

Physicochemical characteristics, thermal stability and antioxidant characteristics of *Trichosanthes kirilowii* maxim seed oil as affected by different extraction methods

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SUMMARY: In conducting this study, the extraction of *Trichosanthes kirilowii* maxim seed oils (TSO) was carried out with the help of cold pressing (CP), hot pressing (HP) and soxhlet extraction (SE). Investigation, together with comparison, was carried out with respect to the physicochemical properties, thermal stability and antioxidant action of TSO. The key ingredients in the seeds consisted of fat, fiber and protein. The physicochemical characteristics of the oils brought to light the fact that CPTSO possessed top oil quality. The findings also suggested that linoleic acid, punicic acid and oleic acid were the leading unsaturated fatty acids in TSO. It was also discovered that TSO had an almost identical chemical composition regardless of the extraction method was used. It was demonstrated by TG/DTG curves that both HPTSO and CPTSO had more thermal stability in comparison with SETSO. Furthermore, the antioxidant activity assessments emphasized that CPTSO had better radical scavenging potential. CP had the ability to deliver an extract with higher quality as well as antioxidant activity in comparison with HP and SE methods and can be taken into consideration as a more suitable method in order to attain high quality oil.

KEYWORDS: Antioxidant activity; Chemical composition; Fatty acid; Thermal stability; *Trichosanthes kirilowii* maxim seed oil

RESUMEN: Características fisicoquímicas, estabilidad térmica y características antioxidantes del aceite de semillas de *Trichosanthes kirilowii* maxim según diferentes métodos de extracción. Se realizó la extracción de aceites de semillas *Trichosanthes kirilowii* maxim (TSO) mediante prensado en frío (CP), prensado en caliente (HP) y extracción mediante soxhlet (SE) y se compararon las propiedades físico-químicas, la estabilidad térmica y la acción antioxidante de TSO. Las semillas estaban compuestas fundamental por grasa, fibra y proteína. Las características fisicoquímicas de los aceites pusieron de manifiesto el hecho de que el aceite de prensado en frío era de una calidad superior. Los estudios también pusieron de manifiesto que los ácidos linoleico, punicílico y oleico eran los principales ácidos grasos insaturados en TSO. Además, se constató que TSO presentaba una composición química casi idéntica, cualquiera que fuera el método de extracción utilizado. Es de destacar, por las curvas TG/DTG que tanto HPTSO como CPTSO tienen más estabilidad térmica que SETSO. Además, la evaluación de la actividad antioxidante determinó que CPTSO tiene un potencial de barrido radical más fuerte. El prensado en frío suministra un aceite con una calidad y actividad antioxidante superior, en comparación con HP, y con el SE, siendo el método más adecuado para obtener un aceite de alta calidad.

PALABRAS CLAVE: Aceite de semillas *Trichosanthes kirilowii* maxim; Ácido graso; Actividad antioxidante; Composición química; Estabilidad térmica

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1. INTRODUCTION

Over 80 species of *Trichosanthes* are found across the globe and, 40 of them are thought to exist in China. Traditional Chinese medicine has been putting the fruits, seeds, and roots to frequent use. Moreover, they are considered as some of 50 core herbs (Wang *et al.*, 2009). A good number of research studies have been carried out to report on the chemical components as well as the biological function of *T. kirilowii* including anti-tumor, anti-HIV, and anti-tyrosinase (Dat *et al.*, 2010). Huang *et al.* (2000) brought forth a report that *T. kirilowii* seeds contribute to an energy-affluent diet that contains 62% oil together with up to 30% proteins in addition to 2.5% mono- and oligosaccharides.

A good amount of attention has been received by TSO due to its high content in conjugated linolenic acids (CLNA) (Jiang *et al.*, 2015). Various health advantages have been shown by CLNA isomers that include anti-carcinogenic, lipid metabolism regulation, anti-inflammatory, anti-obese and anti-oxidant functions (Yuan *et al.*, 2014). Nevertheless, these uncommon fatty acids that are constrained to triacylglycerols are able to be simply oxidized and polymerized to viscous oils, despite being exposed to typical temperatures (Joh *et al.*, 1995). The methods used for oil extraction are likely to alter minor constituents that have functional properties and contribute to oxidation stability. Nowadays, more and more people have focused on cold extracted oils because the oils attained possess optimal nutritive characteristics. Cold pressing is termed to be a technology that does not take into account heat or chemical treatments throughout oil extraction. The fact that there is no refining also is part of cold pressing is likely to result in an optimal degree of lipophilic phytochemicals that includes natural antioxidants (Kiralan *et al.*, 2014).

