J Am Oil Chem Soc (2017) 94:779–786
DOI 10.1007/s11746-017-2983-1

ORIGINAL PAPER

Physicochemical Properties of *Acer truncatum* Seed Oil Extracted Using Supercritical Carbon Dioxide

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Received: 11 November 2016 / Revised: 20 March 2017 / Accepted: 26 March 2017 / Published online: 12 April 2017

Abstract *Acer truncatum* seed oil rich in nervonic acid was extracted using supercritical carbon dioxide. GC (Gas Chromatography) analysis revealed that the oil contained approximately 6.22% nervonic acid. The *sn*-2 compositions were also determined using lipase hydrolysis. A total of 52 triacylglycerides (TAG) were tentatively identified in the oil using an ultra-performance convergence chromatography (UPC^2) coupled with quadrupole time-of-flight mass spectrometry (Q-TOF-MS) for the first time. In addition, the contents of phytosterols (1961.9–2402.8 μmol/kg) and β-carotene (2.09–2.35 μmol/kg) were also quantified for the first time, along with tocopherols (2352.0–2654.3 μmol/kg). The γ-tocopherol (1296.9-1442.3 μmol/kg) was the primary tocopherol, while β-sitosterol (1355.2–1631.3 μmol/kg) was the dominant phytosterol. The physicochemical properties of the oil were also investigated. This study indicated that *A. truncatum* seed oil is rich in nervonic acid and other nutraceutical constituents. It has a high potential in functional foods for improving human health.

Keywords *Acer truncatum* seed oil · Nervonic acid · UPC^2-Q-TOF-MS analysis · *sn*-2 · Nutraceutical constituents · Physicochemical properties

Introduction

Nervonic acid (*cis*-tetracos-15-enoic acid; 24:1 Δ15) is an important long-chain monounsaturated fatty acid beneficial for brain health through improving the biosynthesis and maintenance of nerve cell myelin [1] and enhancing neurodevelopment in premature infants [2]. The seed oils of a few plants such as *Lunaria annua* (Money Plant), *Borago officinalis* (Borage), *Cannabis sativa* (Hemp), *Acer truncatum* (Purpleblow maple), *Tropaeolum speciosum* (Flame flower), *Cardamine graeca* (Bittercress) and *Malania oleifera* have been reported to contain nervonic acid [3, 4]. New natural plant resources rich in nervonic acid for large scale production are in high demand because most of these plants have limited availability and are not suitable for commercial production.

Acer truncatum (*A. truncatum*) is a native plant widely distributed in Northern China. A previous study showed that *A. truncatum* seeds contained more than 42.0% oil, and more than 92% of that is unsaturated fatty acids including 25.8% oleic, 37.3% linoleic, and 5.5% nervonic acids [5]. *A. truncatum* seed oil (ATO) is usually obtained by cold pressing and commercialized as a crude oil. However, cold pressing is known for its relative low oil recovery from the seeds. Reversely, supercritical carbon dioxide ($SC\text{-}CO_2$) is known for its environmental and food safety, inexpensive, nontoxic a high penetrability into solid matrices and easily being removed from the extracts, but its ability to extract ATO has not been investigated [6–8].

Electronic supplementary material The online version of this article (doi:10.1007/s11746-017-2983-1) contains supplementary material, which is available to authorized users.

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It has also been noted that the absorption and metabolism of fatty acids (FA) depend on their *sn*-positions esterified on the TAG backbone. It is well demonstrated that the FA located at the *sn*-2 position are physiologically important, because the *sn*-1 and *sn*-3 positions of triacylglyceride (TAG) can be hydrolyzed by the pancreatic lipase which is regiospecific for the external positions of TAG, thus giving *sn*-2 monoacylglycerols that can be absorbed efficiently [9–11]. Thus, it is interesting to determine the *sn*-2 fatty acid compositions of ATO.

