.54 ±0.01
5.	Sonication	3.02 ± 0.01
6.	Combination (Vortex /Sonication)	3.52 ± 0.02

Table 2: Results of validation parameters for TAA, TPC, and TFC.

TAA			TPC			TFC		
Ascorbic Acid (ppm)	%R	%RSD	Gallic Acid (ppm)	%R	%RSD	Qurectein Conc. (ppm)	%R	%RSD
0.63	98.31	0.913	0.63	100.89	1.18	2.5	92.21	1.41
1.25	98.20	2.791	1.25	100.30	4.84	5	100.32	1.48
1.88	97.76	2.037	1.88	100.23	1.60	10	98.81	0.84
2.50	96.12	1.167	2.50	98.62	2.28	15	98.34	1.33
3.13	94.90	1.092	3.13	98.44	1.00	20	98.48	1.90
3.75	91.25	1.054	3.75	95.02	1.22	-	-	-
6.25	90.45	1.088	6.25	92.46	1.284	-	-	-
Other validation parameters for TAA, TPC, and TFC								
Linear Equation	y = 0.1585x +0.0230		y= 0.1229x + 0.0198			y= 0.0609x - 0.0027		
R^2	0.9991		0.9989			0.9998		
LOD (ppm)	0.019		0.024			0.099		
LOQ (ppm)	0.063		0.081			0.328		

3.6. Validation of Analytical Methods

Table 2 shows the results obtained for the different validation parameters studied using the spectrophotometric methods. The values of the correlation coefficients (R2) obtained in Fig.4 for TAA and TPC, and Fig. 5 for TFC were higher than 0.9987. The limits of detection and quantification obtained for the TAA, TPC, and TFC methods varied between 0.019 and 0.099 ppm for the LOD and between 0.063 and 0.328 ppm for the LOQ.

In the precision study, the % RSD values for the TAA, TPC, and TFC methods ranged between (0.84% and 4.84%), and the low % RSD values were indicated by the high repeatability of the TAA, TPC, and TFC measurements. The recovery of TAA, TPC, and TFC was performed using samples spiked with different amounts of ascorbic acid, gallic acid, and quercetin standard solutions. Our results, expressed as the relative recovery %R, ranged from (90.45% to 98.51%) for TAA, (92.46% to 100.89%) for TPC, and (92.21% to 100.32%) for TFC, as shown in Table 2.

3.7. Results of the Analysis of Almond Samples

3.7.1. Results of the Analysis of Whole Almond Samples

The optimized and valid method was applied to analyze the total antioxidant activity, total polyphenol content, and total flavonoid content of whole, skin, and kernel almond samples. The details of the results are summarized in Tables 4-7. Table 4 shows that, the analysis results of total antioxidant activity in whole almond samples in triplicate. It is clear to see that, in 12 out of 46 samples were commercial variety imported almond samples, the TAA ranged from 1.59±0.01-2.92±0.01mg AAE/g, TPC ranged from 0.30±0.01-0.77±0.01 mg GAE/g, and the TFC ranged from 0.04±0.002 - 0.13±0.012 mg QE/g, whereas the Yemeni almond samples were 34 out of 46, TAA was ranged from 2.54± 0.01–8.20±0.01 mg AAE/g, the TPC ranged from 0.75±0.01-1.91±0.03 mg GAE/g, and TFC ranged from 0.03±0.002-0.24±0.020 mg QE/g. The researchers found that the methanol extract of almond fruit cultivated in Turkey's Sırnak region contains total phenolic content, equal to 0.73±0.13 mg GAE/g, and its total flavonoid content was 0.01±0.00 mg QE/g [43], which is lower than that grown in Yemen. The