This study aims at the extraction of oil from *T. kirilowii* seeds with the help of cold pressing, hot pressing and soxhlet extraction. A comparison study among the three oils with regards to their physicochemical properties and chemical composition in addition to thermal stability was carried out. Furthermore, the antioxidant potential of the oils was assayed by applying the b-carotene bleaching tests; 1-diphenyl-2-picrylhydrazyl (DPPH) and 2-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) radical scavenging examination.

2. MATERIALS AND METHODS

2.1. Specimens and reagents

A collection of *T. kirilowii* seeds was taken from the Jiangsu Provinces (China) from the *T. kirilowii*

seed yield in the month of October, 2015. Prior to analyses, the *T. kirilowii* seeds were persevered at -4 °C. DPPH, ABTS, Trolox, Rutin, Cholesterol, α -tocopherol and γ -tocopherol were secured from the Aladdin Industrial Corporation (Shanghai, China). The purchase of Fatty acid methyl esters (FAMES) was made from Nu-Chek-Prep Inc (Elysian, MN, USA). The procurement of the rest of the chemicals as well as reagents of analytical standard was done from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Chemical characteristics of *T. kirilowii* seeds

The determination of the fat, moisture, protein, ash, and crude fiber contents of the *T. kirilowii* seeds was carried out according to GB/T 14488.1-2008, GB/T 14489.1-2008, GB/T 14489.2-2008, GB/T 5505-2008 and GB/T 5515-2008 (Chinese national standard, 2008).

2.3. Oil extraction

The extraction of *T. kirilowii* seed oils was done with the help of three varied approaches listed to be the Soxhlet approach, Cold pressing and Hot pressing. According to the Soxhlet method, as described by GB/T 14488.1-2008, *T. kirilowii* seeds were directly pressed with a hydraulic press (T100 model, Shandon, China) for Cold pressing and Hot pressing. Cold pressing: *T. kirilowii* seeds were pressed at a temperature of 25 °C with no thermal processing. Hot pressing: *T. kirilowii* seeds were stir-fried 30 min, and pressed at a temperature of 110 °C. The other extraction condition was the same with the exception of a pressure of 50 MPa and pressing for 50 min. Collection and clarification of the pressed oils with fine suspended solids was carried out by centrifuging at 8000g for 15 minutes at 4 °C. Finally, the seed oils were put in dark bottles which were then flashed with nitrogen gas and placed in a freezer at -20 °C temperature for succeeding physicochemical analyses. The oil yield was computed with the help of the following equation:

$$\text{Oil yield (\%)} = [(M_1 - M_2) / M_1] * 100 \quad (1)$$

Where M_1 denotes the weights of *T. kirilowii* seeds (g) and M_2 represents the *T. kirilowii* seeds after extracted weight (g).

2.4. Determination of oil quality indices

The acid value (AV), peroxide value (PV), iodine value (IV), saponification value (SV) and refractive index (RI) shown by the oil specimens were determined according to GB/T 5530-2005, GB/T 5538-2005, GB/T 5532-2008, GB/T 5534-2008 and GB/T 5527-2010.

2.5. Determination of fatty acid (FA) composition

Preparation of the FAME solution of the oil specimens was done as per the bases of the method of Sun *et al.* (2013). 0.1 g of oil specimen was dissolved in 1 mL petroleum ether and 1 mL benzene, adequately shaken for the purpose of dissolution of the oil, and, thereafter, completely blended with 2 mL of a 0.4mol/L KOH-CH₃OH solution. For a time period of ten minutes, the mixture was stored at room temperature, and 6 mL of saturated NaCl solution were added. The upper layer was removed for the gas chromatography (GC) analysis.

The FA composition of the oil specimens was determined by GC instrument (Model GC-7890B, Agilent, USA) equipped with a DB-WAXETR capillary column (30 m×0.25 mm×0.25 μm, Agilent, USA) and a flame ionization detector (FID) in addition to helium as the carrier gas. 1 μL of the FAME solution was injected into the split mode at a ratio of 1:30. The column temperature program was as follows: 140 °C (2 min), 140-210 °C (10 °C/min), 210-250 °C (5 °C/min), 250 °C (5 min). The injector as well as detector temperatures were 280 °C. The identification of fatty acids was done by comparing their respective retention times (Rt) with matching standards. Furthermore, calculation of the composition of Fatty acids was done taking into account the relative FID response regions. All determinations were conducted in triplicate.