The aim of this study is to investigate FA composition, the *sn*-2 fatty acid profile, and triglyceride composition of *A. truncatum* seed oil rich in nervonic acid prepared using SC-CO_2 extraction. The SC-CO_2 extracted ATO was also compared with that obtained by Soxhlet extraction with petroleum ether for their nutraceutical components including tocopherols, phytosterols and β-carotene, as well as physicochemical properties including iodine value, saponification value, and acid and peroxide values. In addition, the thermal properties and the oxidative stability of the ATO were examined. The results from this study may promote the value-added commercial utilization of SC-CO_2 extracted ATO.

Materials and Methods

Plant Materials and Sample Preparation

The seeds of *Acer truncatum* Bunge were collected in Jinyang, Shaanxi Province, China by Shaanxi Nature Fragrance Biotechnology Development Co., Ltd. The seeds were de-shelled and the kernels were pressed to an average thickness of about 400 μm. The pressed kernels were dried at 60 °C for 0.5 h, and stored at 4 °C until used. The moisture content of the flaked kernel was about 4.5% (w/w).

Supercritical Carbon Dioxide (SC-CO_2) Extraction

The SC-CO_2 extraction was carried out in a 100-mL stainless steel vessel (SFT-100 XW model of Supercritical Fluid Technology, Newark, DE, USA) with a maximum operating pressure of 68.9 MPa (10,000 psi). Approximately 5.5 g *A. truncatum* deshelled pressed seeds were used for each extraction. The optimal parameters for SC-CO_2 extraction were determined to be 39 MPa, 44 °C and 10 h resulting in a maximum yield of 43.1% (w/w). The detailed procedure was shown in the Supplementary Data.

Soxhlet Extraction

The ATO was extracted with petroleum ether (60–90 °C) using a Soxhlet apparatus for 6 h. The residual solvent was removed at 90 °C under reduced pressure with a rotary evaporator (Senco R206, Shanghai, China). The oil yield was gravimetrically calculated as an average of three extractions.

GC Analysis of Fatty Acids

Fatty acid composition was determined according to AOCS method Ce 1e-89 [12] and similar to that reported by Adhikari *et al*. [13]. After methylation, methyl esters (0.2 μL) were injected into a gas chromatography (GC) (Agilent 7820A GC System, Agilent Technologies, Little Falls, Del., 93 USA) equipped with a Agilent GC capillary column (CP-sil 88 100 m × 0.25 mm, Agilent Technologies, Little Falls, Del., 93 USA). The initial temperature of oven was 80 °C and held for 2 min. Then, the oven temperature was increased to 120 °C at a rate of 10 °C/min. Following that, oven temperature was increased to 180 °C and held for 2 min then increased to 206 °C at a rate of 2 °C/min and finally increased to 230 °C at a rate of 25 °C/min and held for 5 min. The injector and detector temperatures were set at 250 °C, and 280 °C, respectively. Fatty acid composition at the *sn*-2 position was determined using the pancreatic lipase method (Shanghai Maikun Chemical Co. Ltd. Shanghai, China). Each ATO sample (0.1 g) was placed in a test tube and 0.02 g of pancreatic lipase (Shanghai Maikun Chemical Co. Ltd., Shanghai, China), 2 mL of Tris-buffer (pH 8), 0.5 mL of sodium tauroglycocholate, and 200 μL of saturated $CaCl_2$ were added. The mixture was stirred for 1 min and heated at 40 °C for 3 min. This procedure was repeated 3 times. Thereafter, 1 mL of hydrochloric acid and 1 mL of diethyl ether were added to this mixture. Thin-layer chromatographic (TLC) separation was conducted using hexane/diethyl ether/ethyl acetate/formic acid (60:38:2:1, v/v/v/v) as the mobile phase, and the monoacylglycerol band was collected for methylation, and subjected to GC analysis.

