**NOTE**

**The Effects of Amplitudes Ultrasound-Assisted Solvent Extraction and Pretreatment Time on the Yield and Quality of *Pistacia Khinjuk* Hull Oil**

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Abstract: In this study, the effect of three amplitudes of ultrasound-assisted solvent extraction (UASE) (0, 25 and 50 %; 100 W, 30 kHz; the 0% treatment serving as control) on yield and quality of extracted oil from *P. khinjuk* hull at various pretreatment times (15, 30 and 45 min) was evaluated. The highest oil yields at the three amplitude levels tested were 24.4, 29.8 and 37.8% for 0, 25 and 50 %, respectively. Although increase in pretreatment time increased oil yield, pretreatment time at 30 and 45 min did not significantly different for both 25 and 50% amplitude. Furthermore, UASE did not significantly affect fatty acid composition, peroxide value (PV), conjugated diene value (CDV) and anisidine value (AnV) of extracted oils. UASE also increased tocopherols and tocotrienols content of oils but pretreatment time at 30 and 45 min did not significantly different for them. Therefore, UASE increase yield and quality of extracted oil and reduce extraction time. All these advantages make UASE a good substitute for the extraction of oil.

Key words: *P. khinjuk*, pretreatment time, quality, ultrasound-assisted solvent extraction, yield

1** INTRODUCTION**

The genus *Pistacia* belongs to the Anacardiaceae, a widely world spread plant family which comprise about 70 genera and over 600 species. Among them, *Pistacia vera* L., *Pistacia atlantica* subsp. *mutica* and *Pistacia khinjuk* are the native species to Iran. *P. khinjuk* which naturally grows in Iran is called in Persian language as *Kolkhoung*¹ ². The extract from this plant has been shown to have pharmacological activities such as antidiabetic, antitumor, anticholinesterase, antimicrobial and antifungal activity³ ⁵. In traditional Iranian medicine, *P. khinjuk* was used as helpful remedies for various diseases including stomach discomfort, vomiting, nausea and motion sickness⁶.

The oil from plant is usually extracted either by mechanical pressing or solvent extraction⁷. Mechanical extraction is known as a traditional oil extraction method and a very inefficient process with low oil recovery. On the other hand, although standalone solvent extraction is the industrial standard for the extract of some vegetable oils, this method requires long treatment times and numerous sample preparation steps prior to the actual extraction process⁸. Therefore, different innovative methods have been suggested, that could be used to “assist” the solvent extraction process. Among these, two methods have had significant success; ultrasound and microwaves⁹. Ultrasound-assisted extraction method has been used as an alternative for solvent extraction because of its many advantages as kind of simplicity and solvent amount, temperature and extraction time reduction¹⁰.

Hashemi et al.¹¹ reported pulsed ultrasound assisted solvent extraction increased yield and quality of extracted oil. Li et al.¹² also found soybean oil yield increased with increasing ultrasonic power intensity.

Therefore, the aim of this study were to investigate the effect of ultrasound amplitude and treatment time on the extraction yield and quality of extracted *P. khinjuk* hull oil.

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2 EXPERIMENTAL

2.1 P. khinjuk hulls and chemicals

Dried P. khinjuk fruits (13% moisture content/dry basis, 5 kg) were purchased from the local market in Yasooj city (Kohgilooyleh and Boyerahmad, Iran) in January 2015 and stored at −18°C until initiation of the experiments. After separation of the kernels, hulls were ground into powder using a grinder and passed through a standard sieve to select particles smaller than 1.18 mm. All chemicals and solvents used in this study were of analytical reagent grade and purchased from Merck Co. (Germany) and Sigma Chemical Co. (St., Louis, USA).

2.2 Oil extraction process

For UASE, a Hielscher ultrasonic device (UP100H, Germany; 100 W, 30 kHz) with a titanium horn (M6 × 0.75) was used. The effects of three levels of amplitude (0, 25 and 50%) at three pretreatment times (15, 30 and 45 min) were investigated. The 0% amplitude experiment was the control; basically, ultrasonics was not turned on and the sample-solvent mixture was undisturbed during the process.

