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Research Article

Ultrasound-Assisted Extraction of Micro- and Macroelements in Fruit Peel Powder Mineral Supplement for Osteoporosis Patients and Their Determination by Flame Atomic Absorption Spectrometry

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Osteoporosis is a worldwide disease depicted by the reduced bone mass, an adequate supply of minerals is needed to support bone remodeling, and their deficiency causes bone-related diseases, osteoporosis in particular, and has osteoprotective effects. The aim of this recent research was to quantify the micro- (Mn, Fe, Cu, and Zn) and macroelements (Mg, K, and Ca) in the peel powder of some common fruits (pomegranate, orange, lemon, mango, and grapefruit) by flame atomic absorption spectrometer (FAAS). The extraction of micro- and macroelements in peel powder was done by using dilute acids in an ultrasonic bath. Apple leaves were used as standard reference material (SRM, NIST 1515) to optimize the ultrasound-assisted extraction (UAE) method at varied operating parameters. Maximum response was obtained for extracting of minerals in 500 mg SRM at 60°C temperature, setting a vortexing time of 5 min while using 5.0 mL extracting agent HNO_3 (0.5 M)-H_2O_2 (10%) at 90% sonication amplitude of ultrasound bath for 6 min. While analyzing the SRM, the percentage recovery was obtained in a range between 96.8 and 102.7% to assure the accuracy whereas repeatability ($n = 10$) study in terms of % RSD yielding ≤2.29 supports well the precision of the proposed method, and limits of quantitation (μg/g) were 0.034, 0.061, 0.065, 0.057, 0.017, 0.175, and 0.053 for Mn, Fe, Cu, Zn, Mg, K, and Ca, respectively. The proposed UAE method was reliable, efficient, and advantageous over the conventionally employed acid digestion method with regard to less consumption of reagents and short analysis time for the determination of micro- and macroelements in fruit peel powder.

1. Introduction

Osteoporosis is a worldwide disease depicted by the reduced bone mass followed by the microarchitectural decay of bone tissues which results in the elevated risk of bone fracture. Annually more than 200 million cases of osteoporosis are reported as active cases worldwide, with approximately 2 million cases are of hip fractures [1, 2]. Younger women with estrogen deficiency while older ones who undergone menopause are primarily caught by osteoporosis whereas secondary osteoporosis occurs in younger individuals (both male and female) by chronic use of steroids like

corticosteroids [3]. Its prevalence in Pakistan is also high; according to an estimation, 5.6–17.8% of premenopausal women and 20–49.3% of postmenopausal women become a victim of it [4]. To prevent osteoporosis, maintenance, and development of bone mass, nutrition particularly mineralization plays an important role. Osteoclasts are specialized, multinucleated cells that are involved in bone development and regeneration and also ensure bone mineralization. So an adequate supply of minerals (macroelements: Mg, K, and Ca and microelements: Mn, Fe, Cu, and Zn) and vitamins are needed to support bone remodeling [2, 5]. The importance of these minerals other than Ca for good bone health can hardly be overemphasized. Several studies have been conducted to link these minerals with bone health and their deficiency causes bone-related diseases, osteoporosis in particular, and has osteoprotective effects [5–7].

Most Ca (about 99%) found in the body is associated with bones that plays a major role in bone strength and health throughout life, and bone loss associated with aging is managed by adequate Ca intake. Marginal Ca intake or its deficiency is an important factor underlying osteoporosis and increased bone fracture risk [3, 8]. Hydroxyapatite production is supported by Mg [9], about 60% of its total in the body is stored in the skeleton, and this constitutes about 1% of the total bone mineral content. Mg deficiency via hypocalcemia elevates osteoclast activity while the osteoblastic number and activity decline, so it has a major role in Ca metabolism [10, 11]. Ca hemostasis influenced by potassium particularly excretion and conservation of urinary Ca and bone density is closely related to K intake and its higher intake is associated with less bone loss [12]. Mn is a constituent of some enzymes and is distributed in tissues throughout including bone. It plays an important role in bone formation, the thickness of the trabecular bone area, and its mineralization. Utilization of Mn in combination with Cu, Zn, and Ca is more effective in preventing spinal bone loss than alone using Ca [2, 13]. Fe has a bone protective effect; it plays an enzymatic cofactor to stimulate the synthesis of bone matrix and mineralization via activation of lysyl hydroxylase and 25-hydroxycholecalciferol, respectively [14, 15]. Skeletal abnormalities associated with Cu deficiency in osteoporosis malabsorption of Cu have also its role. Cu removes bone-free radicals that cause osteoclast activation and also inhibit osteoclastic bone resorption directly [2, 16]. A large portion of the total body burden of Zn exists in skeletal, and Zn-related proteins regulate cellular function in osteoblasts and osteoclasts. Zn increases osteoblastic activity and inhibits osteoclastic bone resorption [17, 18].

Fruits are consumed across the world as a rich source of nutrients like minerals, vitamins, fiber, and other bioactive compounds that are required for a healthy human life. As a result, increased consumption of the fruits leads to the wastage of the fruits especially their peels and seed. But the recent studies show that peels and seeds are rich sources of various valuable chemicals as compared to pulp [19]. So the peels of these fruits should be focused on their use in the manufacturing of various cereals used in breakfast, dietary supplements, and the formulation of a nutraceutical for the treatment of bone-related problems especially in aged women [20]. Nutritive values of edible parts of the fruits are focused more, and the peels are usually considered as agrowastes, but the study has revealed that some minerals are even more concentrated in fruit peels than in the edible parts of the fruits [21]. Hundreds of tons of fruit peels are produced each year, and the wastage of peel means the wastage of minerals.