UPC^2-Q-TOF-MS Analysis of TAG

One gram of ATO was dissolved in 10 mL hexane/isopropanol (7:3, v/v) and filtered through a 0.22-μm filter membrane prior to TAG analysis using UPC^2-Q-TOF-MS. The analytical conditions were set according to a laboratory procedure [14]. Briefly, the Waters Acquity UPC^2 system (Acquity UltraPerformance Convergence Chromatography, Milford, MA, USA) was equipped with a binary solvent delivery pump, an autosampler, a column oven and a back pressure regulator. The qualitative analysis was performed at 50 °C using an Acquity UPC^2 BEH 2-EP column (150 × 3.0 mm i.d.; 1.7 μm; Waters, Milford, MA, USA). The elution gradient (eluent A, CO_2; eluent B, acetonitrile: ethanol = 1: 1, v/v) started at 0.2%

B; increased via linear gradient to 0.7% B at 5 min, 0.8% B at 10 min, 1.2% B at 15 min, 2.0% B at 20 min, and 12% B at 25 min. The back pressure was set at 1600 psi. The flow rate was 1.2 mL/min and the injection volume was 1.0 μL.

Analysis of Tocopherols, β-Carotene, and Phytosterols

Tocopherol profile was determined according to AOCS method Ce 8–89 [15] as described by Follegatti-Romero *et al*. [6]. β-Carotene was determined using a procedure described by Gimeno *et al*. [16]. Phytosterols were analyzed according to the AOCS Method Ch 6–91 [17] as described by Adhikari *et al*. [13].

Physicochemical Analyses

The iodine value was estimated according to the AOCS method Cd 1c-85 [15]. The free fatty acid content was determined using the potentiometric titration method (modified AOCS method Ca 5a-40 [15]). Peroxide and saponification values were quantified according to AOCS methods Cd 8–53 [18] and Cc 17–79 [15], respectively.

Oxidative stability of the oil samples was determined as the induction time using the Rancimat method (Rancimat 743, Metrohm, Switzerland) at 120 °C. For oxidative stability, 2.5 g of the sample was weighed into the glass vessel. Conductometric cells were filled with 80 mL of distilled water and air was passed through the heated oils at a flow rate of 20 L/h.

Thermal Properties of ATO

Thermal analysis of ATO was conducted using a differential scanning calorimeter (DSC Q2000, TA Inc., New Castle DE, USA). An empty aluminum pan was used as the reference. Samples (5 mg) were accurately weighed for DSC. The sample was heated to 60 °C and held for 10 min. Thereafter, the temperature was decreased at 5 °C/min to −70 °C. After holding at −70 °C for 10 min, the melting curve was obtained by heating to 60 °C at 5 °C/min.

Statistical Analysis

Data were reported as the means ± standard deviation (SD) for triplicate analyses. Statistical analysis was performed using Statistical Analysis System Software (SAS Institute, Inc., version 9, Cary, NC, USA), and the significance was set at $P < 0.05$.

Table 1 Fatty acid profiles of *A. truncatum* seed oil obtained by SC-CO_2 extraction under the preferred operating conditions (39 MPa, 44 °C, 10 h)

	FAC (g/100 g)		*sn*-2 (g/100 g)	
	Mean value	SD	Mean value	SD
C16:0	3.64	0.07	0.25	0.07
C16:1	0.05	0.05	–	–
C18:0	2.30	0.01	–	–
C18:1	24.03	0.51	29.52	0.05
C18:2	32.90	0.47	65.68	0.01
C18:3	2.61	0.4	3.76	0.51
C20:0	0.24	0.01	–	–
C20:1	7.90	0.05	0.47	0.02
C20:2	0.30	0.01	–	–
C22:0	0.87	0.03	–	–
C22:1	18.25	0.69	0.16	0.02
C24:0	0.35	0.11	–	–
C24:1	6.22	0.28	0.09	0.01
SFA	7.39	0.05	0.25	0.01
MUFA	56.46	0.32	30.24	0.02
PUFA	36.15	0.29	69.44	0.17

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, - not detected

Mean values represent the means of three replicates

Results and Discussion

Fatty Acid (FA) Profiles and *sn*-2 Compositions

The FA profiles of ATO obtained by SC-CO_2 extraction under a preferred operating condition (see Table S1-S2 and Figure S1-S2 in the Supplementary Data) predicted by the response surface methodology (RSM) are presented in Table 1. More than 13 fatty acids were detected in the ATO. The primary fatty acids included linoleic (32.90%), oleic (24.03%), erucic (18.25%), eicosenoic (7.90%), and nervonic (6.22%) acids. This nervonic acid content is greater than that of 5.52% reported by Liu *et al*. [1, 5], suggesting that ATO could serve as a good plant resource of nervonic acid. The difference might be attributed to the different subspecies, geographical, and climatic conditions as well as the harvesting time of *A. truncatum* seeds. The unsaturated fatty acids of ATO were more than 92%—this is greater than that in rice bran oil (75.0%) [19], olive oil (85.1%), sunflower oil (88.6%), and soybean oil (85.1%) [20].