Ground P. khinjuk hull powder was mixed with n-hexane at ratio of 1:4 (w/v). Ultrasound treatment was applied to the sample by inserting the probe 5 cm from the top into the sample-solvent suspension in the cell. To avoid considerable solvent evaporation that could potentially occur at long treatment periods and/or at higher amplitudes, a special evaporation-proof cap was used during the ultrasonic extraction process. A water bath was used to keep the temperature of the extraction medium at 30°C during the process. Additionally, the temperature of the medium was monitored every 30 min to ensure that there was no temperature over run. Each experiment was carried out in triplicate. After ultrasonic extraction, the sample-solvent suspension was allowed to sit at ambient temperature for 15 minutes; the supernatant was then decanted into a separate tube and the residue was washed twice with 5 mL of n-hexane. Next, 15 mL of 10% sodium sulphate solution was added to the decanted suspension, separating it into two phases: an upper hexane phase containing the extracted oil and a lower phase containing all the other cellular components. Phase separation was made more distinct by centrifugation at 3000 g for 15 min. The upper hexane phase was then aspirated into a pre-weighed stainless steel vessel and dried over a stream of nitrogen for 3 hours until constant weight.

2.3 Oil yield determination

The oil yield (Y) was determined gravimetrically:

\[ Y(\%) = \frac{m_o}{m_h} \times 100, \]

Where \( m_o \) is the mass of extracted oil (g) and \( m_h \) the mass of ground P. khinjuk hull (g).

2.4 GC analysis of fatty acid

Fatty acid compositions of the P. khinjuk hull oils were determined by gas chromatography (GC) and were reported in relative area percentages. Fatty acids of the hull oils were transesterified into their corresponding fatty acid methyl esters (FAME) by vigorous shaking of a solution of oil in hexane (0.3 g in 7 mL) with 2 mL 7 N methanolic potassium hydroxide at 50-55°C for 10 min. After shaking, the solution was allowed to settle for 5 min. The upper layer was collected for GC analysis after mixing with anhydrous natrium sulphate and filtering. The FAME were identified using an HP-5890 chromatograph (Hewlett-Packard, CA, USA) equipped with a CP-SIL 88 (Supelco, Bellefonte, PA, USA) capillary column of fused silica, 60 m, 0.32 mm i.d., 0.2 µm film thickness, and a flame ionization detector (FID). Helium was used as carrier gas with a flow rate of 1 mL/min. One µL of FAMEs was injected with split ratio of 1:8. The oven temperature was maintained at 198°C, and that of the injector and the detector at 250°C.

2.5 HPLC analysis of tocopherols and tocotrienols

The content of tocopherols and tocotrienols in the hull oils of P. khinjuk were measured using a high performance liquid chromatography (HPLC) (Waters, Alliance system, USA) with a Spherisorb column (25 cm × 4 mm i.d., Waters, USA) packed with silica (5 µm particle size) and a fluorescence detector operating at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. A total of 10 µL aliquot samples were injected. The mobile phase was hexane–isopropanol (98.5:0.5, v/v) at a flow rate of 1 mL/min. The tocopherols and tocotrienols in the test samples were verified by comparison of their retention times with those of reference standards.

2.6 Oxidation products measurement

Peroxide value (PV) was measured by treating a solution of sample oil (5 ± 0.05 g) in 30 mL acetic acid–chloroform with 0.5 mL saturated potassium iodide solution and titration with 0.1 N sodium thiosulphate. Measurement of anisidine value (AnV) was done by reading the absorbance of a solution of sample oil (0.5–4 ± 0.001 g) in 25 mL isooctane, treated with 1 mL p-anisidine reagent at 350 nm using solvent with p-anisidine reagent as blank in the reference cuvette. Conjugated dienes value (CDV) was determined spectrophotometrically (UV/Visible Philips Cambridge, UK) at 234 nm. The oil samples were diluted with hexane and an extinction coefficient of 29000 mol/L was utilized to quantify the concentration of conjugated dienes formed during oxidation.