2.6. Determination of total phytosterols (TP)

The specimens were prepared in accordance with the approach by Chirinos *et al.* (2013). The TP content was brought to analysis as per the Sulfate-Phosphate-Ferric approach with some modifications. 4 mL of sample extract were placed in a 10 mL test tube in addition to 2 mL of a Sulfate-Phosphate-Ferric chromogenic agent, shaken and cooled to room temperature. Absorption at a value of 480 nm was calculated in a Shimadzu UV-Vis Spectrophotometer (Shimadzu UV-1800, KYOTO, JAPAN) against a blank sample. The TP was described as Cholesterol in milligrams per gram of oil, with the help of a standard curve ($Y = 0.0057X + 0.1084$ $R^2 = 0.9941$) produced with 50-200 μg/mL.

2.7. Determination of total tocopherols (TT)

Total tocopherols were determined with the help of a HPLC system. A Hypersil BDS C18 column (250×4.6 mm, 5 μm) was used with methanol/water (96:4) as the mobile phase with a flow rate of 1 mL/min. Specimen preparation was performed in accordance with the HY/T 1598-2008 (Chinese agricultural standard, 2008).

2.8. Determination of total flavonoids (TF)

The extraction of TF from the oil was done in accordance with the method of Liu *et al.* (2013). 10 mL of 90% alcohol were added to 1 g of oil sample, and extracted for 1 h by ultrasonic. After centrifugation (15 min, 4000 g, ambient temperature), the supernatants were separated, packed and retained in the dark at 4 °C well before the TF assay.

The analysis of TF was carried out in accordance with the method of Xu *et al.* (2015) with some modifications. With the rutin solution (0-0.1 mg/mL) as standard, the standard curve ($Y = 7.3111X + 0.0551$, $R^2 = 0.9981$) was drawn. In accordance with the drawn standard curve, the material of aggregate flavonoids was computed and described as the amount of rutin in weight (mg) in 1 g oil, mg/g.

2.9. Determination of total phenolic compounds (TPC)

Phenolic compounds were extracted from the oil in accordance with the method of Rombaut *et al.* (2015). The analysis of the TPC content was conducted in accordance with the Folin-Ciocalteu reagent process with some modifications. 0.2 mL of specimen extract were poured into a 20 mL test tube together with 1 mL of Folin-Ciocalteu reagent (Singleton *et al.* 1999). After one minute, 18.8 mL of sodium carbonate (4%) were added and mixed. The mixture was then placed in a 75 °C water bath for 10 min. Absorbance was measured at 760 nm. The TP material was expressed as gallic acid equivalents (GAE) in milligrams per gram of oil, with the help of a standard curve ($Y = 0.0484X + 0.0456$, $R^2 = 0.9982$) produced with 0-6 μg/mL.

2.10. Thermal stability of TSO

The evaluation of the thermal stability of the TSO was carried out with the help of the thermogravimetric (TG) method on a thermo-gravimetric analyzer (STA449F5, NETZSCH, Germany) taking into account the nitrogen and synthetic air atmosphere conditions. Ten mg of sample were heated at a rate of 10 °C/min from room temperature to 750 °C. TG curves, together with derivative curves (DTG) were used for analyzing the thermal stability of the TSO.

2.11. β-carotene bleaching test with TSO

Performance of the β-carotene bleaching test was done as stated by Miraliakbari and Shahidi after some modifications. A 3 mL amount of the β-carotene solution together with 40 mg linoleic acid in addition to 400 mg Tween 40 emulsifier were

inserted into a 100 ml flask with a round bottom. The removal of Chloroform was conducted under vacuum with the help of a rotary evaporator, and 100 mL of distilled water were put into the flask and vigorously shaken. Finally, 200 μ L of TSO or methanol (control) were added to trigger the reaction which was computed by examining the absorbance at 470 nm in cycles of 20 min for 120 min. The preparation of blank specimens devoid of β -carotene was performed for background subtraction. Evaluation of the potential of the extracts to resist the oxidation of β -carotene was done as follows:

$$\beta\text{-Carotene retention (\%)} = 100 - \left[\frac{(A_{0 \text{ Sample}} - A_{0 \text{ Blank}}) - (A_{120 \text{ Sample}} - A_{120 \text{ Blank}})}{(A_{0 \text{ Control}} - A_{120 \text{ Control}})} \right] * 100 \quad (2)$$

Where A represents the absorbance at a specific time

2.12. The ABTS scavenging activity of TSO

The aggregate antioxidant function of the oil extracts was calculated with the TEAC test as put forth by Magalhaes *et al.* (2008) after some modifications. The ABTS⁺ solution was mixed with alcohol, to an ultimate absorbance of the control of 0.7 ± 0.02 at 734 nm. 3 mL of ABTS⁺ solution were added to 1 mL of the specimen solution (1-100 mg/mL). The absorbance was measured at a value of 734 nm after 30 min, with ethanol as a blank. All analyses were conducted in triplicate. The aggregate antioxidant function was expressed as TEAC (Trolox equivalent antioxidant capacity), using a standard curve ($Y = 1.0024X + 25.415$ $R^2 = 0.9972$) generated with 4-72 μ mol/L.