The *sn*-2 fatty acid composition analysis showed a small amount of nervonic acid (0.09%) located at the *sn*-2 position. This was consistent with previous findings that long-chain fatty acids such as erucic acid (22:1

Δ13), docosadienoic acid (22:2 Δ5, Δ13) and nervonic acid (24:1 Δ15) were predominantly located at the *sn*-1,3 positions of the glycerol backbone [3]. Linoleic acid tended to distribute more at the *sn*-2 position (65.68%), while eicosenoic and erucic acids were more located at the *sn*-1,3 positions. The type and positional distribution of fatty acids on the glycerol backbone might influence the physical behavior and metabolism of the dietary fats. A previous study showed that unsaturated fats were easily metabolized and absorbed *in vivo* when they were present at the *sn*-2 position [21] These data suggested that the bioavailability and health benefits of nervonic acid from ATO may be limited as it is primarily located at the *sn*-1 and *sn*-3 positions.

Triacylglyceride Compositions

UPC^2 is an emerging analytical method that has been successfully used to analyze the TAG composition in milk fat [14]. In this study, UPC^2 was connected with TOF-MS to detect the TAG composition in ATO for the first time (Table 2). A total of 52 different TAG were tentatively identified in ATO. For each TAG, the relative amount was calculated as the ratio of its peak area to the sum of the fatty acid peak areas. The LLE, OLE, and OOL were the three most abundant TAG and accounted for 23.5% of the total triglycerides. Fifteen monosaturated TAG (29.12% of the total) and eleven disaturated TAG (6.7% of the total) were found. There were no trisaturated TAG. The major TAG containing nervonic acid were NeLL, NeOL, NePO and NeOO, respectively.

Tocopherol, β-Carotene, and Phytosterol Contents in ATO Obtained by SC-CO_2 Extraction and Soxhlet Extraction

Table 3 presents the tocopherol, phytosterol and β-carotene contents in ATO extracted by SC-CO_2 and Soxhlet methods, respectively. Significant differences ($P < 0.05$) were observed between the nutraceutical contents extracted by the two methods. The tocopherol contents obtained by SC-CO_2 extraction varied from 2352.0 to 2654.3 μmol/kg under different processing conditions. The tocopherol content in ATO via Soxhlet extraction was 2403.4 μmol/kg, which was lower than that (3025.2 μmol/kg) by solvent extraction method in a previous report [22]. The variance may be due to different sources of raw materials. However, the total tocopherol contents of ATO obtained by these two methods were much greater than many common vegetable oils such as cotton seed oil (864.5 μmol/kg), tea seed oils (279.0 μmol/kg), and palm oil (152.4 μmol/kg) [22]. γ-Tocopherol was the predominate tocopherol in ATO followed by δ-tocopherol, α-tocopherol and β-tocopherol.

The phytosterol and β-carotene contents of ATO were reported for the first time in this study. Four phytosterols including β-sitosterol, stigmasterol, campestarol and campesterol were detected. The β-sitosterol was the dominant phytosterol in the ATO. The total phytosterol contents of ATO obtained via SC-CO_2 extraction ranged from 1961.9 to 2402.8 μmol/kg depending on the extraction parameters, which was comparable to that of 2181.8 μmol/kg obtained by Soxhlet extraction. The content of β-carotene varied from 1.75 to 2.35 μmol/kg in the ATO extracted by SC-CO_2, whereas the β-carotene amount was 2.09 μmol/kg in that obtained by Soxhlet extraction. In brief, the SC-CO_2 extraction had a significant difference in extracting these health beneficial components from *A. truncatum* seeds as compared to the Soxhlet technique.