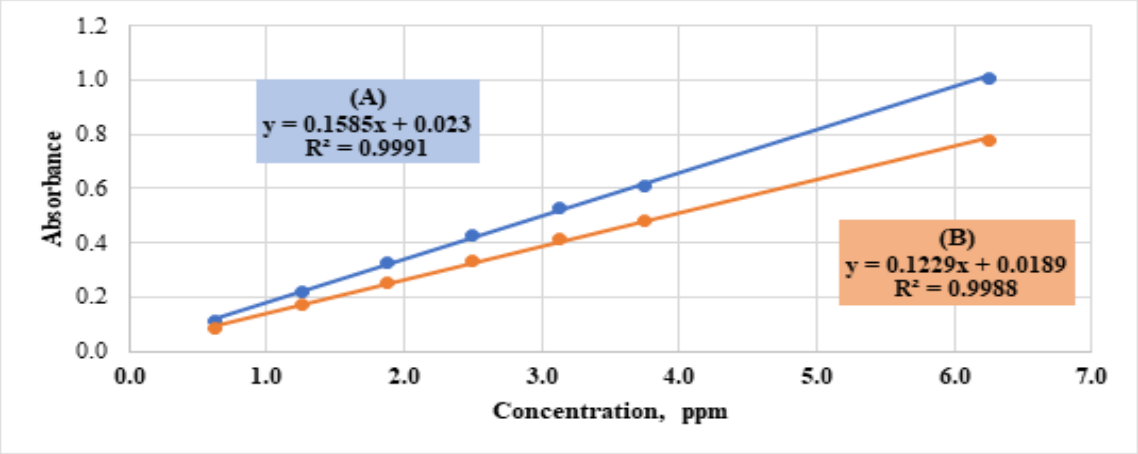

Figure 4. Calibration Curve of Ascorbic acid (A) and Gallic acid (B) Standards in Spiked Almond Sample.

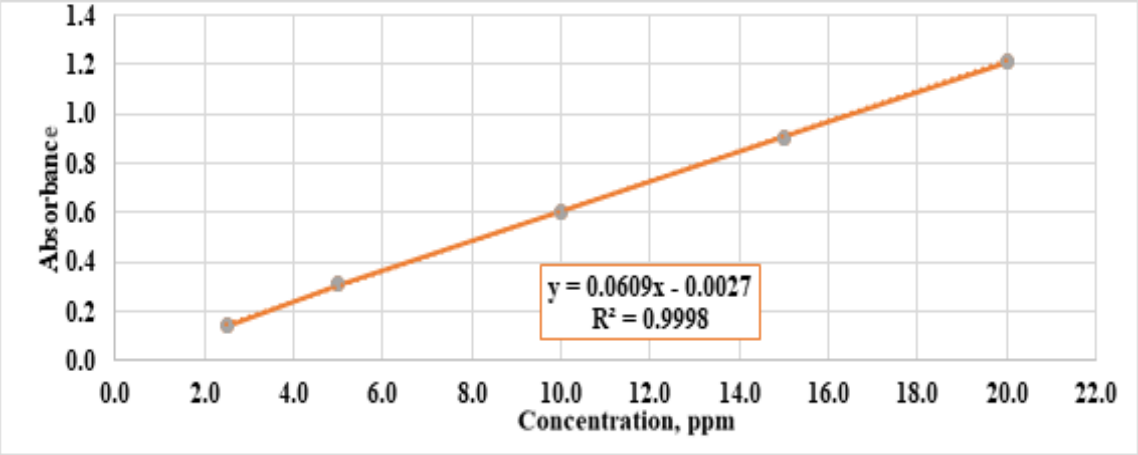

Figure 5. Calibration Curve of Quercetin Standard in Spiked Almond Sample.

data clearly show that the Yemeni almond has a higher content of antioxidants rather than that imported; so which means a higher nutrient value for Yemeni almonds. The higher value of antioxidant content may depend on the cultivar and harvest year.

3.7.2. The Results of the Analysis of Skin Almond Samples

Table 5 shows that, the results of the analysis of TAA in skin almond samples in triplicate. It is clear to see that, in 6 out of 11 samples, were commercial variety imported almond samples, the TAA ranged from 15.51±0.02-48.28±0.01mg AAE/g, TPC ranged from 10.08±0.02 – 15.06±0.01mg GAE/g, and TFC ranged from 4.22±0.01-7.98±0.01mg QE/g, while the Yemeni almond samples analysis show that, it contains higher values of total antioxidant in almond skin than that, imported. The TAA ranged from 64.82±0.04 – 85.16±0.02 mg AAE/g, the TPC ranged from 25.80±0.03- 32.93±0.01mg GAE/g, likewise, the TFC ranged from 5.57±0.01 - 8.85±0.01 mg QE/g.

