Comparison of the Physicochemical Characteristics of *Pinus koraiensis* L. Nut Oils from Different Extraction Technologies

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Abstract: In this study, the physicochemical properties, fatty acid profiles, and tocopherol compositions of *Pinus koraiensis* L. nut oils were evaluated, and the impact of different extraction technologies on the overall quality of pine nut oil was assessed. All the pine nut oils had pleasant and characteristic odor and flavor of the pine nuts and the flavor of the oil from hot-pressing was strongest. The oils obtained by hexane extraction and sub-critical extraction had less deterioration, indicating hexane extraction and sub-critical extraction preserve the oil quality well. Gas chromatography showed that the predominant fatty acid was linoleic acid (45.36%–45.91%), followed by oleic acid (26.91%–27.10%), and pinolenic acid (13.33%–13.63%), respectively. The oils from hexane extraction of cold-pressed cake and sub-critical extraction were richer in tocopherols and tocotrienols, namely, 37.52 and 36.18 mg/100g, respectively, with α -tocopherol most abundant. The pine nut oils from hexane extraction of cold-pressed cake, hexane extraction, cold pressing, and sub-critical extraction had better oxidation stability with the oxidation induction times of 6.91, 5.27, 4.96 and 4.93 h, respectively. Based on its features, *P. koraiensis* nut oil may have the multiple application in edible oil, cosmetic, and pharmaceutical industry. *P. koraiensis* can be one of the good woody candidates for closing the gap between the demand and production of vegetable oils.

Keywords: *Pinus koraiensis* nut oil; Physicochemical properties; Fatty acid composition; Tocopherols

1 Introduction

Woody oil plants don't occupy the cultivated land needed for crops production, and thus the development of the woody oil industry has become one of the important ways to close the gap between supply and demand for oil.

About 20 000 tons of pine nuts are produced yearly in the world. China, Russia (Siberia), Korea and Pakistan are the main exporting countries^[1], and *Pinus koraiensis* L. is the chief pine species in China. Pine nuts are rich in protein and fat, with unsaturated fatty acid in the pine nut up to 85%. Laboratory studies showed that *P. koraiensis* nut oil increases the antioxidant activity of rat serum and decreases lipid peroxidation^[2], which may be ascribed to fatty acid composition and other minor components in pine nut oil. Currently, the industrial oil extraction technologies include cold or hot pressing, solvent extraction and hexane extraction of cold-pressed cake, and the specifics depend on the kind of oilseeds and the ultimate use of the oil. Subcritical extraction technology, also called low temperature pressure extraction technology, is carried out at lower pressure and temperature, and obtains a higher extraction yield compared with supercritical fluid extraction^[3]. Subcritical extraction technology also has the advantages of short extraction time, low environmental impact and low residual solvent^[4].

Some efforts have been made to understand the physicochemical

properties of pine nut oil^[5–7], and the pine nut oils from different kinds^[8–9], different mountains^[10] have been compared. Bao et al.^[11] optimized ultrasonic-assisted aqueous enzymatic extraction of pine nut oil, and the effect of the two methods of extraction including the aqueous enzymatic and the solvent methods were compared^[12]. In this study, *P. koraiensis* nut oils were obtained by different extraction technologies including hot-pressing, cold-pressing, hexane extraction, hexane extraction of cold-pressed cake, and sub-critical extraction. The physicochemical properties, fatty acid profiles, and tocopherol compositions of the different oils extracted were evaluated, and the impact of different extraction technologies on the overall quality of pine nut oils was assessed. The information obtained has valuable implications for assessing the *P. koraiensis* the potential as one of the woody candidates for closing the gap between the demand and production of vegetable oils.

2 Materials and Methods

2.1 Materials

Fresh *P. koraiensis* seeds were bought from the “Ruyi Local Speciality Store” in Heilongjiang Province, China. The seeds were dehulled manually. The dehulled pine seeds were packed in plastic bags and stored at 4 °C. Oil was extracted from seeds within 5 days of being put into storage.

2.2 Oil Extraction

Oil samples, in duplicate, were prepared using five different methods of extraction, as described below.