Mixtures of concentrated acids alone or in combination with hydrogen peroxide are widely used as extracting agents for the extraction of metallic elements in different food matrices by employing microwave-assisted digestion or conventional acid digestion methods. High pressure and temperature along with a large volume of reagents (acids and hydrogen peroxide) are required for getting adequate results by using these digestion methods [22–24]. Ultrasound-assisted extraction (UAE) is a promisingly alternative method to microwave-assisted digestion or conventional acid digestion methods to get adequate results and enough potential for extracting metals in different food matrices, sediments [25], and animal and plant tissues [26, 27] where ultrasonic energy is used to make the process of extraction in the presence of matrix analyte easier.

Also keeping in view the principles of green chemistry, the UAE method in combination with dilute acids to extract the metals is generating less waste which is harmful to the environment; also, this method has advantages over the conventional digestion/extractions methods related to operating costs, safety, simplicity, and reduced extraction time [28, 29]. The main target of the current research is to investigate the micro- (Mn, Fe, Cu, and Zn) and macroelements (Mg, K, and Ca) composition in the peel's powder of some common fruits (pomegranate, orange, lemon, mango, and grapefruit). The UAE method was optimized prior to the determination of their concentration by flame atomic absorption spectrometer (FAAS). Consequently, this fruit peel powder is a nonchemical/organic source of micro- and macroelements having nutritional benefits for the patients of osteoporosis to overcome skeletal abnormalities and improve bone health.

2. Materials and Methods

2.1. Reagents and Solutions. Hydrochloric acid (37% m/m), nitric acid (65% m/m), and hydrogen peroxide (30% v/v) of reagent grade (Sigma Aldrich, USA) for digestion of fruit peel powder samples and preparation of extracting agents were purchased from Falcon Chemicals, Lahore-Pakistan. Standard solutions of each metallic element to construct respective calibration curve were prepared by diluting the certified reference standard, 1000 mg/L of each metal (Merck, Germany) with HNO_3 (2% v/v) whereas glassware employed in this procedure was soaked in HNO_3 (20% v/v) to avoid any contamination. 18 MΩ cm resistivity quality water was prepared in our own lab by GenPure water system (Thermo Scientific, USA) for diluting the solutions. Apple leaves were used as a standard reference standard (NIST 1515) for FAAS method validation.

2.2. Collection of Samples. Fresh fruit samples including pomegranate, orange, lemon, mango, and grapefruit were collected from one supplier located in the provincial capital of Punjab province of Pakistan, namely, Lahore. Three packets were collected randomly; from each pack of fruit samples, three samples were collected for each fruit, and in total, 45 fruit samples (3 packets *x* 3 fruits *x* 5 fruit species) were used in this study. The plant material was authenticated by a plant taxonomist from the Institute of Agriculture Sciences, University of the Punjab, Lahore, Pakistan. A voucher specimen (DCUET-020219) was deposited at the Institute's herbarium. After collection, the fruit samples were washed with ultrapure water and then peeled off. The fruit peels were dried for 72 h at 60°C after washing with ultrapure water; the dried peels were ground by using a knife mill and screened through 100 US mesh, then packed in an airtight glass jar, and stored at 4°C till further analysis. The plant used in this research complies with the criteria and policy established by the "Convention on the Trade in Endangered Species of Wild Fauna and Flora and the IUCN Policy Statement on Research Involving Species at Risk of Extinction".

2.3. Extraction and Digestion of Samples

2.3.1. Ultrasound-Assisted Extraction. Apple leaves as SRM were used to optimize the UAE method for extraction of micro- and macroelements, and extraction was performed by using HNO_3 [EA-1], HNO_3–HCl (1 : 3) [EA-2], and HNO_3–H_2O_2 (2 : 1) [EA-3] as extracting agents. Pre-sonication of the sample was done by using a pulse-type vortex mixer (Digital Vortex Mixer, Thermo Scientific™, UK) for 5–15 min whereas sonication time (90% sonication amplitude) and temperature of the ultrasonic bath were varied from 2 to 20 min and 30 to 90°C, respectively. 5.0 mL of extracting agent and 500 mg of finely ground SRM were transferred to a round bottom flask and vortex for 5, 10, and 15 min. Then, the sample was placed in an ultrasonic bath at 30, 60, and 90°C temperatures for different intervals (2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 min.). The resulting suspension was centrifuged (Sorvall™ ST 8, Thermo Scientific™, UK) at 3000 rpm for 3 min. Then, the supernatant was diluted with ultrapure water and made up to volume in 25 mL of volumetric flask. The final solution was stored at 4°C till further analysis. The optimized UAE method was applied for the determination of micro- and macroelements in the fruit peel powder under investigation.

2.3.2. Conventional Acid Digestion. The finely ground powder of fruit peels was transferred to a glass digester containing concentrated HNO_3 (15 mL); the sample was initially heated at 100°C for 15 min. Then, before adding the H_2O_2 (2 mL), it was kept at 120°C for 2 h, and after the addition of H_2O_2, it was further heated for 20 min at the same temperature. This conventional acid digestion method was also applied to SRM for the comparison of UAE with it.