3.7.3. Results of the Analysis of Kernal Almond Sample

To compare the content of antioxidants between the kernel almonds cultivated in Yemen and those that imported only one sample of each was analyzed. The results in Table 6 show that, the TAA, TPC, and TFC of the Afghani1kernel almond were 0.26±0.01 mg AAE/g, 0.08±0.01 mg GAE/g, and 0.018±0.01 mg QE/g respectively. Whereas the TAA, TPC, and TFC in the Khawlan1 Yemeni kernel almonds were 0.77±0.02 mg AAE/g, 0.23±0.01 mg GAE/g, and 0.02±0.01 mg QE/g respectively.

3.7.4. Effected of Prolonged Storage on TAA, TPC, and TFC Contents

To compare the effect of prolonged storage time on almond TAA, TPC, and TFC Contents, an Alhimah 2 almond sample was used and the obtained results in Table 7 were shown that the TAA, TPC, and TFC for the AL-Haimah 2 whole almond sample decreased with prolonged storage time compared with those shelling and analyzed directly.

Table 4: Results of Whole Almond Samples Analysis.

No	Sample code	Average n =3			No	Sample code	Average n =3		
		TAA mg AAE/g	TPC mg GAE/g	TFC mg QE/g			TAA mg AAE/g	TPC mg GAE/g	TFC mg QE/g
1	USA 1	1.59±0.01	0.52±0.02	0.09±0.01	24	Khawlan 3	4.61±0.01	1.43±0.01	0.08±0.02
2	USA 4	1.67±0.01	0.51±0.01	0.07±0.01	25	AL-Himah 9	4.61±0.04	1.91±0.03	0.08±0.01
3	USA 3	1.70±0.01	0.53±0.01	0.11±0.01	26	Bani-Matar1	4.80±0.04	1.84±0.01	0.11±0.01
4	Cheli 2	1.78±0.01	0.56±0.01	0.06±0.01	27	AL-Himah 4	4.85±0.01	1.33±0.01	0.07±0.01
5	Syrian 1	2.00±0.02	0.77±0.01	0.04±0.01	28	Khawlan 14	4.88±0.01	1.31±0.02	0.21±0.02
6	USA 2	2.10±0.01	0.31±0.01	0.11±0.01	29	Bani-Matar4	4.88±0.01	1.26±0.01	0.09±0.01
7	Pakistani 1	2.23±0.02	0.69±0.01	0.12±0.04	30	Khawlan 16	4.90±0.01	1.55±0.01	0.10±0.01
8	Afghani 1	2.29±0.01	0.32±0.01	0.10±0.01	31	Khawlan 5	5.12±0.02	1.43±0.01	0.06±0.01
9	Cheli 1	2.32±0.01	0.30±0.01	0.13±0.01	32	Khawlan 18	5.51±0.01	1.75±0.01	0.07±0.01
10	Afghani 2	2.34±0.01	0.58±0.01	0.09±0.01	33	Khawlan 7	5.62±0.02	0.75±0.01	0.16±0.01
11	Afghani 3	2.45±0.02	0.76±0.01	0.10±0.01	34	Bani-Matar5	5.65±0.01	1.75±0.01	0.14±0.02
12	Khawlan 11	2.54±0.01	0.81±0.02	0.14±0.02	35	AL-Himah 6	5.70±0.03	1.70±0.02	0.24±0.02
13	Irani 1	2.92±0.01	0.40±0.01	0.09±0.01	36	Khawlan 12	5.91±0.04	1.61±0.02	0.13±0.02
14	Khawlan 19	3.21±0.02	0.89±0.01	0.03±0.01	37	Khawlan 2	6.06±0.02	1.74±0.01	0.09±0.01
15	Khawlan 15	3.48±0.01	1.02±0.02	0.13±0.01	38	Khawlan 10	6.17±0.02	1.53±0.03	0.07±0.01
46	Khawlan 6	3.49±0.03	1.20±0.01	0.09±0.01	39	Khawlan 9	6.22±0.03	1.63±0.01	0.09±0.01
17	Khawlan 20	3.60±0.01	1.07±0.02	0.03±0.01	40	Khawlan 1	6.31±0.02	1.53±0.01	0.09±0.01
18	Khawlan 13	3.78±0.03	0.97±0.02	0.15±0.01	41	AL-Himah 3	6.39±0.01	1.73±0.02	0.10±0.01
19	Bani-Matar2	3.83±0.02	1.15±0.02	0.09±0.01	42	AL-Himah 2	8.20± 0.01	1.89± 0.02	0.14±0.02
20	AL-Himah 7	4.03±0.01	1.17±0.02	0.06±0.01	43	Khawlan 17	6.62±0.02	1.75±0.01	0.07±0.02
21	Khawlan 4	4.04±0.02	1.21±0.01	0.07±0.01	44	AL-Himah 1	6.64±0.01	1.53±0.01	0.10±0.01
22	Khawlan 8	4.14±0.02	1.27±0.02	0.09±0.01	45	AL-Himah8	6.84±0.02	1.84±0.02	0.13±0.02
23	AL-Himah 5	4.34±0.02	1.18±0.01	0.07±0.01	46	Bani-Matar 3	6.85±0.02	1.50±0.01	0.09±0.01