2.13. DPPH scavenging capacity of TSO

The antioxidant activities of the oils were determined according to Sun *et al.* (2005) and Dalonso *et al.* (2012) with some modifications. First, 2 mL of 0.2 mmol/L of DPPH in ethanol were blended with 2 mL of the specimen solution (5-100 mg/mL). The absorbance A_i was measured at a value of 517 nm after incubation for 20 min at 25°C. 2 mL ethanol were taken to replace the sample solution to measure the absorbance A_0 . The specimen solution was

added with 2 mL of ethanol, and the absorbance A_j was measured. All measurements were carried out in triplicate. The calculation of the inhibition by DPPH radicals was made according to the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{[A_0 - (A_i - A_j)]}{A_0} * 100. \quad (3)$$

2.14. Statistical analysis

The entire number of trials was performed in triplicate and data were expressed as the means \pm standard deviations (SD). SPSS Version 19.0 software was used and the statistical analysis was conducted by one-way analysis of variance. Significance was stated at $p < 0.05$.

3. RESULTS

3.1. Chemical characteristics of *T. kirilowii* seeds

The average proximate composition of *T. kirilowii* seeds together with some literature references are shown in Table 1. The ash content (3.28%) together with the oil content (31.85%) were slightly higher in comparison with those previously reported in the literature (Solati *et al.*, 2013) whereas moisture content (5.77%) was lower and the crude fiber (20.61%) contained in the seeds was in agreement with those earlier described in the literature (Hu, 2004). This type of difference in nutrient concentrations among classes is likely to be attributed to changes in harvest areas, storage conditions and maturity stage. It may also be due to regional as well as climatic dissimilarities where *T. kirilowii* seeds are grown (Solati *et al.*, 2013). The present findings suggest that *T. kirilowii* seeds are a suitable means of protein as well as lipids with respect to human consumption.

3.2. Physicochemical Characteristics of TSO

The oil yield together with the physicochemical characteristics of TSO following different extraction

TABLE 1. Chemical characteristics of *T. kirilowii* seeds

Component	%	% Values in the literature		
		<i>T. uniflora</i> ^a	<i>T. truncate</i> ^a	<i>T. kirilowii</i> ^a
Oil	31.85 \pm 0.16	26.46	30.68	31.22
Moisture	5.77 \pm 0.12	7.65	5.98	6.88
Protein	20.24 \pm 0.35	18.35	20.46	18.48
Ash	3.28 \pm 0.13	2.25	2.14	1.98
Fibre	20.61 \pm 0.39	21.11	20.55	20.46

^a Hu *et al.* 2004.
Values are means \pm SD in triplicate.

methods are presented in Table 2. Soxhlet extraction produced the maximum oil yield (31.85%), followed by hot pressing (29.49%) and cold pressing (28.66%). Both AV and PV are considered the most important factor regarding the seed oil standard. The AV of TSO values obtained from the cold pressing, hot pressing and soxhlet methods were 0.45, 0.51 and 0.57 (mg KOH/g), respectively. In comparison with the other oils, a higher stability of the oil extracted by cold pressing was suggested by its low AV. The AV of CPTSO was lower as compared with those reported by Yan *et al.* (2008) and Jiang *et al.* (2015). The PV of cold pressing (4.56 meq/kg) together with that of hot pressing (4.86 meq/kg) were discovered to be exceptionally low as compared with that of the soxhlet extract (6.17 meq/kg). The PV showed by the entire amount of the extracted samples in this study was lower in comparison with those reported by Jiang *et al.* (2015). The IV suggests the level of

instauration and the value of 134.15 (g I₂/100 g) was found for cold pressing, 132.79 (g I₂/100 g) for hot pressing and 131.75 (g I₂/100 g) for the soxhlet extract, which is higher compared to the IV reported by Yan *et al.* (2008) regarding the soxhlet extract (120.90 g I₂/100 g). The SV displayed by the soxhlet extract was a bit lower (149.89 mg KOH/g) in comparison with the other extraction methods. All the corresponding values were exceptionally low in comparison with those reported by Yan *et al.* (2008) and Zeng *et al.* (2007). The RI shown by the TSO was identical to that reported by Yan *et al.* (2008). Furthermore, these findings were lower in comparison with those reported by Zeng *et al.* (2007).