The results were presented in mg $100\,g^{-1}$ of dry powder mass while the analysis was carried out in triplicate whereas stock solutions of real samples and SRM were further diluted as per the requirement of analysis.

2.4. Instrumentation. Air circulation oven (Bio-Science, China) and knife mill (GRINDOMIX GM 300, Thomas Scientific, USA) were used for drying and grinding of fruit peels, respectively. Ultrasonic bath (Model 1800, Thomas Scientific, USA) and glass digester (Hach DRB 200 Dry Thermostat Reactor, Thomas Scientific, USA) were employed for UAE and CAD, respectively. The composition of Mn, Fe, Cu, Zn, Mg, K, and Ca present in SRM and fruit peel powder was determined by using FAAS equipped with an autosampler (Model: PG-990, PG-instruments, UK). AA WinLab® software was used to interpret the data whereas experimental design and data for optimization of FAAS are explained in Table 1 [30, 31]. Operational parameters of FAAS in the present study are presented in Table 2.

Wavelength accuracy and its reproducibility were checked to optimize the FAAS by using a hollow cathode lamp of mercury (HCL, Hg) while searching the peaks at 253.7, 546.1, and 871.6 nm whereas bandwidth was fixed at 0.2 nm. The wavelength accuracy was measured by the difference between the standard value and the calculated value. For resolution studies of peaks obtained for a particular element, the minima and maxima were calculated by using manganese (Mn) HCL; details of parameters are provided in Table 1.

2.5. Validation of Proposed Method. For the analytical validation study of the proposed UAE method, percentage recovery was calculated to assess the accuracy of the method after analyzing the SRM whereas paired *t*-test at 95% confidence of interval was performed to check the efficiency of UAE as compared to CAD. Precision was evaluated by the repetition of extraction procedure in replicates of ten ($n = 10$) under the same conditions, and results of repeatability are expressed in % RSD (100 x σ/μ, where σ is SD and μ is mean value). Limit of detection (LOD = 3σ/S) and limit of quantitation (LOQ = 10 σ/S) were also estimated where standard deviation (SD) of analytical blank measurement ($n = 18$) was presented by σ while S is the slope of the calibration curve ($y = mx + b$) [32, 33].

3. Results and Discussion

3.1. Optimization of FAAS. The peaks were scanned in triplicate at three different wavelengths (253.7, 546.1, and 871.6 nm) by using HCL of Hg; the results of obtained wavelength are presented in Table 3; the difference between set and obtained wavelength represents the wavelength accuracy whereas maximum/minimum values were reported at the interval of 6 h to check the wavelength reproducibility which are in compliance with user requirement limit of ±0.25 nm and ±0.10 nm; respectively.

HCL of Mn was used to check the resolution of peaks after scanning at 279.5 nm in triplicate; the peaks were obtained in a range between 279.45 and 279.71 nm. The ratio of the maxima and minima values at the first two peaks is

Table 1: FAAS optimization.

Parameters	Wavelength accuracy and reproducibility	Resolution
HCL	Mercury	Manganese
Lamp current (mA)	3.0	2.0
Bandwidth (nm)	0.2	0.2
Negative voltage (V)	300	300
Gas flow (acetylene) (mL min. $^{-1}$)	1200	1700
Flame height (mm)	5.0	5.0
Flame position (mm)	0.0	0.0

Table 2: FAAS operational parameters.

Parameters	Element						
	Mn	Fe	Cu	Zn	Mg	K	Ca
Wavelength (nm)	279.5	248.3	324.7	213.9	285.2	766.5	422.7
Slit width (nm)	0.4	0.2	0.4	0.4	0.4	0.4	0.4
Lamp current (mA)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Sample flow (mL min.$^{-1}$)	6.0	6.0	6.0	6.0	6.0	6.0	6.0
[a] gas flow (mL min. $^{-1}$)	1700	1700	1700	1700	1700	1700	1700
Air pressure (psi)	40	40	40	40	40	40	40

[a]Acetylene (99.998%, Linde, Pakistan).

Table 3: Wavelength accuracy and reproducibility.

Set wavelength (nm)	Obtained wavelength ($n = 3$)	Wavelength accuracy	Maximum (nm)	Minimum (nm)	Wavelength reproducibility
253.700	253.685	−0.015	253.715	253.675	0.040
546.100	546.015	−0.085	546.125	546.090	0.035
871.600	871.625	+0.025	871.650	871.585	0.065

0.191 ($n = 3$) which is less than 0.30 as recommended by the manufacturer. The resolution data is summarized in Table 4.

3.2. Optimization of UAE. SRM (NIST 1515) was used to optimize ($n = 6$, see supplementary data, Tables 1S–8S) the UAE method by using three different extracting agents whereas operating parameters including presonication, sonication, and temperature were also varied (Table 5). The proposed UAE method was evaluated on the basis of the recovery studies of seven elements present in the SRM (microelements: Mn, Fe, Cu, and Zn and macroelements: Mg, K, and Ca) and compared with certified value.