It was also noted that the tocopherol, phytosterol and β-carotene contents in the ATO extracted by SC-CO_2 decreased as a function of increasing yield (Table 3). Follegatti-Romero *et al*. [6] reported similar results for the tocopherol content in the Sacha inchi oil extracted by SC-CO_2. This phenomenon can be explained by the greater selectivity of SC-CO_2 in extracting tocopherols than triglycerides. This indicates that the tocopherol concentration in ATO was higher during the initial extraction phase and diluted during the subsequent extraction phases [23]. This theory may also explain the similar results in extraction of phytosterol and β-carotene.

Physicochemical Characteristics of ATO Obtained by SC-CO_2 Extraction and Soxhlet Extraction

Physicochemical properties of ATO extracted by SC-CO_2 or Soxhlet method were also determined and compared as shown in Table 4. There was no significant difference ($P > 0.05$) in the saponification value (SC-CO_2: 183.0 mg KOH/g oil; Soxhlet: 179.0 mg KOH/g oil) and iodine value (SC-CO_2: 111.8 g I_2/100 g oil; Soxhlet: 108.7 g I_2/100 g oil) for the ATO extracted via the two methods, consistent with the previous observations [6]. However, there were significant differences ($P < 0.05$) between the acid values (SC-CO_2: 1.33 mg KOH/g oil; Soxhlet: 1.04 mg KOH/g oil) and the peroxide values (SC-CO_2: 1.21 meq/kg oil; Soxhlet: 2.69 meq/kg oil) in the oils extracted by the two methods. ATO extracted by SC-CO_2 showed a higher acid value than that obtained by Soxhlet extraction, which was similar to the result of Sacha inchi seed oil from SC-CO_2 extraction [6]. This might be explained by the fact that free fatty acids are more soluble in SC-CO_2 than the mono-, di-, and tri-acylglycerides [24]. However, the peroxide value of ATO extracted by SC-CO_2 was lower than that obtained by Soxhlet extraction, indicating that a lower extraction temperature and oxygen-free extraction condition might play a role. In

Table 2 Tentative identification of TAG in *A. truncatum* seed oil by UPC^2-Q-TOF-MS

Peak no.	Structure	Retention time (min)	Exact mass $([M + NH_4])^+$	Calculated mass	Relative percentage (%)
1	PPO	18.38	850.7874	850.7864	0.13
2	POO	19.37	876.8018	876.8020	1.15
3	PSLn	20.23	874.7795	874.7864	0.44
4	POL	20.23	874.7867	874.7864	2.45
5	PSO	20.55	878.8091	878.8177	0.31
6	OLnLn	20.55	894.7065	894.7551	0.18
7	LLLn	20.55	894.7567	894.7551	0.40
8	POLn	20.69	872.7707	872.7707	2.20
9	PLLn	20.86	870.7045	870.7551	0.12
10	OOO	21.32	902.8186	902.8177	5.51
11	LnLnG	21.40	922.7385	922.7864	0.37
12	PPL	21.47	848.7711	848.7707	0.27
13	SOLn	21.47	900.7952	900.8020	1.17
14	OOL	21.47	900.8030	900.8020	5.72
15	SSO	21.47	906.7624	906.8490	1.91
16	PPE	21.47	906.8424	906.8490	0.23
17	SSL	21.71	904.7470	904.8333	1.49
18	POG	21.71	904.8348	904.8333	1.91
19	OLL	21.74	898.7872	898.7864	5.56
20	OLLn	21.84	896.7192	896.7707	0.31
21	LLL	21.84	896.7716	896.7707	2.09
22	PLE	21.88	930.8502	930.8490	5.11
23	PSE	22.06	934.8375	934.8803	0.28
24	SSG	22.06	934.8735	934.8803	0.10
25	OLG	22.10	928.8284	928.8333	0.98
26	SLnG	22.10	928.8340	928.8333	4.37
27	POE	22.13	932.8663	932.8646	1.94
28	LLG	22.30	926.8196	926.8177	4.48
29	NePO	22.34	960.8098	960.8959	2.08
30	LLnG	22.45	924.8038	924.8020	0.71
31	NePL	22.45	958.8135	958.8803	0.43
32	SLE	22.45	958.8740	958.8803	1.65
33	OOE	22.45	958.8823	958.8803	5.36
34	NePS	22.55	962.9057	962.9116	0.17
35	SLnE	22.62	956.8576	956.8646	2.10
36	OLE	22.62	956.8665	956.8646	8.30
37	LLE	22.69	954.8505	954.8490	9.43
38	SOE	22.79	960.8894	960.8959	1.87
39	LLnE	22.90	952.8362	952.8333	1.62
40	OGE	22.90	986.9055	986.9116	0.98
41	NeOO	22.90	986.9148	986.9116	1.81
42	NeSS	22.94	990.9166	990.9429	1.35
43	NeOL	23.01	984.8983	984.8959	3.97
44	LGE	23.01	984.8900	984.8959	0.94
45	NeLL	23.08	982.8832	982.8803	3.41
46	PEE	23.15	988.9213	988.9272	0.57
47	NeLLn	23.22	980.8680	980.8646	0.40
48	OEE	23.22	1014.9374	1014.9429	0.21