Hot pressing: About 500 g of dehulled pine nuts were roasted at 180 °C for 25 min, cooled to (130±5) °C, and then pressed under hydraulic pressure of 50 MPa for 25 min using hydraulic press 180 (Henan Weihang Machinery Co. Ltd.,

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Zhengzhou, China). The obtained oil was filtered through a double layer of Whatman filter paper (0.45 μm), and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

Cold pressing: About 500 g of dehulled pine nuts were directly pressed using the hydraulic press 180 under hydraulic pressure of 50 MPa for 25 min at room temperature of $(25\pm 5)\text{ }^{\circ}\text{C}$. For each sample, the extraction procedure was repeated three times, and the resultant oil was collected together and filtered.

Hexane extraction: About 500 g of dehulled nuts were ground to a powder and sieved to a mesh size of 35 then added to a beaker with hexane (1:1, V/V), and stirred thoroughly; the hexane extraction procedure was repeated three times. The solvent was removed by using the rotary evaporation apparatus RE52-86A (Shanghai Yarong Biochemical Instrument Co. Ltd., China).

Hexane extraction of cold-pressed cake: The pine nut cake produced by cold-pressing was pulverized, and subjected to hexane extraction as described in "Hexane extraction".

Sub-critical extraction: The oil from approximately 200 g of pulverized pine nuts was extracted using sub-critical extraction apparatus CBE-5L (Henan Sub-critical Biotechnology Co. Ltd., Anyang, China). The extraction conditions were as follows: extraction solvent, *n*-butane; flow rate, 77 L/h; extraction pressure, 0.5 MPa; temperature, $(21\pm 5)\text{ }^{\circ}\text{C}$. The extraction was repeated three times.

2.3 Analytical Methods

The determination of protein, moisture, ash, and fat contents was performed using the methods of Association of Official Analytical Chemists^[13].

The fiber content was measured according to Van Soest et al.^[14].

The total sugar content was determined color-metrically based on absorption at 490 nm using glucose as the standard^[15].

The relative densities of the oils were measured using pycnometer method according to the National Standards of the People's Republic of China^[16]. The determination of refractive index was performed at room temperature using an Abbe refractometer (Beijing Winxinyio Science and Technology Development of Co. Ltd., China).

The color was determined using a Lovibond Tintometer WSL (Zhengzhou Zhonggu Mechanical Equipment Co. Ltd., China). The viscosity of the oil samples was determined using a digital viscometer DJ-5S (Shanghai Hengping Scientific Instrument Co. Ltd., China). The rotor (no. 1) and the temperature probe were immersed in the oil sample. The speed of rotation was then adjusted so that the measured value was within 20%–90% of the measuring range, and the rotation speed and the reading were recorded.

The odor and flavor were determined by five trained panelists as follows: after being heated to $50\text{ }^{\circ}\text{C}$, an oil sample of 10 g was whisked by glass bar, smelled, and tasted.

Iodine value, saponification value, acid value, and peroxide value were determined as described by the Association of Official Analytical Chemists^[13].

Oxidative stability of the pine nut oils was measured with the 743 Rancimat (Metrohm Co., Basel, Switzerland). The conditions were set at a temperature of $110\text{ }^{\circ}\text{C}$, and an air flow rate of 20 L/h

with sample of 3 g. Oxidative stability was expressed as the oxidative induction time^[17].

Fatty acid composition: Fatty acid methyl esters were prepared and analyzed as described by Rezig et al.^[18] using Gas chromatography (GC) with slight modifications. Briefly, the pine nut oil samples were methylated using 0.5 mol/L NaOH in methanol solution and boron trifluoride-methanol, successively. The fatty acid methyl esters (FAME) in chloroform was analyzed by GC 7890B (Agilent Technologies, Wokingham, UK) equipped with FID detector under the following conditions: the temperatures of inlet, column, and detector were $210\text{ }^{\circ}\text{C}$, $230\text{ }^{\circ}\text{C}$, and $300\text{ }^{\circ}\text{C}$, respectively. The flow rates of the nitrogen gas, the air, and the hydrogen gas were set as 1.0 mL/min, 400.0 mL/min, and 35.0 mL/min, respectively. The fatty acids were identified and quantified by comparing their retention times and areas with those of the FAME standards of known composition under the same conditions.