3.3. Fatty acid composition of TSO

This study reported eleven fatty acids (Table 3). The following order as per the ranking of the major

TABLE 2. Effects of extraction on physicochemical parameters of TSO

Physicochemical indexes	CPTSO	HPTSO	SETSO
Oil yield (%)	28.66 ± 0.12a	29.49 ± 0.07b	31.85 ± 0.13c
AV (mg KOH/g)	0.45 ± 0.02a	0.51 ± 0.02b	0.57 ± 0.01c
PV (meq/kg)	4.56 ± 0.25a	4.86 ± 0.19b	6.17 ± 0.08c
IV (g I ₂ /100 g)	134.15 ± 1.52c	132.79 ± 1.77b	131.75 ± 1.02a
SV (mg KOH/g)	165.07 ± 2.43b	160.62 ± 3.13b	149.89 ± 2.68a
RI at 25°C	1.41 ± 0.00a	1.43 ± 0.00b	1.46 ± 0.00c

The results represent the mean of three replicates (Mean ± SD); the same superscripts in a same row do not differ significantly (p > 0.05).

TABLE 3. Effects of extraction on fatty acid composition (% of total fatty acids) of TSO

Compound	CPTSO	HPTSO	SETSO
C14:0	0.04 ± 0.00b	0.04 ± 0.00a	0.04 ± 0.00b
C16:0	3.94 ± 0.01a	3.92 ± 0.01a	3.91 ± 0.01a
C18:0	2.40 ± 0.00b	2.38 ± 0.00a	2.38 ± 0.00a
C18:1n-9	21.83 ± 0.06c	21.51 ± 0.06b	21.34 ± 0.06a
C18:1n-7	0.69 ± 0.01a	0.69 ± 0.01a	0.69 ± 0.00a
C18:2	41.02 ± 0.06b	40.80 ± 0.06a	40.78 ± 0.06a
C20:0	0.54 ± 0.00a	0.54 ± 0.54a	0.55 ± 0.02a
Punicic acid	25.57 ± 0.30a	25.57 ± 0.33a	26.01 ± 0.32a
α-Eleostearic acid	2.40 ± 0.08a	2.70 ± 0.04b	2.59 ± 0.03b
Catalpic acid	0.87 ± 0.07a	1.12 ± 0.05b	0.98 ± 0.04a
C20:1	0.70 ± 0.09a	0.73 ± 0.09a	0.73 ± 0.09a
SFA	6.92 ± 0.02a	6.89 ± 0.02a	6.88 ± 0.03a
MUFA	23.22 ± 0.15b	22.92 ± 0.16ab	22.76 ± 0.16a
PUFA	69.86 ± 0.16a	70.19 ± 0.18ab	70.36 ± 0.19b
Unsaturates	93.08 ± 0.02a	93.11 ± 0.02a	93.12 ± 0.03a
PUFA/SFA	10.09	13.52	10.22

The results represent the mean of three replicates (Mean ± SD); the same superscripts in the same row do not differ significantly (p > 0.05). SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids.

fatty acids was determined: linoleic acid (LA, C18:2), punicic acid (PA), oleic acid (OA, C18:1n-9), palmitic acid (C16:0), α -Eleostearic acid, stearic acid (C18:0). Palmitic acid and stearic acid were the key saturated fatty acids (SFA) in TSO whereby their contents were in the range of 3.91 to 3.94% and 2.38 and 2.40%, respectively. The palmitic acid and stearic acid contents of CPTSO had higher values in comparison with HPTSO and SETSO. LA, PA, and OA were the prominent unsaturated fatty acids (UFA) in TSO, and accounted for 40.78 to 41.02%, 25.57 to 26.01% and 21.34 to 21.83%, respectively. OA was the most abundant monounsaturated fatty acid (MUFA) in the TSO. The material of OA categorization leading to CPTSO, HPTSO, SETSO, PA, α -Eleostearic acid and catalpic acid contained three isomers of CLNA found in TSO, which is in agreement with the findings of Joh *et al.* (1995). PA appeared as the major CLNA isomer; the content of PA reported by Joh *et al.* (1995) and Yang *et al.* (2011) were higher in comparison with what we found. Nevertheless, Yang *et al.* (2011) reported no catalpic acid content in TSO, and the LA content was higher compared to the findings of Joh *et al.* (1995) and Yang *et al.* (2011). CPTSO showed the highest LA content (41.02%), while SETSO possessed the highest PA content (26.01%). CPTSO had the maximum MUFA material while showing the least amount of PUFA material. The PUFA material of TSO stood considerably higher as compared with 49.7% as reported by Wang *et al.* (2009). The PUFA/SFA ratio came out to be 10.09 (CPTSO) to 13.52 (SETSO), which was consistent with Yang *et al.* (2011). A PUFA to SFA ratio of more than 1.5 is linked with goodness of fit, and this is why TSO is considered healthy. The fatty acid profile together with high quantities of PUFA makes the TSO a prime constituent for nutritional applications.

