J Am Oil Chem Soc (2016) 93:995–1005
DOI 10.1007/s11746-016-2835-4

ORIGINAL PAPER

Extraction and Characterization of Montmorency Sour Cherry (*Prunus cerasus* L.) Pit Oil

Nathan M. Korlesky[1] · Lucas J. Stolp[1] · Dharma R. Kodali[1] · Robert Goldschmidt[2] · William C. Byrdwell[2]

Received: 15 July 2015 / Revised: 1 April 2016 / Accepted: 15 April 2016 / Published online: 30 April 2016

Abstract Montmorency sour cherry (*Prunus cerasus* L.) pit oil (CPO) was extracted and characterized by various methods including: GC, LC–MS, NMR, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and X-ray powder diffraction (XRD). The oil gave an acid value of 1.45 mg KOH/g, saponification value of 193 mg KOH/g and unsaponifiable matter content of 0.72 %. The oil contained oleic (*O*) and linoleic (L) acids as the major components with small concentrations of α-eleostearic acid (El, 9*Z*,11*E*,13*E*-octadecatrienoic acid) and saturated fatty acid palmitic (P) acid. The CPO contained six major triacyglycerols (TAG), OOO (16.83 %), OLO (16.64 %), LLO (13.20 %), OLP (7.25 %), OOP (6.49 %) and LElL (6.16 %) plus a number of other minor TAG. The TAG containing at least one saturated fatty acid constitute 33 % of the total. The polymorphic behavior of CPO as studied by DSC and XRD confirmed the presence of α, β′ and β crystal forms. The oxidative induction time of CPO was 30.3 min at 130 °C and the thermal decomposition temperature was 352 °C.

Keywords *Prunus cerasus* L. · Sour cherry · Fatty acids · XRD · Triacylglycerol · Oxidative stability · Thermal stability · Eleostearic acid · Polymorphism

✉ Dharma R. Kodali
dkodali@umn.edu

[1] Department of Bioproducts and Biosystems Engineering, University of Minnesota, 2004 Folwell Ave., St. Paul, MN 55108-1038, USA

[2] Food Composition and Methods Development Lab, USDA, ARS, BHNRC, 10300 Baltimore Ave., Beltsville, MD 20705-2350, USA

Abbreviations

Fatty acids

M	Myristic (14:0)
P	Palmitic (16:0)
Po	Palmitoleic (16:1*n*-7)
L	Linoleic (18:2*n*-6)
El	α-Eleostearic (18:3*n*-5)(9Z,11E,13E-octadecatrienoic acid)
Ln	Linolenic (18:3*n*-3)
S	Stearic (18:0)
O	Oleic (18:1*n*-9)
A	Arachidic (20:0)
G	Gadoleic(20:1*n*-11)
B	Behenic (22:0)
Lg	Lignoceric (24:0)
APCI	Atmospheric pressure chemical ionization
CPO	Sour cherry (*Prunus cerasus* L.) pit oil
DAG	Diacylglycerol(s)
DSC	Differential scanning calorimetry
OIT	Oxidative induction time
TAG	Triacylglycerol(s)
TGA	Thermogravimetric analysis
XRD	X-ray diffraction

Introduction

Montmorency variety sour cherries (*Prunus cerasus* L.) are grown worldwide, with Russia accounting for nearly 16 % of total production and the United States accounting for around 10 % [1]. Recent statistics report sour cherry production at about 290 million pounds in the United States for 2014

Table 1 Summary of literature reports on sour (*P. cerasus*) and sweet (*P. avium*) cherry species