Tocopherol composition: Tocopherol composition was analyzed according to Przygoda et al.^[19] using HPLC (Waters e2695, USA) equipped with a fluorescence detector. The analysis conditions were as follows: column temperature, $25\text{ }^{\circ}\text{C}$; hexane/isopropanol (99:1, V/V) as the mobile phase; excitation wavelength, 295 nm; and emission wavelength, 330 nm. The vitamin E were quantified based on an external calibration method using α -, β -, γ -, δ -tocopherols, and α -, β -, γ -, δ -tocotriphenols (Sigma Chemicals, US) as the standards.

2.4 Statistical Analyses

Microsoft Excel 2010 was used to carry out statistical analyses. The data were expressed as the mean $\pm$ standard deviation (SD) of three independent experiments.

3 Results and Discussion

3.1 Chemical Composition

Table 1 shows that the pine nuts analyzed here had 17.41% protein, a value lower than that reported for *P. halepensis* Mill. (26.62%), but comparable to *P. pinaster* (16.25%) and *P. canariensis* (16.71%), and higher than *P. pinea* L. (14.25%)^[20]. The variation in the protein content is most likely due to differences between the plant species and/or climate, geography and cultivation practices. The contents of crude fiber, total ash and total sugars in the *P. koraiensis* nuts were 10.78%, 2.81% and 0.02%, respectively.

The *P. koraiensis* nuts has 68.67% crude fat, which is in accordance with that reported by Wolffet al.^[21], 0.53–0.58 times higher than that of *P. pinea*^[22], 0.40–1.22 folds higher than those reported for *P. pinaster*, *P. nigra*, *P. griffithii*, *P. sylvestris*, and

Table 1 Chemical composition of *P. koraiensis* nuts

Component	% (on dry weight basis)
Crude fat	68.67 $\pm$ 0.93
Protein	17.41 $\pm$ 0.16
Crude fibre	10.78 $\pm$ 0.09
Total ash	2.81 $\pm$ 0.02
Total sugars	0.02 $\pm$ 0.001
Moisture content	7.30 $\pm$ 0.02

P. mughus^[21], and 0.91–2.47 times more than that of *P. halepensis*^[23].

The crude fat content of the pine nuts is also higher than that reported for the *Cucurbita maxima* L. seed^[18], *Ximenia caffra* L. and *Riciodendron rautanenii* L. nuts from Zimbabwe^[24], *Arachis hypogaea* L.^[25], and *Torreya grandis* L. kernel^[26], and approximately comparable to *Corlylus avellana* L.^[27]. Combining its high yield and oil content, the *P. koraiensis* nuts have a great advantage as the promising resource for being used in the oil industry.

3.2 Oil Yield

Oil yield of pine nuts is illustrated in Table 2. The extraction rates for different technologies range from 45.91% to 55.38%, in accordance with that summarised by Xie et al.^[28]. The hexane extraction yielded the most oil of all the methods compared, and sub-critical extraction obtained the medium yield. The oil yield for different extraction processes in decreasing order is: hexane extraction > hexane extraction of cold-pressed cake > sub-critical extraction > hot pressing > cold pressing.

3.3 Physicochemical Properties of Pine Nut Oils

The color of the oil obtained by hexane extraction (HEO) and the oil obtained by sub-critical extraction (SEO) was pale yellow (Y30 R1.0 and Y30 R0.6, respectively) and transparent, while the oil obtained by hexane extraction of cold-pressed cake (HECCO) and the oil obtained by hot pressing (HPO) was yellow, and the latter was reddest of all the oils. The HPO had a strong, pleasant and characteristic odor and flavor of pine nuts, while that of the other oils was only slight, albeit characteristic. The oil obtained by cold pressing (CPO) had more water and unsaponifiable matter than oils from the other extraction processes, which render the oil opaque.

The relative density, viscosity, refractive index, saponification value and iodine value are important for assessing the quality of an oil. Relative densities and refractive indexes of the obtained oils were similar. HEO, HECCO and SEO had lower viscosity ranging from 80.77 to 85.03 MPa·s.