Table 5: Results of the Analysis of Skin Almond Samples.

No	Sample code	TAA as mg AAE/g	TPC as mg GAE/g	TFC as mg QE/g
1	USA 2	25.58±0.01	10.08±0.02	6.05±0.01
2	Afghani 1	48.28±0.01	15.06±0.01	4.22±0.01
3	Pakistani	27.91±0.03	10.25 ±0.01	5.44±0.01
4	Irani	39.40±0.09	11.20±0.01	6.53±0.04
5	Syrian	15.51±0.02	11.93±0.02	7.98±0.01
6	Cheli 2	27.81±0.03	10.39±0.03	6.24±0.01
7	AL-Himah 3	69.63±0.02	28.1±0.01	7.84±0.01
8	Bani-Matar 5	85.16±0.01	32.93±0.01	7.15±0.01
9	Khawlan 12	76.88±0.02	32.36±0.01	5.57±0.01
10	Khawlan 18	64.82±0.04	24.25±0.01	6.95±0.01
11	khawlan 1	78.04±0.01	25.80±0.03	8.85±0.01

Table 6: Results of Kernel Almond Samples Analysis.

No	Sample code	TAA mg AAE/g	TPC mg GAE/g	TFC mg QE/g
1	khawlan 1	0.77±0.02	0.23±0.01	0.022±0.01
2	Afghani 1	0.26±0.01	0.08±0.01	0.018±0.01

Table 7: Results of the Whole Almond Prolonged Storage Affected TAA, TPC, and TFC Contents.

Items	Almond post-harvest days (Shell removing)	Values
TAA mg AAE/g	0	8.20± 0.011
	7	7.89±0.01
	60	6.34±0.01
	0	1.89± 0.015
TPC mg GAE/g	7	1.45±0.01
	60	1.15±0.010
	0	0.14±0.015
TFC mg QE/g	7	0.12±0.01
	60	0.11± 0.015

4. CONCLUSION

In this study, an extraction method was optimized to analyze the total antioxidant activity of almonds, including polyphenols and flavonoids. Control of all the parameters affecting the extraction process of total antioxidants in almonds was studied; As a result, sonication combined with the vortex extraction method is a high-potential technique to recover total antioxidants compounds from almonds including (whole almond, skin, and kernel), obtaining valuable information about the extraction process. The present study reflects the importance of controlling the extraction temperature, solvent concentration, and extraction time to obtain an extract rich in TAA, TPC, and TFC with good antioxidant activity. The developed extraction method has high linearity and good sensitivity with lower LOD values. The results of this study showed that Yemeni almonds had higher TAA, TPC, and TFC values than other commercially imported almonds available in the Sana'a market.

AUTHOR CONTRIBUTIONS

A. A Performed and designed the experiment; A, A, methodology, F. A. M and D. A.; analyzed the data A. A and M.A.; writing-original draft preparation, F. A. M and D. A; writing-review and editing A. A and M. M. A.; supervision, A. A. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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