The results of Mn, Fe, Cu, Zn, Mg, K, and Ca present in SRM obtained by UAE were compared with CAD method, and significance difference at 95% confidence of interval ($p = 0.05$) was assessed by comparing the $t_{critical}$ (cut-off point on the t distribution) and $t_{experimental}$ (experimentally compare the means of two groups) for both the methods (CAD and UAE). The value of $t_{critical}$ (2.57) was more than $t_{experimental}$ at five degrees of freedom (n-1 = 5) which indicates no significant difference in obtained values of Mn, Fe, Cu, Zn, Mg, K, and Ca present in SRM by using both the methods as shown in Table 6.

3.2.1. Vortexing and Sonication Time Influence on Extraction Recoveries. The required quantity of SRM and each fruit peel powder along with extracting agent was added to vortexing tube separately and subject to presonicated (vortexing) for different time intervals (5–15 min.). After vortexing, the tube was placed in an ultrasonic bath; 5 min vortexing was noted as the optimum time for extracting both the micro- and macroelements from SRM and all samples (pomegranate, orange, lemon, mango, and grapefruit) understudy to achieve excellent recoveries. An increase in vortexing time (10 or 15 min.) did not show any increment in recoveries (supplementary data, Tables 9S–11S). UAE efficiency increased while increasing the sonication time (Figure 1(a)) but the maximum recoveries of micro- and macroelements from SRM were obtained at 6 min sonication time by using HNO_3–H_2O_2 as extracting agents. It was observed that the sonication of more than 6 min did not show any increase in recovery of micro- and macroelements from SRM. So the optimum recoveries were achieved by using dilute acid with hydrogen peroxide as extracting which offers practical advantages over CAD methods where an extended time is required to digest the sample [34].

3.2.2. Extracting Agent Influence on Extraction Recoveries. The influence of extracting agents including HNO_3 [EA-1], HNO_3–HCl (1 : 3) [EA-2], and HNO_3–H_2O_2 (2 : 1) [EA-3] was also studied over the extraction of micro- and macroelements from SRM and fruit peel powder. A vortexing and sonication time was fixed at 5 and 6 min, respectively, at a temperature of 60°C while using the extracting agents, and higher recoveries were obtained by using EA-3, which is an acid-oxidant mixture (Figure 1(b)). The extraction

Table 4: Resolution studies.

Set wavelength for peak searching (nm)	Obtained peaks after searching (nm)	Maxima/minima ratio
279.5	279.60, 279.48, 279.45	14.8/78.1 = 0.189
279.5	279.64, 279.59, 279.71	15.0/78.0 = 0.192
279.5	279.59, 279.71, 279.68	14.9/78.1 = 0.191
Average		0.191

Table 5: Optimized conditions for extraction of micro- and macroelements in fruit peel powder by UAE method.

Variables	Values	Optimized obtained value
Presonication time (min.)	5–15	5
Sonication time (min.)	2–20	6
Temperature (°C)	30–90	60
Extracting agents		
HNO_3 (0.5 M), HNO_3 (0.5 M)-HCl (1.0 M) [1 : 3] and HNO_3 (0.5 M)-H_2O_2 (10%) [2 : 1]		HNO_3 (0.5 M)-H_2O_2 (10%) [2 : 1]

Table 6: Extraction and digestion method validation by using SRM ($n = 6$).

Element	SRM value	UAE ($\bar{x} \pm ts/\sqrt{n}$)	CAD ($\bar{x} \pm ts/\sqrt{n}$)	% Recovery (UAE/CAD)	$t_{experimental}$
Microelements ($\mu g\ g^{-1}$)					
Mn	54.1 ± 1.1	53.77 ± 1.01	53.77 ± 1.34	99.4/99.4	−0.226
Fe	82.7 ± 2.6	82.8 ± 0.64	82.8 ± 0.60	100.1/100.1	−0.421
Cu	5.69 ± 0.13	5.65 ± 0.36	5.79 ± 0.32	99.3/101.8	−1.505
Zn	12.45 ± 0.43	12.05 ± 0.58	11.95 ± 0.48	96.8/96.0	0.604
Macroelements (mg g^{-1})					
Mg	2.71 ± 0.12	2.67 ± 0.23	2.75 ± 0.18	98.5/98.5	−0.672
K	16.08 ± 0.21	16.52 ± 0.36	16.63 ± 0.24	102.7/103.4	−0.798
Ca	15.25 ± 0.10	15.34 ± 0.38	15.45 ± 0.41	100.6/101.3	−1.154

($\bar{x} \pm ts/\sqrt{n}$) = mean ± CI ($p < 0.05$), CI = confidence interval.