The saponification values reflect the C-chain length of the fatty acids. For the oils tested here, saponification values ranged from 167.34 mg KOH/g to 185.19 mg KOH/g, generally lower than those reported for *Torreya grandis* kernel oils^[26], indicating that the triacylglycerols of the pine nut oils had higher molecular weights,

and HECCO (185.19±6.45 mg KOH/g) had the smaller molecular weights than the oils from other extraction methods, while SEO (167.34 mg KOH/g) the highest molecular weights. Iodine value is an indicator of the degree of saturation of the fatty acids, and no significant difference was observed among the oil samples. The obtained oils had iodine values within the range of 137.14 and 141.61 g I₂/100g, higher than that of soybean oil^[29], *T. grandis* kernel oils^[26], and sunflower oil^[30]. The results indicate that pine nut oils have more unsaturated fatty acids.

Acid value and peroxide value are the indicators of the oils deterioration. The acid values of the pine nut oils were within the range of 0.36–0.99 mg KOH/g, reaching the National Standard of the People's Republic of China for third-class soybean oil^[31]. The acid values in decreasing order were HECCO > HPO > SEO > HEO > CPO. HECCO had a significant higher acid value than HEO ($P < 0.05$), indicating that pressing may increase the acid value, which is consistent with the finding of Kasote et al.^[32] The results may be attributed to the long extraction process including the cold-pressing extraction followed by the hexane extraction, in which cold pressing caused cells to break down, free fatty acids were released through enzymatic hydrolysis, and the acid value was increased.

While in terms of the peroxide values of 2.47–4.34 mmol/kg, the pine nut oils meet the National Standard of the People's Republic of China for the first-class soybean oil^[31]. The observed peroxide values were in decreasing order of CPO > HECCO > SEO > HPO > HEO. Combining the acid value and peroxide value, we can see that hexane extraction and sub-critical extraction yielded the oil with decent quality.

Based on the results, the *P. koraiensis* nut oils meet the National Standard of the People's Republic of China for the soybean oil^[31], indicating the oil is a good source of the edible oil. Furthermore, HEO and SEO was pale yellow and transparent, and had a pleasant and slight odor, meet the standard of Light industry Standard of the People's Republic of China for massage base oil and massage oil^[33], indicating that the pine nut oils obtained by hexane extraction, and sub-critical extraction may expand their application to the cosmetic industry.

3.4 Fatty Acid Profile

Table 3 shows the fatty acid compositions of pine nut oils. Overall, *P. koraiensis* nut oil has a high content of unsaturated

Table 2 Physicochemical parameters of pine nut oils

Parameters	CPO	HPO	HEO	HECCO	SEO
Oil yield (%)	45.91±2.01	46.09±1.08	55.38±2.15	52.71±1.93	49.71±1.26
Color	Y5, R0.5	Y30, R3.2	Y30, R1.0	Y40, R2.0	Y30, R0.6
Relative density (d ₄₂₅)	0.9269±0.0011	0.9275±0.0013	0.9225±0.0025	0.9234±0.0022	0.9205±0.0019
Viscosity (MPa·s)	104.00±3.23	115.84±3.78	81.81±1.66	80.77±4.72	85.03±3.51
Refractive index (n _D ²⁵)	1.4718±0.0026	1.4720±0.0041	1.4729±0.0035	1.4713±0.0086	1.4704±0.0024
Saponification value (mg KOH/g)	184.31±6.79	183.08±7.23	178.33±5.64	185.19±6.45	167.34±5.01
Iodine value (g I ₂ /100g)	137.14±3.29	140.72±4.33	138.89±3.55	141.03±4.63	141.61±4.89
Acid value (mg KOH/g)	0.36±0.01	0.83±0.01	0.51±0.02	0.99±0.01	0.64±0.02
Peroxide value (mmol/kg)	4.34±0.15	2.72±0.11	2.47±0.16	3.66±0.13	2.92±0.20
Water and unsaponifiable matter (%)	0.07±0.01	0.03±0.00	0.05±0.01	0.03±0.01	0.03±0.01

Note: CPO: the pine nut oil obtained by cold pressing; HPO: the pine nut oil obtained by hot pressing; HEO: the pine nut oil obtained by hexane extraction; HECCO: the pine nut oil obtained by hexane extraction of cold-pressed cake; SEO: the pine nut oil obtained by sub-critical extraction. The same below.