Reference	Species	Common name	Location	El	TAG composition	Comment
Farrohi and Mehran (1975) [6]	*P. avium*, *P. cerasus*	Sweet cherry, sour cherry	Iran	Not reported	Not reported	Reported fatty acids represent both species
Takagi and Itabashi (1981) [7]	*P. species*	Hood river cherry (sweet cherry)	USA	10.6 %	Not reported	A cross reference indicates *Prunus yedoensis* Matsum
Comes *et al.* (1992) [8]	*P. avium*	Sweet cherry	France	10–13 %	Were reported	The main fatty acids reported were L, O, El, and P
Chandra and Nair (1993) [9]	*P. cerasus*	Sour cherry (Montmorency)	USA	Not reported	Reported a single TAG OLO	Reported O and L
Kamel and Kakuda (1992) [10]	*P. avium*, *P. cerasus*	Sweet cherry, sour cherry	Canada	Not reported	Not reported	Reported fatty acids represent both species
Matthaus and Ozcan (2009) [11]	*P. cerasus*	Sour cherry	Turkey	Not reported	Not reported	The main fatty acids reported were O, L, and P
Popa *et al.* (2011) [12]	*P. cerasus*	Sour cherry	Romania (Banat)	Not reported	Not reported	The main fatty acids reported were O, L, and S
Yilmaz and Gokmen (2013) [13]	*P. cerasus*	Sour cherry	Turkey	Not reported	Not reported	The main fatty acids reported were O, L, P and Ln by different extraction methods
Vidrih *et al.* (2014) [14]	*P. avium*	Sweet cherry	Slovenia and Norway	Norway: 11.5 %, Slovenia: 8.7 %	Not reported	Location significantly influenced the fatty acid distribution
Ozcan *et al.* (2015) [15]	*P. avium*, *P. cerasus*	Sweet cherry, sour cherry	Turkey	Not reported	Not reported	Reported fatty acids O, L, and P individually for both species

[2], resulting in pit byproduct amounts of 35–44 million pounds [3]. Michigan is the largest domestic sour cherry producer, followed by Utah. The cherry flesh is nutritious and primarily used in food products such as pie fillings, cherry preserves, and juice concentrates. The pits, however, currently have few value-added uses with the majority being disposed of in landfills. Small amounts of pits are utilized in therapeutic products such as heating and cooling packs, used as a heating fuel [4, 5], or added as filler in cement manufacturing. Given the large amount of sour cherry fruit produced for food consumption, finding value-added applications for this undervalued, abundantly available pit byproduct is an economic necessity. Like other nut oils, the sour cherry (*P. cerasus*) pit oil (CPO) can become a value added product if its composition and properties are fully understood.

The cherry species belong to the genus *Prunus* that includes plums, peaches, nectarines, apricots and almonds. The kernel oils from two cherry species, sour cherry (*P. cerasus*) and sweet cherry (*Prunus avium* L.), have been extensively studied, as reported in the literature, including a very recent report that appeared during the publication of the current study [6–16]. The oil characteristics from these two species differ from each other in terms of fatty acid composition, including the presence of a physiologically important fatty acid, α-eleostearic acid (El), as well as differing triacylglycerol(s) (TAG) compositions. In some literature reports there is ambiguity and overlap between these two cherry species. The findings of these earlier studies are summarized in Table 1.

The present study is concerned with the characterization of CPO including its properties and composition. In contrast to Chandra and Nair, who identified oleic and linoleic acids and 1,3-dioleoyl-2-linoleoyl glycerol (OLO) in the oil, five other studies including a recent publication reported different fatty acid compositions [11–13, 15, 16]

without any TAG compositions. These studies reported the presence of other fatty acids including saturated fatty acids (6–19 %), in addition to oleic and linoleic acids. Some of these studies also identified minor components like tocopherols in the CPO [11, 13, 16]. Recently a report by Górnás *et al.* submitted after, but published before this current report showed the fatty acid, tocopherol, and sterol compsitions of six cultivars of sour cherry from Baltic countries and Russia [16]. However, the oil composition including the specific TAG, minor components, and the oil properties were not fully investigated. In the present study we report a thorough evaluation of the CPO, with extensive analysis of the oil's fatty acid, TAG and diacylglycerol(s) (DAG) compositions, along with other minor components such as tocopherols and sterols. The oil's physical and functional properties were also evaluated in order to find value added applications for this undervalued resource.

Materials and Methods

Materials

Hexane, ethyl alcohol, toluene, potassium hydroxide, sodium hydroxide, hydrochloric acid, *n*-butyl alcohol, isopropyl alcohol, phenolphthalein, and $CDCl_3$ were all ACS grade and used as received from Sigma Aldrich (St. Louis, MO). Thin layer chromatography was carried out on Analtech (Newark, NJ) Uniplate 250 μm silica gel plates coated with fluorescent indicator. The plates were sprayed with a 50 % H_2SO_4 solution and charred on a hot plate for visualization. Commercial oils, canola oil from Roundy's (Milwaukee, WI), soybean oil from Cargill (Minnetonka, MN), and high oleic soybean oil from Du Pont (Wilmington, DE), were used for comparison. Eleostearic acid (9Z, 11E, 13E octadectrienoic acid) for fatty acid confirmation was ordered as the methyl ester in ethanol solution, ≥98 % purity, from Cayman Chemicals, Inc (Ann Arbor, MI) and as the free fatty acid, ≥97 % purity, from Larodan Fine Chemicals (Solna, Sweden).