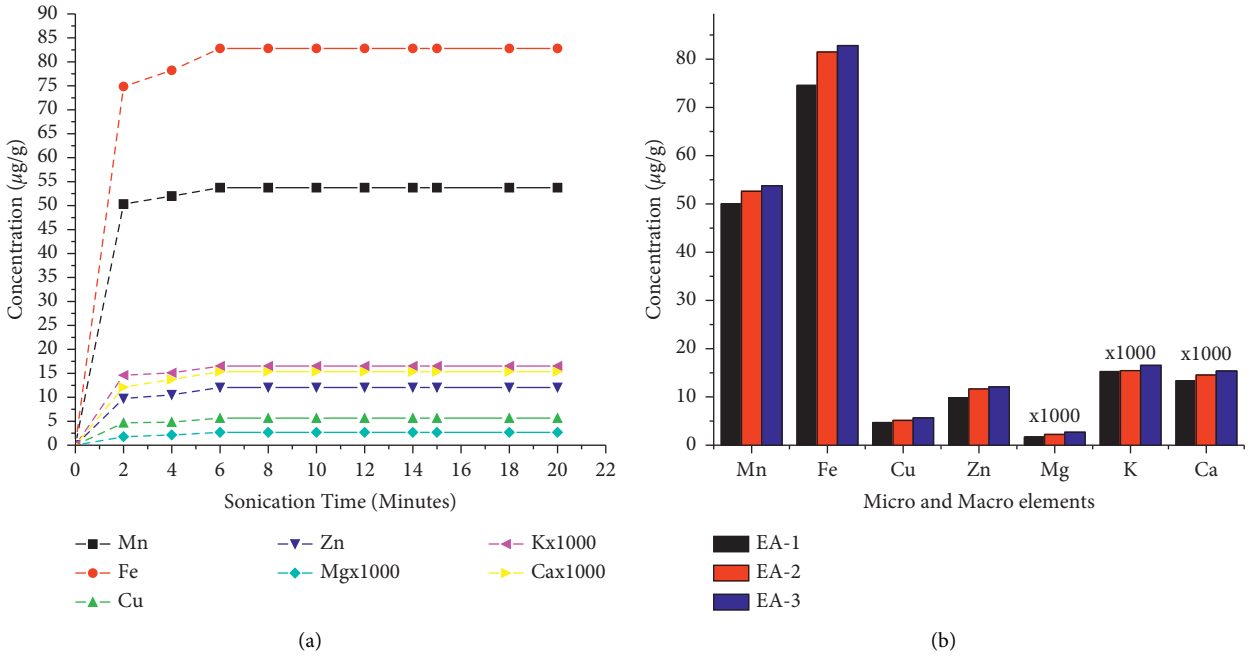

Figure 1: Sonication time (a) and extracting agent (b) influence on micro- and macroelements recovery from SRM (NIST 1515).

recoveries of micro- and macroelements from SRM by using the rest of two extracting (single acid, EA-1, and combination of two acids, EA-2) agents were comparatively lower (supplementary data, Tables 5S and 6S). Traditionally strong oxidizing acid ($HClO_4$) alone or in combination with other oxidants gave excellent extraction as compared to weak

oxidizing acid (HNO_3) because organic matter present in plant material has an important role in releasing metals. So, the combination of oxidizing acid (HNO_3) with oxidizing agent H_2O_2 dissociates hydroxyl (OH^-) radicals on heating that could attack organic matter; hence, the efficiency of extraction increases [22, 34].

3.2.3. Temperature Influence on Extraction Recoveries. Variation in temperature (30, 60, 90°C) of the ultrasonic bath was demonstrated for extraction of micro- and macroelements in fruit peel powder to assess the effect of temperature on extraction performance. Increasing the temperature of extracting agents along with increment in sonication time resulted in the formation of free radicals which accelerate the reaction involved in the digestion of samples. Extraction recoveries were also assessed by increasing the time of vortexing along with varied conditions of temperature (30, 60, and 90°C). The data showed that (Figure 2 and supplementary data, Tables 9S–11S) the extraction recovery did not increase while increasing the vortexing time and temperature. To release the micro- and macroelements from SRM, 60°C temperature was optimum, so a temperature higher than 60°C was necessary for efficient extraction of metals from SRM and real samples under study as compared to extraction at lower or room temperature [34, 35].

3.3. Validation of Proposed Method. The errors in the analytical methods determine the accuracy of results on the basis of the difference between the actual values and determined values of SRM which enable us to make the decision about the method adopted for analysis. The magnitude of the sample matrix, purity of reference standard, environmental condition of the laboratory, and stability of instrument play a vital role to get accurate results, so to ensure accuracy during the analysis of micro- and macroelements in real samples, SRM was analyzed and results are presented in terms of percentage recovery studies (Table 6) which are obtained in ranges between 96.8 and 102.7%, and these best recovery values determine the excellent extraction efficiency of UAE method. A linear calibration curve ($y = mx + b$) was obtained by plotting the nominal concentration (x) of each element against the relevant peak height (y) while selecting a dynamic range of 0.2–1.0 μg/mL for Mg, Mn, and Zn, 10–30 μg/mL for Ca, and 1–5 μg/mL for Fe, Cu, and K. Each analyte under investigation has a nominal concentration $x = 0.2$, 0.4, 0.6, 0.8, and 1.0 μg/mL for Mg, Mn, and Zn, $x = 10$, 15, 20, 25, and 30 μg/mL for Ca, and $x = 1$, 2, 3, 4, and 5 μg/mL for Fe, Cu, and K, where "m" and "b" represent the slope and intercept, respectively. The necessary parameters of the linear calibration curve are shown in Table 7.

The lower values of detection limits (LOD and LOQ) indicate that the method provided adequate sensitivity as shown in Table 7. The repeatability ($n = 10$) was performed to check the precision of the method, and results were presented in % RSD which were obtained in the range of 0.43 to 2.29 (Table 7) during the analysis of SRM which is good enough and in compliance with the FDA manual (% RSD <7).