Table 3 Fatty acid compositions of the pine nut oils

Fatty acid (%)	CPO	HPO	HEO	HECCO	SEO
C16:0	5.21	5.20	5.16	5.48	5.20
C18:0	2.41	2.40	2.36	2.32	2.35
C18:1	27.10	27.09	26.91	26.71	27.07
C18:2	45.91	45.36	45.85	45.90	45.68
C18:3 (Pinolenic acid)	13.53	13.50	13.63	13.33	13.61
C18:3 (linolenic acid)	1.46	1.48	1.49	1.60	1.51
C20:0	0.68	0.67	0.68	0.74	0.68
C20:2	1.02	1.05	1.05	1.11	1.04
Trans-C18:2	2.23	2.23	2.24	2.09	2.24
Poly-unsaturated fatty acid (PUFA)	61.92	61.39	62.02	61.94	61.85
Unsaturated fatty acid (UFA)	89.02	88.48	88.93	88.65	88.92
Saturated fatty acid (SFA)	8.30	8.27	8.20	8.54	8.23

fatty acids (88.48%–89.02%), and among these, linoleic acid is most abundant (45.36%–45.91%), followed by oleic acid (26.71%–27.10%), comparable to that reported by Wang et al.^[51] It is generally recognized that unsaturated fatty acids can influence the fluidity and permeability of the cell membrane. Linoleic acid (C18:2) is important for the healthy growth of human skin, and oleic acid (C18:1) has positive effects on cardiovascular diseases prevention^[34]. Palmitic acid (C16:0) and stearic acid (C18:0) are the dominant saturated fatty acids, which is in good agreement with that reported by Imbs et al.^[35]. These two saturated fatty acids comprise 8.20%–8.54% of total fatty acids, render the oil resistant to oxidative rancidity. The fatty acid profile of *P. koraiensis* nut oil makes it desirable with respect to nutrition and it may be used in food industry, for example, as edible cooking oil, as frying oil, as salad oil, or for the manufacture of margarine.

According to classification of vegetable oils by Dubois et al.^[36], the *P. koraiensis* nut oil, containing a high content of linoleic and oleic acid, is classified as a polyunsaturated oil. This class includes a large quantity of vegetable oils such as sunflower, corn, sesame and wheat germ oils. Besides being used in the food industry, the oils with a high content of polyunsaturated fatty acid, such as *P. koraiensis* nut oil, have the potential to be used in the cosmetics industry^[37].

Pinolenic acid, typically present only in conifer nut oil, is 13.33%–13.63% in *P. koraiensis* nut oil, similar to that reported by Xie et al.^[28]. Lee et al.^[38] enriched the content of the pinolenic acid in *P. koraiensis* nut oil up to 45.1% using urea complexation and this concentration may enhance hepatic low-density lipoprotein (LDL) uptake and thus have LDL-lowering properties, indicating *P. koraiensis* nut oil may be a valuable source for pharmaceutical application.

The fatty acid compositions differed only slightly between the different extraction methods, indicating that the extraction method has little impact on the lipid profiles of the pine nut oil produced.

3.5 Content and Composition of the Tocopherols and Tocotrienols

Tocopherols and tocotrienols are natural antioxidants in most vegetable oils. Table 4 shows that the total contents of tocopherols and tocotrienols of the pine nut oils ranged from 27.65 to 37.52 mg/100 g, lower than that reported for *Pinus sibirica* nut oils (46.6–49.6 mg/100 g)^[39], *Curcubita maxima* seed oil (418.66 mg/100 g)^[18], *Chenopodium Quinoa* Willd. seed oil (72.78–336.00 mg/100 g)^[19], and *Punica granatum* L. seed oils (135.3–524.6 mg/100 g)^[40], but higher than that of walnut oil (24.9 mg/100 g), almond oil (25.0 mg/100 g), and peanut oil (4.8 mg/100 g)^[41].