CPO Extraction and Purification

Sour cherry pits (*P. cerasus*) were provided by Cherry Central (Traverse City, Michigan) from fruit grown in Payson, Utah. The pits were cracked using a hydraulic press, with the kernels separated from the shells using a brine solution (specific gravity = 1.3). The resulting kernels were rinsed with water and dried overnight at 105 °C. The dried kernels were pulverized in a mill and the oil was extracted using hexane in a Soxhlet apparatus for 24 h. The solvent was removed *in vacuo* to afford the crude CPO. The crude CPO was purified by eluting through a 60 Å (mesh 200–425) silica column to remove polar components.

Physical Properties

The oil was analyzed in triplicate for the acid value (AOCS Official Method Cd 3d-63), saponification value (AOCS Official Method Cd 3-25) and unsaponifiable matter (AOCS Official Method Ca 6a-40) [17].

Fatty Acid Distribution

An Agilent Technologies, Inc. (Santa Clara, CA) 6890 N gas chromatograph (GC) with a flame ionization detector (FID) was used for analysis of the fatty acid methyl esters (AOCS Official Method Ce-1b-89), using the column and conditions previously described [18]. The saponification temperature was lowered to 50 °C to minimize isomerization of El, confirmed by analysis of the pure fatty acid. The retention times and monoisotopic masses of El and all other fatty acid methyl esters were confirmed by GC–MS on an Agilent 7890A GC with 5975C MSD, using the same column and conditions as used for GC-FID. Chemical ionization with methane gas was employed for GC–MS.

TAG and DAG Distribution

An Agilent 1200 liquid chromatography (LC) system using OpenLab Chemstation software was used for the reversed-phase analysis of DAG and TAG. Two Inertsil ODS-2 columns (25 cm × 4.6 mm, 5 μm particles) in series, maintained at 10 °C, were employed, as described elsewhere [19]. A column-switching dual liquid chromatography with quadruple parallel mass spectrometry (LC2/MS4) system was used for this analysis, as a proof of concept experiment. An Agilent 1290 LC system using OpenLab Chemstation software was used as a second LC system operating in normal-phase mode for analysis of the polar bolus switched from the Agilent 1200 reversed-phase LC system. However, since the oil was purified on a silica column no substantial polar components were detected.

Effluent from the reversed-phase LC system was monitored using atmospheric pressure chemical ionization (APCI) MS on a TSQ Vantage EMR mass spectrometer, atmospheric pressure photoionization on a TSQ Quantum Access Max mass spectrometer, and electrospray ionization on a Q Exactive high-resolution, accurate-mass orbitrap mass spectrometer, all from Thermo Scientific, Inc. (San Jose, CA) in a 'triple parallel mass spectrometry' configuration (LC1/MS3). Effluent from the normal-phase LC system was monitored using an LCQ Deca XP ion trap mass spectrometer operated in APCI mode (LC1/MS1). LC and MS instruments were coordinated using the wireless communication contact closure system recently described with minor modifications [20].

Table 2 CPO physical properties of current and previous studies

Assay	Current	Popa *et al*. [12]	Ozcan *et al*. [15]
Phosphorus (ppm)	443	–	–
Acid value (mg KOH/g)	1.45	1.0	2.5 (calc)
Saponification value (mg KOH/g)	193	183	187
Unsaponifiable matter (%)	0.72	–	0.93

APCI-MS response factors for TAG were calculated from fatty acid molar response factors as previously described [21] and the resultant fatty acid distribution from the response-factor-adjusted TAG composition was calculated. The spreadsheet to perform these calculations is available online at: http://www.ars.usda.gov/services/software/software.htm?modecode=80-40-05-05.

Oil Stability

The thermal decomposition temperature of the CPO was determined by thermogravimetric analysis (TGA) carried out on a TA Instruments Q50. A 10-mg sample in an aluminum pan was heated from 25 to 500 °C at 10 °C/min under an N_2 atmosphere and the decomposition temperature of the sample was determined by the onset temperature of the major weight loss.