Table 2 continued

Peak no.	Structure	Retention time (min)	Exact mass $([M + NH_4])^+$	Calculated mass	Relative percentage (%)
49	GGE	23.22	1014.9462	1014.9429	0.32
50	NeLE	23.22	1040.9634	1040.9585	0.20
51	LEE	23.25	1012.9303	1012.9272	0.88
52	LnEE	23.39	1010.9185	1010.9116	0.09

P palmitic acid, *O* oleic acid, *Ln* linolenic acid, *S* stearic acid, *L* linoleic acid, *G* eicosenoic acid, *E* erucic acid, *Ne* nervonic acid

Table 3 Oil yield (w/w), tocopherol, phytosterol, and β-carotene content (μmol/kg) of *A. truncatum* seed oil obtained by SC-CO_2 extraction under different extraction conditions and Soxhlet extraction

	A[a]	B	C	D	Soxhlet
Oil yield	29.05	29.79	39.76	43.07	45.98
α-Tocopherol	362.9 ± 2.3a	344.3 ± 0.9b	316.7 ± 1.2c	313.7 ± 1.4c[b]	332.7 ± 1.6b
β-Tocopherol	78.5 ± 1.4a	77.8 ± 1.0a	78.2 ± 1.4a	75.1 ± 1.2a	76.5 ± 0.5a
γ-Tocopherol	1442.3 ± 6.0a	1375.6 ± 3.4b	1310.8 ± 3.1c	1296.9 ± 3.6c	1336.0 ± 6.5bc
δ-Tocopherol	770.6 ± 4.5a	695.9 ± 3.6b	668.6 ± 3.6c	666.3 ± 4.2c	658.1 ± 2.5c
Total tocopherol	2654.3 ± 11.0a	2493.6 ± 6.2b	2374.3 ± 4.6d	2352.0 ± 3.6e	2403.4 ± 8.4c
Campesterol	147.5 ± 1.5a	135.5 ± 1.7b	132.5 ± 1.7b	130.0 ± 2.2b	147.0 ± 1.5a
Campestarol	78.3 ± 1.5a	70.5 ± 0.8b	60.7 ± 1.0c	58.2 ± 1.5c	61.0 ± 1.8c
Stigmasterol	545.7 ± 3.4a	490.9 ± 5.8b	438.6 ± 1.0c	418.5 ± 5.6c	458.7 ± 2.7c
β-Sitosterol	1631.3 ± 5.1a	1555.6 ± 2.7b	1376.2 ± 2.7c	1355.2 ± 4.6c	1515.1 ± 3.6b
Total phytosterol	2402.8 ± 6.9a	2252.5 ± 5.4b	2008.0 ± 6.4d	1961.9 ± 5.7e	2181.8 ± 5.9c
β-Carotene	2.35 ± 0.06a	1.86 ± 0.04c	1.79 ± 0.06c	1.75 ± 0.06c	2.09 ± 0.04b