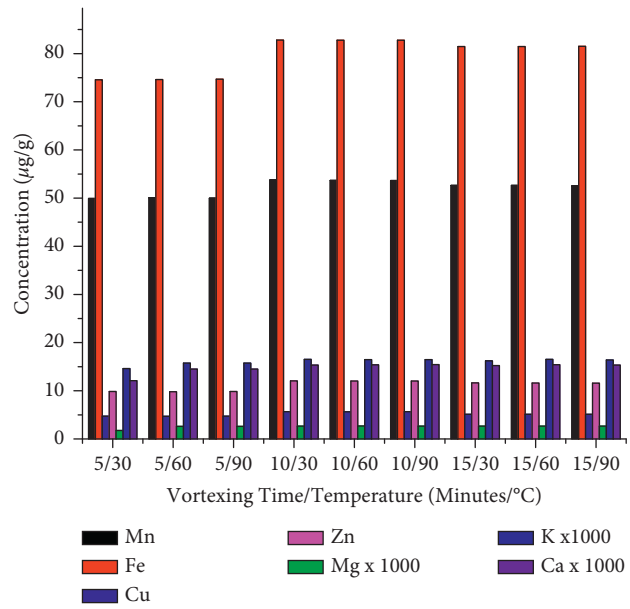

Figure 2: Vortexing time and temperature influence on micro- and macroelements recovery from SRM (NIST 1515).

Table 7: Parameters of the calibration curve, results of detection limits, and precision studies.

Metal	m	b	r^2	LOD ($\mu g\ g^{-1}$)	LOQ ($\mu g\ g^{-1}$)	(%) RSD
Mn	0.1338	0.1532	0.9987	0.012	0.040	1.41
Fe	0.0982	0.1384	0.9991	0.017	0.056	0.43
Cu	0.7552	0.1973	0.9989	0.019	0.063	1.38
Zn	0.1231	0.1452	0.9981	0.018	0.059	2.29
Mg	0.4293	0.2180	0.9998	0.006	0.017	1.67
K	11.200	9.110	0.9978	0.058	0.175	0.59
Ca	0.3168	0.2314	0.9991	0.023	0.053	0.87

3.4. Analysis of Fruit Peel Powder Samples. The optimized UAE procedure was applied to fruit peels for extraction of the micro- (Mn, Fe, Cu, and Zn) and macroelements (Mg, K, and Ca), and subsequently, these were determined by FAAS; results are presented in Table 8.

The highest Mn content was found in mango peel that ranged between 0.49 and 0.60 mg/100 g (Table 8S, supplementary data) which is about 4% of dietary reference intake (DRI, 8–13 mg/day) [36] while the lowest concentration was found in orange peel that ranged between 0.19 and 0.22 mg/100 g (2% of DRI). The concentration of Mn reported by Czech et al. in the peels of orange (0.13 mg/100 g), lemon (0.05 mg/100 g), and red grapefruit (0.10 mg/100 g) from Turkey [20] is less than that found in our study (Table 8), while 0.21–0.34 mg/100 g Mn was found in orange cultivar (lima and pera orange) from Brazil [37] which is comparable with concentration found in our samples under investigations.

Our study showed that mango peel contained the highest Fe concentration (6.5 mg/100 g) that is 80.2% of the estimated average requirement (EAR, 5.0–8.1 mg/day) and 36.1% of recommended dietary allowance (RDA, 8–18 mg/day), so both parameters cover the DRI as 5.0–18 mg/day for

Table 8: Contents of micro- and macroelements in fruit peel samples using the UAE method.

Fruit sample	Micro- and macroelement concentration (mg/100 g) $\overline{x} \pm$ CI on a dry basis						
	Mn	Fe	Cu	Zn	Mg	K	Ca
Pomegranate	0.41 ± 0.04	1.36 ± 0.05	0.06 ± 0.02	0.85 ± 0.12	49.33 ± 3.79	728.00 ± 3.79	277.33 ± 9.39
Orange	0.22 ± 0.06	2.22 ± 0.10	0.13 ± 0.04	0.82 ± 0.13	48.67 ± 5.16	680.00 ± 6.56	471.33 ± 13.65
Lemon	0.47 ± 0.09	1.32 ± 0.12	0.12 ± 0.04	0.84 ± 0.08	74.00 ± 4.96	867.00 ± 4.96	795.66 ± 18.28
Mango	0.54 ± 0.04	6.50 ± 0.16	0.29 ± 0.05	1.03 ± 0.15	141.33 ± 5.16	1501.67 ± 10.02	454.00 ± 9.92
Grapefruit	0.36 ± 0.06	3.53 ± 0.09	0.14 ± 0.04	0.84 ± 0.12	79.33 ± 3.78	984.33 ± 13.66	801 ± 17.88

$(\overline{x} \pm ts/\sqrt{n})$ = mean ± CI ($p < 0.05$, $n = 3$), CI = confidence interval.

both males and females. Grapefruit also contained an appreciable amount of Fe (3.53 mg/100 g) while lemon and pomegranate have almost the same concentration (Table 8). The concentration of Fe reported for the peels of orange (0.51 mg/100 g), lemon (0.34 mg/100 g), and red grapefruit (0.23 mg/100 g) from Turkey [20] is less than that found in our study (Table 8), while 1.01 mg/100 g Fe was found in orange cultivar (lima orange) from Brazil [37].