3.4. Bioactive compound of TSO

The bioactive compounds (TF, TP, TT and TPC) of the extracted oils of the three methods are given in Table 4. Except for TP, there was no significant difference in the different bioactive compounds of the TSO extracted by different methods ($p < 0.05$). Phenolics as well as flavonoids were the key constituents of

non-nutritive compounds and presented antioxidant, anti-mutagenic, anti-inflammatory, and anti-carcinogenic properties, which aid in the prevention of atherosclerosis (Lee *et al.* 2006). HPTSO had the highest TF content (1.33 ± 0.06 mg RT/g), followed by CPTSO. However, the content of TPC in CPTSO was higher than HPTSO, and the content of TF and TPC in SETSO was the lowest. Phytosterols are the major constituents of the unsaponifiable matter in vegetable oils and fats. This fraction, possessing an intricate composition, can reach up to 10% or more in particular plants. A sterol analysis provides useful information with regards to both the quality and the identity of the probed oil and can be considered as a fingerprint. CPTSO had the highest TP content (5.80 ± 0.29 mg/g), and the lowest TP content was found for HPTSO. In this way, high temperatures are likely to damage the flavonoids in the oil. The tocopherols contained in vegetable oil are thought to protect the polyunsaturated fatty acids from peroxidation. The highest TT content (9.89 ± 0.18 mg/100g) was SETSO, and the TT content of CPTSO was less than SETSO. The TT content of HPTSO was the lowest, possibly because of the difference in the solubility of tocopherols due to different systems. In summary, the four kinds of bioactive compounds were found to have high contents in CPTSO in comparison with HPTSO and SETSO, especially in TP and TPC, and have a direct relationship with the antioxidant activity of TSO.

3.5. Thermal stability analysis

The TG/DTG curves of TSO showed the thermal events presented in Table 5 whereby, an observation of 10% weight loss in the was made at 389.50 °C (CPTSO), 391.86 °C (HPTSO) and 389.41 °C (SETSO) in a static climate. On the other hand, regarding oxidative atmosphere, this weight loss was observed at 369.90 °C (CPTSO), 395.69 °C (HPTSO) and 356.82 °C (SETSO). This behavior remained constant with respect to weight losses of 50 and 90%, suggesting that air present in the combustion of triacylglycerides in an oxidizing climate, resulted in a rapid thermal decomposition of the oil.

The TG/DTG curves of TSO in a still N₂ atmosphere (A and C) as well as in an air atmosphere