Determination of the oxidative induction times (OIT) of sour cherry oil, canola oil, soybean oil, and high oleic soybean oil was carried out on a differential scanning calorimetry (DSC) instrument (TA Instruments Q2000) following a modified version of ASTM International Methods D3895-07 [22] at 130 °C.

Minor Component Analysis

Analysis of tocopherols, sterols, and phosphorus content of CPO was performed by POS Bio-Sciences (Saskatchewan, Canada). Phosphorus analysis followed AOCS Ca 20-99 [17]. Tocopherols and sterol analysis followed a method based on a previous publication [23].

Polymorphic Behavior

The polymorphic behavior was studied by DSC. Approximately 5.0 mg of CPO sample in an aluminum hermetically sealed pan was loaded at room temperature. The sample was cooled from 25 to −80 °C, incubated for 10 min at −80 °C, and heated back to 25 °C. The rate of cooling and heating was 2.5 °C/min.

The polymorphs were identified by wide angle x-ray powder diffraction (XRD) on a Bruker D8 Advance instrument with a Cu source of 40 kV and 40 mA using a Lynx-eye detector. The scans were taken from 5 to 35° of the 2θ range with a step size of 0.02°, using dwell times of 0.5 s at a wavelength of $\lambda = 1.54059$ Å. The sample was cooled on the XRD stage to −80 °C and the scans were recorded at −80 °C, −35 °C, −18 °C, and 0 °C. The XRD scan temperatures were adapted from the DSC analysis for the different polymorphs.

Spectroscopy

^{1}H and ^{13}C NMR spectra were obtained on a Varian Inova 500 MHz spectrometer running VnmrQ 2.2 software. The spectra were recorded at 25 °C in $CDCl_3$. An IR spectrum was recorded on a Thermo Fisher Scientific Nicolet iS5, of a neat sample between two NaCl plates.

Results and Discussion

The cherry pits were cracked, the kernels separated and pulverized, and the oil extracted by hexane using a Soxhlet apparatus. The weight of the kernels was found to be 22.4 % of the pits. The oil yield from kernels was 30.9 wt% similar to the earlier reported values of oil content by Ozcan *et al*. [15]and recently reported values of Górnás *et al* [16]. The overall oil content of the pits was 6.9 %. These results are similar to those of Popa *et al*. [12], but differed from other previous reports [9–11, 13]. This variability in oil content of the kernels is partially due to the influence of various geographic locations and conditions [9, 10, 12, 14, 16].

The extracted CPO was subjected to various wet chemical analysis methods to determine the properties, and the results are presented in Table 2. The oil contained 443 ppm phosphorus, higher than the value of 263 ppm reported by Kamel and Kakuda for sweet cherry kernel oil [10]. The phospholipid content of the CPO can be estimated by assuming a similar phospholipid distribution as soybean oil. Using the phospholipid to phosphorus weight ratio of 31.7 for soybean oil [24], the estimated phospholipid content of the CPO was 1.4 %. The acid value of the oil depends upon the age and moisture content of the kernels, along with extraction conditions. The acid value of CPO was 1.45 mg KOH/g, higher than most commercial oils, which may require refining before utilization in value added applications. The saponification value of 193 mg KOH/g and unsaponifiable matter of 0.72 % compare reasonably with the previously reported values [12, 15] (Table 2).

The fatty acid distribution of the CPO was determined using GC-FID and compared to previously reported data (Table 3). The recent results of Górnás *et al* [16] are not included as they reported the fatty acid compositions of

Table 3 Fatty acid distribution (%) of CPO of the current study compared with previously reported values

Fatty acid	Current[a]	Chandra and Nair [9]	Matthäus and Ozcan[b] [11]	Popa *et al*. [12]	Yilmaz and Gokmen [13]	Ozcan *et al*. [15]
M (14:0)	0.07	–	–	0.5	–	–
P (16:0)	8.18	–	5.3	11	6.23	6.08
Po (16:1n-7)	0.63	–	–	–	–	–
S (18:0)	2.46	–	1.5	6.4	1.33	–
O (18:1n-9)	47.62	63.6	63.9	42.9	48.80	43.55
L (18:2n-6)	33.47	31.5	27.0	38.2	40.58	44.20
Ln (18:3n-3)	0.12	–	0.1	–	5.06	5.70
El (18:3n-5)[c]	5.72	–	–	–	–	–
A (20:0)	0.90	–	0.1	0.9	–	–
G (20:1n-11)	0.41	–	–	–	–	–
B (22:0)	0.20	–	–	–	–	–
Lg (24:0)	0.21	–	–	–	–	–