α -tocopherol is the most abundant, accounting for 46.87%–54.42% of the total tocopherols and tocotrienols, followed by γ -tocopherol. While α -tocopherol is considered to be of most biological importance as a the vitamin E analogue, γ -tocopherol is reported to be more effective in preventing cellular damage induced by cytokines^[42].

The total contents of tocopherol and tocotrienol in descending order is HECCO > SEO > CPO > HEO > HPO. The oils from hexane extraction of cold-pressed cake and sub-critical extraction are richest in vitamin E, while the oil from hot pressing has the lowest contents of tocopherols and tocotrienols, which may be attributed that the high-temperature roasting involved in the hot pressing rendered the vitamin E destroyed.

3.6 Oxidation Stability

Rancimat test was carried out to measure the oxidation stability of the pine nut oils, and the oxidation induction time was determined (Table 5).

The oxidation induction time of the oils is 3.12–6.91 h, comparable to the walnut oil^[41], and the values in descending order is HECCO > HEO > CPO > SEO > HPO. The pine nut oils from hexane extraction, cold pressing, and sub-critical extraction

Table 4 Contents of tocopherols and tocotrienols in pine nut oils

Tocol (mg/100 g)	CPO	HPO	HEO	HECCO	SEO
α -Tocopherol	16.14±0.02	12.96±0.05	15.59±0.04	18.25±0.09	19.69±1.02
γ -Tocopherol	11.29±0.09	10.29±1.01	11.72±0.06	14.46±2.03	12.40±0.07
(β + γ)-Tocotrienol	4.39±0.01	4.49±0.01	4.26±0.01	4.81±0.01	4.09±0.02
Total tocol content	31.82±2.12	27.65±1.35	31.57±1.68	37.52±3.01	36.18±2.26

Table 5 The oxidation induction time of the pine nut oils

	CPO	HPO	HEO	HECCO	SEO
Oxidation induction time (h)	4.96±0.02	3.12±0.02	5.27±0.03	6.91±0.02	4.93±0.01

has better oxidation stability, while the oil from hot pressing had the shortest oxidation induction time, showing the similar trend with the content of vitamin E. The result might be due to that the vitamin E are the main contributors to the antioxidant stability of the pine nut oils. Arranz et al.^[41] also found that a significant correlation between 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging values of non-polar fraction and tocopherol content.

3.7 Potential Application of *P. koraiensis* Nut Oils Obtained by Different Extraction Technologies

Based on the above physicochemical and antioxidant evaluations, the *P. koraiensis* nut oils could be a good source of edible oil. Considering the increased need for affordable sources of healthy plant edible oil in developing countries, the *P. koraiensis* could be one of the good woody candidates for closing the gap between the demand and production of vegetable oils.

Furthermore, the various features of the resultant oils from the different extraction procedures may enable the pine nut oil to find a wide range of utilization. The oils from cold-pressing and sub-critical extraction can be used in cosmetology. Additionally considering the high pinolenic acid content, the relatively high yield and low cost, the oil obtained by hexane extraction may be used as raw material to obtain products with further enriched content of pinolenic acid for pharmaceutical application.

4 Conclusions

As we know, little detailed research has been focused on physicochemical properties, fatty acid profiles, and tocopherol compositions of *P. koraiensis* nut oils obtained by different extraction technologies. The results show that *P. koraiensis* nuts consist of high level of oil (68.67%). The pine nut oils are rich in linoleic acid, oleic acid, and pinolenic acid, and contained about 36 mg/100 g vitamin E, indicating that *P. koraiensis* nut oil has a high nutritive value and potential health benefits, and may be suitable for a wide range of edible oil application.

Furthermore, various physicochemical properties of resultant oils from different extraction procedures and different features of the extraction techniques allow the multiple utilization of *P. koraiensis* nut oil in edible oil, cosmetic, and pharmaceutical industry. Thus industrial scale production of the *P. koraiensis* nut is herein recommended.

Conflict of Interest

The authors declare that there is no conflict of interest.

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