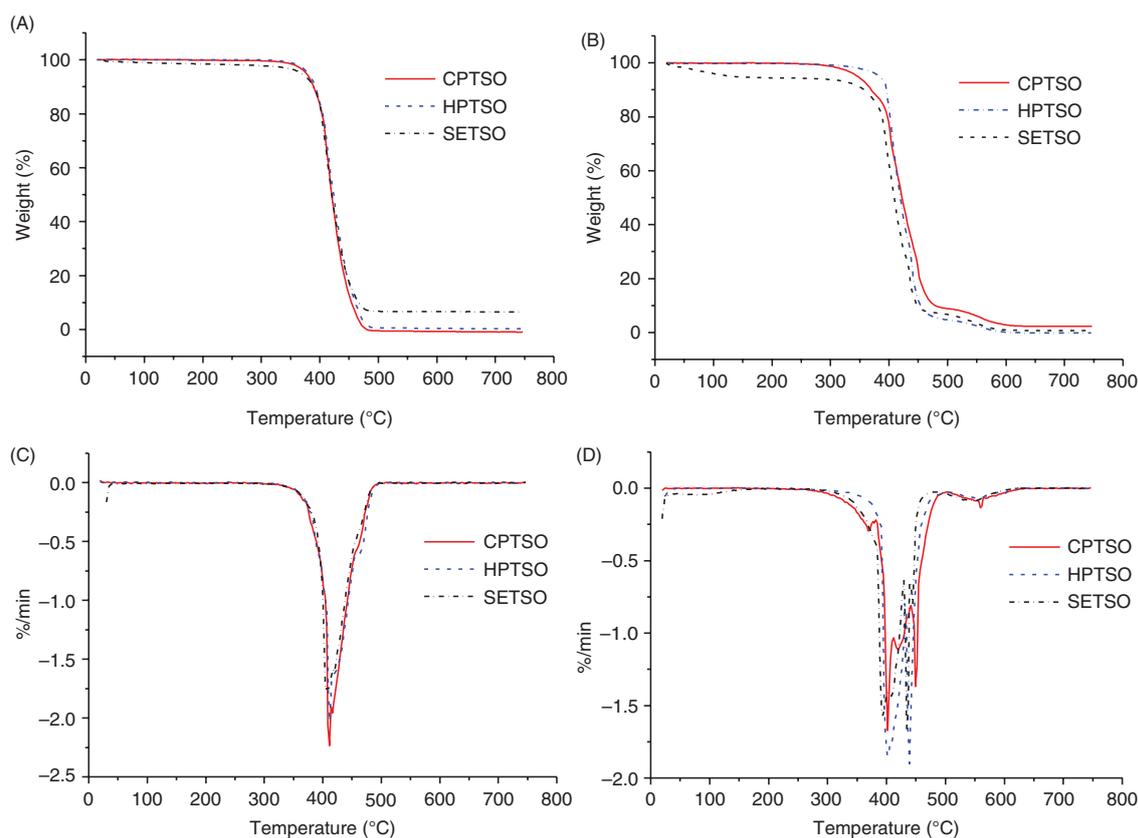
TABLE 4. Bioactive substances in TSO

Compounds	CPTSO	HPTSO	SETSO
TF (mg RT/g)	$1.24 \pm 0.07a$	$1.33 \pm 0.06a$	$1.16 \pm 0.09a$
TP (mg/g)	$5.80 \pm 0.29b$	$4.57 \pm 0.40a$	$5.71 \pm 0.47b$
TT (mg/100 g)	$9.78 \pm 0.17a$	$9.71 \pm 0.17a$	$9.89 \pm 0.18a$
TPC (mg GAE/100 g)	$1.97 \pm 0.05a$	$1.95 \pm 0.19a$	$1.87 \pm 0.33a$

The results represent the mean of three replicates (Mean \pm SD); the same superscripts in the same row do not differ significantly ($p > 0.05$). TF total flavonoids, TP total phytosterol, TT total tocopherol, TPC total phenolic compounds.

TABLE 5. Temperatures of mass loss (10, 50 and 90%) of the TSO under N₂ and air atmospheres.

Sample	Temperature of Mass loss (%)					
	N ₂ atmosphere (%)			Air atmosphere (%)		
	10	50	90	10	50	90
CPTSO	389.50°C	420.43°C	454.10°C	369.90°C	422.67°C	478.95°C
HPTSO	391.86°C	423.39°C	460.96°C	395.69°C	419.25°C	453.74°C
SETSO	389.41°C	420.62°C	466.41°C	356.82°C	406.83°C	447.95°C

FIGURE 1. TG/DTG curves of CPTSO, HPTSO and SETSO at 10 °C/min in N₂ (A, C) and air atmosphere (B, D).

(B and D) are presented in Figure 1. The extrapolated onset temperature was held at 411.56 °C for CPTSO in nitrogen. In addition, HPTSO came out to be the highest, having a temperature of 412.14 °C, which was almost 3 °C higher in comparison with that of SETSO. As the evaluations of the oil in air atmosphere were made, HPTSO showed the top thermal stability, followed by CPTSO and SETSO. The final decomposition temperature of CPTSO as well as that of SETSO was almost 508 °C, which is higher than that of HPTSO (497 °C). Only one stage of weight loss was found under nitrogen in all the oils and decomposition took place at a temperature of 400-450 °C, which is in agreement with the thermal decomposition shown by the oils (Garcia 2007). In accordance with oxidizing climate, the mechanism

was found to be intricate due to the reaction of oils as well as the presence of oxygen. It took four phases for the decomposition of all the oils to take place. The weight loss of SETSO occurred at the temperature about 50-100 °C, because of the residual organic solvents in the oil. CPTSO and SETSO possessed quite an identical decomposition temperature at 400 °C in the foremost phase. HPTSO and SETSO had same decomposition temperature at 436 °C in the second phase and at 550 °C in the 4th stage, while CPTSO met decomposition at the temperature of 450 °C in the third stage and at 560 °C in the fourth stage. The results indicated that CPTSO may be for use as frying oil. The four discrete steps for the purpose of oil degradation in normal air consist of decomposition of PUFA, MUFA, SFA, in addition to the oxidation of

TABLE 6. Antioxidant activity of TSO

Property	CPTSO	HPTSO	SETSO
β -carotene (%)	28.49 \pm 1.40a	29.61 \pm 0.22b	45.03 \pm 0.36c
ABTS (μ mol Trolox/L)	67.87 \pm 1.22a	63.78 \pm 1.45b	57.12 \pm 1.98c
DPPH (IC50 mg/mL)	18.68 \pm 0.68a	20.27 \pm 0.35a	23.51 \pm 0.41b

The results represent the mean of three replicates (Mean \pm SD); the same superscripts in the same row do not differ significantly ($p > 0.05$).

carbonaceous residue, respectively. The rule governing vegetable oil decomposition takes into account the oxidation of fatty acid constituents. In this way, the loss of weight by oils arising out of elevated temperatures was balanced by the absorption as well as reaction of fatty acids with atmospheric oxygen (Solís-Fuentes 2010).