2.7 Statistical analysis

The results were analyzed using two-way ANOVA (factorial experimental design with two factors: amplitude and time), and significant differences between groups were de-
Amplitudes Ultrasound-Assisted Solvent Extraction Yield and Quality Pistacia Khinjuk Hull Oil

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3 RESULTS and DISCUSSION

Ultrasonic amplitude and pretreatment time had significant ($p \leq 0.05$) effects on the oil yield. The highest oil yields at the three amplitude levels tested were 24.4, 29.8 and 37.8% for 0, 25 and 50%, respectively (Fig. 1). Both amplitude and pretreatment time had a positive effect on extraction oil yield from $P. khinjuk$ hull. Although increase in pretreatment time increased oil yield, pretreatment time at 30 and 45 min did not significantly different for both 25 and 50% amplitude. Li et al.\textsuperscript{12} used continuous high-intensity UASE coupled with magnetic stirring to extract oil from soybeans. The authors observed that the oil yield increased with increasing ultrasonic power intensity. Hashemi et al.\textsuperscript{11} found increase in ultrasound amplitude increased oil yield. This increase in yield for UASE is the effect of ultrasonic cavitation. When ultrasound waves pass through a processing medium, their alternating compression and expansion cycles cause the molecules of the medium to vibrate at such low pressures that the intermolecular forces are overcome and microscopic gas filled bubbles are formed. These cavities contract and expand with the alternating pressure cycles, expanding in size until the point where they fall down on themselves. This happening is called cavitation and is the key principle behind ultrasound-assisted cell disruption. High intensity ultrasound waves cause a more rapid form of cavitation where the cavities expand after just a few cycles (transient cavitation). The physical effects of transient cavitation form a hot spot in its immediate vicinity\textsuperscript{17, 18}. The physical effects of cavitation immediately disrupt intact biological cells in these hot spots by rupturing biological membranes and cell walls. Thus, cellular material pours out into the liquid medium made up of the solvent, and lipids are selectively dissolved in it. This process forms the basis for ultrasound-assisted solvent extraction and is responsible for the much higher oil yields from ultrasonic treatment in comparison with other methods\textsuperscript{19}.

The data shown in Fig. 2 indicate that using ultrasonication as a pretreatment allows one to cut down the process time to about 3.5 h without reducing the overall yield of 37.8%. Results also indicate by increasing pretreatment time, the optimum extraction duration can be reduced as reaction occurs faster; however pretreatment time at 30 and 45 min are not significantly different for both 25 and 50% amplitude. The extraction rate is fast at the beginning of the extraction but gets slow gradually. The reason is that when the meal is exposed to the fresh solvent, the free oil

Fig. 1 Yield of $P. khinjuk$ hull oil samples at different extraction conditions. 1: control, 2: extraction at 25% amplitude and pretreatment at 15 min, 3: extraction at 25% amplitude and pretreatment at 30 min, 4: extraction at 50% amplitude and pretreatment at 45 min, 5: extraction at 50% amplitude and pretreatment at 15 min, 6: extraction at 50% amplitude and pretreatment at 30 min, 7: extraction at 50% amplitude and pretreatment at 45 min. All values are means of three determinations. Means with the same superscript letters are not significantly different at $p < 0.05$. 735

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on the surface of seeds is solubilized and oil gets extracted rapidly inducing a fast increase in the extraction rate. Additionally, since the oil concentration is low in the solvent at the beginning of the extraction process, the oil diffuses rapidly from the meal to the liquid phase due to the mass transfer effect. As the time passing by, the concentration of oil increases in the solvent resulting in a decrease in the diffusion rate. These findings are in agreements with the results of previous studies which reported that the fatty acid composition of oil extracted with UASE was comparable to other conventional methods. For instance, Cravotto et al. found that UASE produced a higher oil yield, but the FAME profiles of oil from UASE and Soxhlet extraction were almost comparable.