[a] Fatty acid composition mole percentages by GC-FID. $n = 15$

[b] Reported vaccenic acid (18:1 *t*-11) at 1.0 %

[c] 18:3 is the sum of all isomers of 18:3 except Ln, identified by GC–MS and quantified by GC-FID. El (9(Z),11(E),13(E)-Octadecatrienoic acid) was most abundant, representing 87.83 % of 18:3

six different cultivars of sour cherry. However, our current results show good agreement with that of Górnás *et al*. Results show the major fatty acids are oleic and linoleic acids and also contain small amounts of C18 trienoic acids as well as other saturated fatty acids. The C18 trienoic acids (18:3) contained a majority of conjugated El. The presence of El was confirmed by GC–MS retention time and mass spectra by comparison with an authentic sample. Heating at 100 °C for 1 h according to AOCS Ce-1b-89 gave results that indicated that 18:3 was composed of 65.71 % El, 2.34 % Ln (0.14 % of total FAME), and 31.94 % other 18:3 isomers. Reducing the temperature to 50 °C for 1 h reduced isomerization of El and gave results that indicated that 18:3 was composed of 87.83 % El, 2.10 % Ln (0.12 % of total FAME), and 10.07 % other 18:3 isomers. Using even milder conditions of derivatization overnight at room temperature provided no additional substantial benefit.

The presence of El in the oils of sweet cherry (*P. avium*) cultivars has been reported, but its content varied according to cultivar and location [7, 14]. The fatty acid composition found in this study is more extensive and includes El. The general distribution of most other fatty acids compared reasonably well with previously reported data [11–13, 15, 16]. The saturated fatty acids content of 12 % is comparable to the literature reported values of 6–19 % given in Table 3 [11–13, 15, 16].

Conjugated C18 fatty acids, dienoic acids, conjugated linoleic acids, and the trienoic acid, El, are extensively studied due to their health benefits. The presence of El in CPO was not reported until very recently [16]. However El is present in larger concentrations in various other sweet cherry cultivars of *P. avium* species (see Table 1) and considered to be a nutritionally important fatty acid [7, 14]. Previous research has shown that El quickly converts to conjugated linoleic acid, (9Z11E-C18:2) in rats [25]. Also El has shown promise in suppressing tumor growth in mice [26].

The infrared spectral properties of CPO: IR ν_{max} (liquid film) indicate the presence of unsaturation (3007.2 cm^{-1}), long hydrocarbon chains (2924.92, 2853.97 cm^{-1}) and ester function (1745.69 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of CPO in $CDCl_3$ are shown in Fig. 1.

The ^{13}C-NMR spectrum confirmed the presence of allylic and bis-allylic carbons at 27.20 and 25.63 ppm, respectively, and showed ester carbonyl carbon peaks at 173.1 and 172.79 ppm, glycerol carbons at 68.87 and 62.09 ppm, unsaturated carbons from 127 to 130 ppm, and the fatty acid methylene $(CH_2)_n$ carbons with multiple peaks from 29.0 to 29.8 ppm, corresponding to the literature reported values [27].

The unsaturated fatty acid composition as determined by GC can be further supported by ^{1}H-NMR integration values of the allylic and bis-allylic proton mole percentages. Allylic refers to the protons located on the methylene $C\underline{H}_2$ adjacent to the double bonds of all unsaturated fatty acids, and bis-allylic refers to the methylene hydrogens located on the $C\underline{H}_2$ between the double bonds. The mole percentages were calculated from the ^{1}H-NMR integration values and compared to the fatty acid composition from GC. The allylic proton mole percent matched well with the GC data, giving a value of 10.53 compared to 10.57 from NMR. The bis-allylic mole percent values compared reasonably with those of 1.97 from GC and 1.77 from ^{1}H NMR. The variation in bis-allylic protons is likely due to the presence of