The Cu content in all of the fruit peels was found in the range between 0.06 and 0.29 mg/100 g (Table 8S, supplementary data) while the highest concentration was found in mango peel that ranged between 0.27 and 0.31 mg/100 g which contribute 32.2% of DRI (0.7–0.9 mg/day) including the EAR, 0.70 mg/day, and RDA, 0.90 mg/day, for both males and females with the aged group rangig between 18 and 70 years. The Cu content found in lima and pera orange was 0.06 mg/100 g and 0.09 mg/100 g originating in Brazil [37] while the concentration of Cu was reported for the peels of orange (0.15 mg/100 g), lemon (0.04 mg/100 g), and red grapefruit (0.08 mg/100 g) from Turkey [20].

The Zn content in all the fruit peels was found in the range between 0.78 and 1.10 mg/100 g (Table 8S, supplementary data) while the highest concentration was found in mango peel (1.03 mg/100 g) which contribute 9.4% of DRI (6.8–11.0 mg/day) including the EAR, which ranged between 6.8 and 9.4 mg/day, and RDA, which ranged between 8 and 11 mg/day for both males and females with the aged group ranging between 18 and 51 years. The Zn content found in lima and pera orange was 0.35 mg/100 g and 0.21 mg/100 g, respectively, originating in Brazil [37] while the concentration of Zn was reported for the peels of orange (0.25 mg/100 g), lemon (0.28 mg/100 g), and red grapefruit (0.33 mg/100 g) from Turkey [20].

The highest content of Mg was found in mango peel that ranged between 133 and 141 mg/100 g while the least concentration was found in pomegranate (45–51 mg/100 g) and orange (47–51 mg/100 g) which are comparable (Table 8S, supplementary data). The DRI contribution by mango peel is 33.6% including the EAR, which ranged between 265 and 350 mg/day, and RDA, which ranged between 320 and 420 mg/day for both males and females with the aged group ranging between 18 and 70 years. The Mg content found in lima and pera orange was 23.8 mg/100 g and 27.8 mg/100 g, respectively originating in Brazil [37] while the concentration of Mg was reported for the peels of orange (13.2 mg/100 g), lemon (11.5 mg/100 g), and red grapefruit (10.0 mg/100 g) from Turkey [20].

The present study indicated that the mango peel is rich in K (1501.67 mg/100 g) content while the least amount was found in orange peel (680 mg/100 g). The K content found in lima and pera orange was 258.7 mg/100 g and 266 mg/100 g, respectively, originating in Brazil [37] while the concentration of K reported for the peels of orange (154 mg/100 g), lemon (127 mg/100 g), and red grapefruit (132 mg/100 g) from Turkey [20]. The DRI contribution of K by mango peel is 31.9% as AI (adequate intake, 4700 mg/day), for both genders with the aged group ranging between 18 and 70 years.

Among all the fruit peels, the highest Ca content was found in grapefruit peel (801 mg/100 g) while the least concentration was found in pomegranate (277.33 mg/100 g). Liu et al. reported the highest Ca content (40 mg/100 g) in orange peel [38]. The DRI contribution of Ca by mango peel is 66.8% as AI, 1000–1200 mg/day, for both genders with the aged group ranging between 18 and 70 years. The Ca content found in lima and pera orange was 145.2 mg/100 g and 165.4 mg/100 g, respectively, originating in Brazil [37] while the concentration of Ca was reported for the peels of orange (41.9 mg/100 g), lemon (31.8 mg/100 g), and red grapefruit (36.0 mg/100 g) from Turkey [20].

In conclusion, mango peel comprised the highest concentration of Mn, Fe, Cu, Zn, Mg, and K, while the Ca content was found less than orange, lemon, and pomegranate peels. Special attention should be paid to such a nutritionally rich peel powder of mango for its potential use as a component of functional food. Different food cereals can be fortified with these fruit peel powders especially mango peel powder to meet the body's ongoing demand for individual minerals. Mineralization (Figure 3) is an important modifiable factor in the development and maintenance of bone mass and the prevention of osteoporosis. Concerning nutrition and health, this research showed that fruit peels of pomegranate, orange, lemon, mango, and grapefruit contain an appreciable amount of micro- and macroelements that are good for bone health and could have osteoprotective effects.

To see more clearly the advantages of the method, a comparison of the proposed method with other methods in the literature for different metals' determination in vegetable samples is given in Table 9, with an emphasis on the procedure execution time, type, and concentration of the extractor used for the metals under investigation. Ultrasound-assisted extraction has been successfully used in the extraction of metals in different matrices like fruits, vegetables, and milk samples with the advantages of less extraction time, low energy consumption, and high