[a] A to D means *A. truncatum* seed oil exacted under different conditions. *A* 25 MPa, 35 °C, 8 h; *B* 25 MPa, 55 °C, 8 h; *C* 35 MPa, 45 °C, 8 h; *D* 39 MPa, 44 °C, 10 h

[b] Values with different letters in the same row indicate significant differences ($P < 0.05$); the mean values represent the means of three replicates

Table 4 Physicochemical properties of *Acer truncatum* seed oil extracted using supercritical carbon dioxide and Soxhlet

Extraction method	SV	IV	AV	PV	Oxidative stability
SC-CO_2	183.0 ± 2.0a[a]	111.8 ± 2.4a	1.325 ± 0.04a	1.21 ± 0.06a	6.64 ± 0.09a
Soxhlet	179.0 ± 1.0a	108.7 ± 1.6a	1.04 ± 0.03b	2.69 ± 0.05b	6.57 ± 0.21a

ATO samples were obtained by SC-CO_2 extraction under the optimal conditions (39 MPa, 44 °C, 10 h) as predicted by RSM and Soxhlet extraction

The data represent the means of three replicates ± standard deviation

[a] Values with different letters within columns indicate significant differences ($p < 0.05$)

addition, there was no significant difference ($P > 0.05$) on induction period during the Rancimat tests with IP values of 6.64 and 6.57 h for SC-CO_2 and Soxhlet extracted ATO, respectively. The oxidative stability of the ATO was similar to that of the crude rice bran oil [25] and greater than that of commercial soybean (IP, 4.00 h) and rapeseed oils (IP, 4.10 h) under the same testing conditions [26]. The result indicated that ATO has a good oxidative stability even though the content of unsaturated fatty acids was as high as 92%. This might be due to the high content of monounsaturated fatty acids, tocopherol, phytosterol and β-carotene.

Table 5 The melting enthalpy and melting peaks of ATO obtained by SC-CO_2 extraction

Sample	ΔH (J/g)	Tpeak1 (°C)	Tpeak2 (°C)
ATO	54.3	−28.8	−19.2

Under the optimal operating conditions: 39 MPa, 44 °C, and 10 h

Thermal Properties of ATO

DSC was used to evaluate the thermal properties of the ATO obtained by SC-CO_2 extraction under the optimal

operating conditions (39 MPa, 44 °C, and 10 h) and the data are shown in Table 5. In general, saturated fatty acids have strong molecular interactions and stacked linear structures that require much energy to melt. On the contrary, unsaturated fatty acids showed a bent structure with weak molecular interactions requiring less energy to melt [27]. Via DSC analysis, the melting behavior of the crude ATO showed a melting enthalpy of 54.3 J/g, which is similar to that of a rice bran oil (59.6 J/g) [27] and much lower than palm kernel oil (114.1 J/g) and a palm oil (86.3 J/g) with their relatively high contents of saturated fatty acids [28]. Together, the results might be attributed mainly to the high content of unsaturated fatty acids in ATO.

DSC showed two broad endothermic peaks at −28.8 and −19.2 °C, respectively. In general, this phenomenon could be ascribed to the diversity of TAG and the melting-recrystallization of the original TAG [28]. Considering the 52 TAG identified in ATO by UPC^2-Q-TOF-MS, the appearance of multiple peaks may further demonstrate the diversity of TAG in the ATO. On the other hand, the oils melted completely above 0 °C, which could also indicate a high content of unsaturated fatty acids. The result was very consistent with the FA and TAG compositions of the ATO.

Conclusion

In summary, the ATO extracted from *A. truncatum* seeds using SC-CO_2 contained approximately 6.22% nervonic acid primarily located at the *sn*-1,3 position. The oil also contained significant levels of tocopherols, phytosterols and β-carotene. The triglyceride profile, phytosterols, β-carotene concentration, thermal properties, and oxidative stability of ATO were reported for the first time. These results suggested that ATO may serve as a dietary source of nervonic acid, and warrant additional research in enhancing the bioavailability of this nutraceutical fatty acid through different food and lipid chemistry approaches.

Acknowledgements This research was financed in the foundation program of industry research from the Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd. The authors wish to thank the QC group for providing technical support.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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