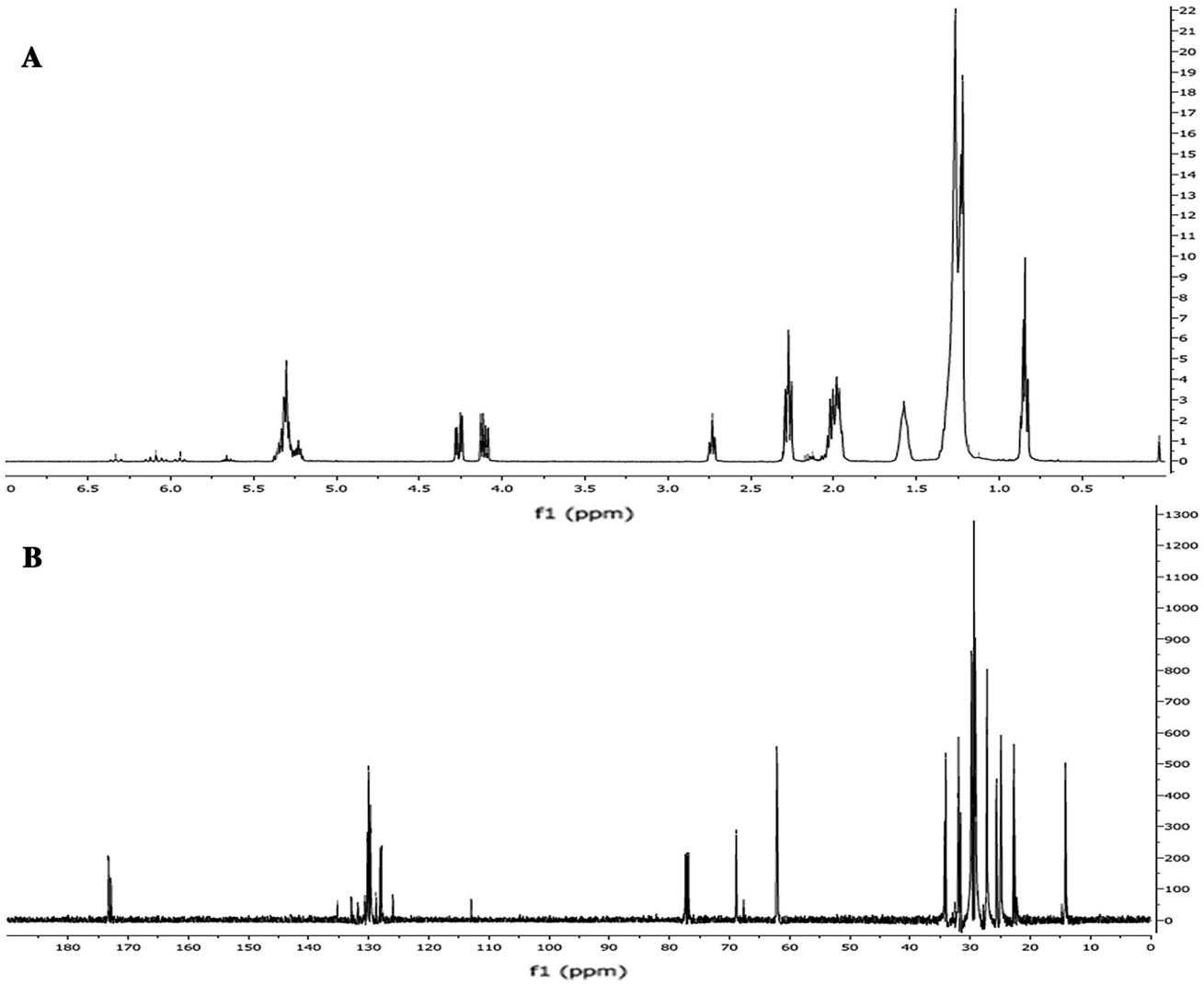

Fig. 1 **a** ^{1}H and **b** ^{13}C NMR spectra of CPO

Table 4 CPO contained 0.87 % of DAG

DAG	Retention time[a] (s)	Average (%)
LEl	30.67	1.47
LL	35.77	19.72
OEl	36.73	2.92
PoO	39.22	0.18
OL	39.78	26.24
PL	40.23	12.05
OO	42.97	19.37
OP	43.86	11.19
SL	44.21	3.05
OS	48.82	3.82

The DAG response-factor-adjusted molar percentage composition and retention times by LC coupled with atmospheric pressure chemical ionization mass spectrometry

[a] Average of two DAG regioisomer peaks

El and other conjugated polyunsaturated fatty acids which contain no bis-allylic protons. Additionally, the discrepancy in quantifying the bis-allylic protons may partially be due to the oil sample representing all the fatty acids in the oil rather than an individual fatty acid.