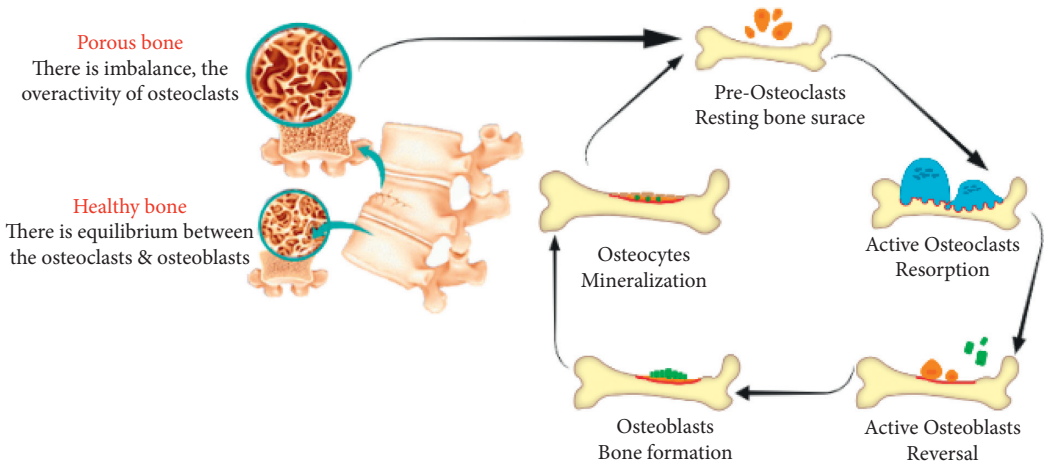

Figure 3: A proposed model for the potential osteoprotective effects of minerals [39].

Table 9: Comparison of the analytical features of the proposed method with those of other reported methods for the determination of metallic elements in fruit peel samples.

Element	Technique	Time	Extracting solvent	Reference
Na, K, Ca, Mg, Fe, Zn, Cu, Mn, Se	MW, FAES and ICP-OES	12 min	HNO_3	[20]
Ca, Fe, Mn Zn,	AD, FAAS	3 h	HCl	[40]
K, Ca, Mg, Fe, Zn, Mn, B, Cu, Mo, Na	MW, ICP-OES	—	$HNO_3 : H_2O_2$ (5 : 2)	[41]
Ca, Na, Fe, K, Mg	AD, FAAS	—	HCl	[42]
P, K, Ca, Mg	AD, FAAS	—	$H_2SO_4 : H_2O_2$	[43]
Mn, Fe, Cu, Zn, Mg, K, Ca	UA extraction, FAAS	6 min	HNO_3 (0.5 M) : H_2O_2 (10%) (2 : 1)	Present study

AD: acid digestion, MW: microwave, ICP-OES: inductively coupled plasma-optical emission spectrometry, FAAS: flame atomic absorption spectrometry, FAES: flame atomic emission spectrometry, UA: ultrasound-assisted.

extraction yield as compared with conventional extraction methods.

4. Conclusion

In this study, an ultrasound-assisted extraction method was optimized by varying the parameters of extraction and using different extracting agents for the micro- (Mn, Fe, Cu, and Zn) and macroelements (Mg, K, and Ca) composition in the peel powder of some common fruits including pomegranate, orange, lemon, mango, and grapefruit. The composition of mentioned elements was consequently determined by the optimized and validated FAAS method. SRM was also analyzed by CAD method to check the performance and comparison of UAE to determine the significant difference between both methods. The significant difference at 95% confidence of interval ($p = 0.05$) was assessed by comparing the $t_{critical}$ (cut-off point on the t distribution) and $t_{experimental}$ (experimentally compare the means of two groups) for both methods (CAD and UAE). The value of $t_{critical}$ (2.57) was more than $t_{experimental}$ at five degrees of freedom ($n-1 = 5$) which indicates no significant difference in obtained values of Mn, Fe, Cu, Zn, Mg, K, and Ca present in SRM by using both methods. Concerning nutrition and health, fruit peel powder is a very valuable source of micro- and macroelements for patients with osteoporosis to overcome skeletal abnormalities and improve bone health. On the basis of the literature, a hypothesis was established for the management of osteoporosis by minerals, and fruit peels are a rich source of minerals and are a potential organic source of minerals rather to use their salts as a source of minerals. This research is just to explain the role of mineralization for the development and maintenance of bone mass and the prevention of osteoporosis, and fruit peels are the rich source of minerals and suggest the utilization of their powder as a multielement source along with other medicines used to manage osteoporosis. The management of osteoporosis is not solely explained to utilize the fruit peel powder as a remedy but their use along with other medication is recommended.

Data Availability

The data are provided in the manuscript and the supplementary file.

Disclosure

The manuscript has been presented in Research Square, https://www.researchsquare.com/article/rs-733194/v1.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Table 1S: validation of UAE and CAD methods against CRM (apple leaves, NIST 1515). Table 2S: validation of UAE and CAD methods against CRM (apple leaves, NIST 1515). Table 3S: validation of UAE and CAD methods against CRM (apple leaves, NIST 1515). Table 4S: validation of UAE and CAD methods against CRM (apple leaves, NIST 1515). Table 5S: validation of UAE and CAD methods against CRM (apple leaves, NIST 1515). Table 6S: validation of UAE and CAD methods against CRM (apple leaves, NIST 1515). Table 7S: precision studies of UAE methods for CRM (apple leaves, NIST 1515). Table 8S: contents of micro- and macronutrients in fruits peels ($n = 3$) by UAE. Table 9S: validation of UAE methods against CRM (apple leaves, NIST 1515). Table 10S: validation of UAE methods against CRM (apple leaves, NIST 1515). Table 11S: validation of UAE and CAD methods against CRM (apple leaves, NIST 1515). (*Supplementary Materials*)

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