The tocopherol and tocotrienol compounds of all extracted oil samples are shown in Table 3. Oils obtained by ultrasound compared with untreated samples demonstrated higher levels for tocopherol and tocotrienol compounds, whereas pretreatment time at 30 and 45 min for both amplitudes did not significantly affect their concentrations in the oils. Results also indicated increase in ultrasound amplitude increased tocopherol and tocotrienol compounds. These findings are in agreements with the results of

Table 1  Fatty acid composition of extracted P. khinjuk hull oil samples.

| Time (min) | Ultrasound amplitude % | C12:0 | C14:0 | C16:0 | C18:0 | C18:1 | C18:2 | C18:3 | C20:0 | C22:0 |
|-----------|------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1         | 0                      | 0.1 ± 0.1” | 0.64 ± 0.1” | 19.4 ± 0.3” | 0.42 ± 0.1” | 63.2 ± 1.2” | 13.6 ± 0.5” | 1.5 ± 0.1” | 0.1 ± 0” | 0.87 ± 0.2” |
| 2         | 15                     | 0.11 ± 0.1” | 0.63 ± 0” | 19.3 ± 0.4” | 0.43 ± 0.1” | 62.2 ± 1.4” | 14.1 ± 0.3” | 1.8 ± 0.2” | 0.11 ± 0” | 0.86 ± 0.1” |
| 3         | 30                     | 0.12 ± 0” | 0.64 ± 0.1” | 19.3 ± 0” | 0.45 ± 0.1” | 63.3 ± 1” | 13.65 ± 0.4” | 1.4 ± 0” | 0.12 ± 0” | 0.88 ± 0” |
| 4         | 45                     | 0.1 ± 0” | 0.61 ± 0.2” | 19.41 ± 0.5” | 0.43 ± 0.1” | 63.4 ± 0.9” | 13.5 ± 0.6” | 1.55 ± 0.1” | 0.11 ± 0” | 0.87 ± 0.1” |
| 5         | 50                     | 0.12 ± 0” | 0.64 ± 0” | 19.21 ± 0.3” | 0.44 ± 0.1” | 63.3 ± 1.5” | 13.3 ± 0.7” | 1.75 ± 0” | 0.12 ± 0” | 0.86 ± 0.1” |
| 6         | 30                     | 0.12 ± 0” | 0.64 ± 0” | 19.2 ± 0” | 0.46 ± 0” | 63.2 ± 0.8” | 13.75 ± 0.6” | 1.6 ± 0.1” | 0.11 ± 0” | 0.83 ± 0.2” |
| 7         | 45                     | 0.11 ± 0.0” | 0.63 ± 0.0” | 19.18 ± 0.2” | 0.45 ± 0” | 63.5 ± 1.7” | 13.56 ± 0.8” | 1.6 ± 0” | 0.11 ± 0.0” | 0.84 ± 0.1” |

All values are means of three determinations. Means within a column with the same superscript letters are not significantly different at $p < 0.05$.

P. khinjuk hull

Fig. 2  Amount of extracted oil versus time at different extraction conditions.

**Table 1** Fatty acid composition of extracted *P. khinjuk* hull oil samples.
Table 2  Oxidation parameters of extracted P. khinjuk hull oil samples.

| samples* | Time (min) | Ultrasound amplitude % | Peroxide value (meq/kg oil) | Conjugated diene value (mmol/L) | Anisidine value |
|----------|------------|------------------------|----------------------------|---------------------------------|----------------|
| 1        | 0          | 0                      | 1.8 ± 0.1*                 | 2.54 ± 0.4*                     | 2.46 ± 0.5*    |
| 2        | 15         | 25                     | 1.84 ± 0.3*                | 2.52 ± 0.3*                     | 2.48 ± 0.2*    |
| 3        | 30         | 25                     | 1.65 ± 0.2*                | 2.6 ± 0.1*                      | 2.43 ± 0.3*    |
| 4        | 45         | 25                     | 1.82 ± 0.2*                | 2.58 ± 0.1*                     | 2.41 ± 0.2*    |
| 5        | 15         | 50                     | 1.85 ± 0.1*                | 2.54 ± 0.2*                     | 2.49 ± 0.4*    |
| 6        | 30         | 50                     | 1.63 ± 0.3*                | 2.57 ± 0.3*                     | 2.45 ± 0.5*    |
| 7        | 45         | 50                     | 1.74 ± 0.1*                | 2.47 ± 0.2*                     | 2.44 ± 0.1*    |

All values are means of three determinations. Means within a column with the same superscript letters are not significantly different at \( p < 0.05 \).