3.6. Inhibition of β -carotene bleaching

The β -carotene bleaching test is considered to be a convenient test when it comes to measuring the capacity of a compound or a blend for the inhibition of the oxidation of β -carotene (Miraliakbari *et al.* 2008). Our findings brought to light the fact that the SETSO had the highest antioxidant function, with a 45.03% reduction in β -carotene following an assessment for 120 minutes (Table 6). The HPTSO showed the second highest activity (29.61% of β -carotene remaining after 120 min assay), leading to CPTSO (28.49% of β -carotene remaining after 120 min assay). The β -carotene bleaching test shares similarities with an oil-in-water emulsion system; dissimilarities in the solubility of antioxidant compounds pose impact on their function in this assay (Miraliakbari *et al.*, 2008). Hydrophobic antioxidants are suggested to deliver a more efficient performance in comparison with hydrophilic antioxidants in the β -carotene bleaching test by focusing on the lipid phase as well as the lipid-water interface, and in this way, fighting lipid radical formation in a direct manner together with β -carotene oxidation (Frankel 2000).

3.7. ABTS free radical scavenging activity

The ABTS test provides the most frequent, convenient and straightforward approach for the purpose of estimating the scavenging potential of free radicals. It has the bases of the reduction of the absorbance of the solution of the radical ABTS⁺, falling within the range of 710-760 nm, relying on the solvent, because of its inactivation from antioxidants. It is applied to examine both lipophilic and hydrophilic substances for their antioxidant properties (Christodouleas *et al.*, 2014).

The scavenging capacity of TSO with regards to ABTS free radicals is presented in Table 6. All the scavenging actions showed a dose-dependent

approach at saturations falling in the ranges of 0 to 100 mg/mL. In addition, all the specimens displayed powerful radical scavenging functions when given higher doses. CPTSO showed the most powerful ABTS scavenging function among the analyzed specimens. When given the dose of 40 mg/mL, the ABTS free radical scavenging capacities of TEAC value of CPTSO, HPTSO, and SETSO were determined as 67.87, 63.78 and 57.12 μ mol/L, respectively. The findings suggested that TSO possesses a sizeable scavenging ability against the ABTS free radical, and CPTSO possesses higher ABTS free radical scavenging abilities than HPTSO and SETSO.

3.8. DPPH scavenging ability

The impact posed by antioxidants on DPPH radical scavenging is typically ascribed to a hydrogen-donation capacity (Birasuren *et al.*, 2013). For the purpose of evaluating this action, the DPPH test makes use of an extensive and user-friendly protocol, despite the fact that it does not take into account an oxidizable substrate (Grajeda-Iglesias *et al.*, 2016). Findings from the computation of the scavenging operation of TSO are presented in Table 6 whereby every specimen showed a concentration-dependent scavenging action against the DPPH radical. The IC50 values for the DPPH radical stood at 18.68, 20.27 and 23.51 mg/mL for CPTSO, HPTSO and SETSO, respectively. CPTSO exhibited better antiradical activity than HPTSO and SETSO.

4. CONCLUSION

The key composition in *T. kirilowii* seed consisted of crude fat (31.85%) and fiber (20.61%) in addition to some quantities of protein, moisture and ash. Carrying out the comparison of the three extraction approaches, the oil extracted by cold pressing presented the lowest oil yield; although it showed improved values with respect to AV, PV, IV and SV. Nevertheless, the extraction approach had no influence on the preservation of the functional compounds, for example, fatty acids. The major fatty acids in CPTSO included linoleic acid together with punicic acid and oleic acid. Unsaturated fatty acids accounted for 93.08% of the aggregate fatty acids. The results of our study in bioactive compounds, thermal stability and antioxidant activity

of TSO showed that the extraction method affected the quality of the oils. HPTSO displayed the optimal thermal stability, followed by CPTSO and SETSO. CPTSO had higher active substances and antioxidant capacity than HPTSO and SETSO. This study showed that good quality TSO can be extracted using cold pressing and the CPTSO could be explored as use in medicine or functional foods.

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