* P. khinjuk hull

Table 3  Tocopherol and Tocoterienol content (mg/kg) of extracted P. khinjuk hull oil samples.

| samples* | Time (min) | Ultrasound amplitude % | \( \alpha \)-Tocopherol | \( \beta \)-Tocopherol | \( \delta \)-Tocopherol | \( \gamma \)-Tocopherol | \( \alpha \)-Tocoterienol | \( \beta \)-Tocoterienol |
|----------|------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| 1        | 0          | 0                      | 37.4 ± 1.1*            | 52.31 ± 1.3*          | 4.0 ± 0.5*             | 55.45 ± 1.3*          | 119.2 ± 3.1*          | 73.15 ± 2.2*          |
| 2        | 15         | 25                     | 41.1 ± 1.2*            | 57.4 ± 1.4*          | 4.33 ± 0.4*           | 61.33 ± 1.4*          | 136.5 ± 4.4*          | 78.77 ± 1.3*          |
| 3        | 30         | 25                     | 45.24 ± 1.1*           | 63.72 ± 1.8*        | 4.3 ± 0.4*             | 70.44 ± 1.2*          | 144.83 ± 2.1*        | 84.4 ± 1.4*            |
| 4        | 45         | 25                     | 46.7 ± 1.3*            | 64.4 ± 1.9*         | 4.4 ± 0.3*            | 69.2 ± 1.7*           | 145.64 ± 5.6*        | 83.52 ± 2.5*          |
| 5        | 15         | 50                     | 50.36 ± 1.5*           | 70.5 ± 1.7*         | 4.45 ± 0.5*            | 76.6 ± 1.7*           | 154.5 ± 3.3b         | 92.34 ± 2.3b           |
| 6        | 30         | 50                     | 59.1 ± 1.6*            | 75.54 ± 1.2*        | 4.24 ± 0.6*            | 83.42 ± 1.6*          | 160.9 ± 4.2*         | 98.4 ± 2.4*            |
| 7        | 45         | 50                     | 60.58 ± 1.4*           | 76.8 ± 2.1*         | 4.42 ± 0.2*            | 84.5 ± 2.4*           | 162.5 ± 4.8*         | 99.73 ± 1.9*           |

All values are means of three determinations. Means within a column with the same superscript letters are not significantly different at \( p < 0.05 \).

* P. khinjuk hull

Hashemi et al., who found application of ultrasound in extraction of oil enhanced tocopherol and tocoterienol compounds of extracted oil. This could be due to the fact that during UASE, the collapse of cavitation bubbles generates short-lived localized hot-spots with extremely high local temperature and pressure that can influence the stability of the tocopherol compounds, which also depends on their chemical structure. P. khinjuk hull oil is considered a valuable source of tocopherols because the tocopherols content reported for this oil is much higher than that of common oils such as sunflower, cottonseed, soybean, canola, and palm oil. P. khinjuk hull oils contain a high level of tocoterienols. Tocoterienols are structural analogues of the tocopherols but they have a higher antioxidant activity than the tocopherols. Consequently, UASE by increasing these compounds has a positive effect on P. khinjuk hull oil extraction.

4 CONCLUSION

The UASE technique was shown to improve the extraction of oil from P. khinjuk hull and increased oil yield. Furthermore, the fatty acid compositions and oxidation of the oil were not affected significantly by the application of ultrasound, whereas UASE increased tocopherol content of extracted oil. The UASE method offers main advantages over traditional alternatives, namely: shorter extraction times and increased oil yield. Furthermore, the reduced cost of extraction is noticeably valuable for the proposed UASE process in terms of time and energy. All these advantages make UASE a good substitute for the extraction of oil.

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