The oil contained 0.87 % DAG relative to TAG. The DAG and TAG were separated on the LC column and the individual molecular species were identified semi-quantitatively by percentage composition. The DAG composition with retention times and relative concentrations is given in Table 4. The most common DAG were found to be oleoyl linoleoyl (OL) glycerol and dioleoyl (OO) glycerol.

The TAG present in the CPO, along with their retention times on the column and relative percentages as determined by LC/APCI-MS, are presented in Table 5. The order of the fatty acids in the TAG in this table indicates the identities of the most abundant regioisomers, based on literature

Table 5 TAG response-factor-adjusted molar percentage composition of CPO and retention time (min) by LC coupled with atmospheric pressure chemical ionization mass spectrometry

TAG[a,b]	Retention time (min)	Average (%)[c]	TAG[a,b]	Retention time (min)	Average (%)[c]
ElElL	52.42	0.64	OOO	80.43	16.83
LElL	55.16	6.16	LLA	81.05	0.61
ElElO	57.47	0.17	LOS	82.52	2.05
LLL	58.17	3.77	OOP	84.14	6.49
ElLO	60.78	4.73	SLP	86.40	0.34
ElLP	63.15	2.17	OOG	87.41	0.29
LLO	64.44	13.20	POP	88.17	0.59
OLPo	65.61	0.67	LLB	88.59	0.17
LLP	67.12	3.57	OLA	90.46	0.86
OElO	67.58	2.03	OOS	92.38	2.57
PoLP	68.27	0.22	PAL	94.74	0.20
LLG	70.16	0.39	SSL	94.88	0.14
ElOP	70.53	0.59	LLLg	96.37	0.17
OLO	71.91	16.64	SOP	96.96	0.43
OOPo	73.35	0.72	LOB	98.55	0.18
LLS	73.85	1.02	OOA	100.87	0.69
OLP	74.95	7.25	AOP	105.63	0.17
POPo	76.47	0.22	SOS	105.83	0.17
ElSO	77.78	0.16	LOLg	106.75	0.20
OLG	78.12	0.42	OOB	109.46	0.14
PLP	78.40	0.58	OOLg	114.97	0.15

[a] TAG name indicates predominant regioisomer

[b] See fatty acid distribution Table 3 for abbreviations

[c] TAG below 0.10 % not included here. The sum of these 42 TAG was 98.76 %. 83 TAG were identified at ≥0.001 %, 77 TAG identified at ≥0.01 %

trends for the recently-described TAG critical ratio 2 [28]. For type II TAG (2 FA, ABA/AAB/BAA), critical ratio 2 is $[AA]^+/[AB]^+$, and correlates the relative abundances of fragments observed in APCI-MS mass spectra to the amount of 'B' in the *sn*-2 position by comparison to that ratio observed from pure regioisomers. For Type III TAG (3 FA, ABC/CBA/ACB/BCA/BAC/CAB), the fatty acid in the *sn*-2 position, which was lost to yield the $[1,3\text{-AC}]^+$ fragment, was identified by the $[DAG]^+$ fragment having the lowest abundance [29] after isotope correction [30], which was used in the numerator of the type III critical ratio 2, $[AC]^+/([AB]^+ + [BC]^+)$. The $[1,2\text{-AB}]^+$ and $[2,3\text{-BC}]^+$ $[DAG]^+$ fragments cannot be differentiated by APCI-MS, but instead follow other trends [28]. The TAG composition shows six major TAG present in concentrations higher than 5 % with triolein (OOO) as the largest contributor at 16.83 % followed by OLO at 16.64 %. The other major TAG are LLO (13.20 %), OLP (7.25 %), OOP (6.49 %) and LElL (6.16 %) along with a number of other TAG at smaller percentages. Additionally, there are a number of TAG that contain one or two saturated fatty acids, which constitute 33 % of the oil. This study also reports the presence of the conjugated fatty acid El along with TAG containing El for the first time. Based on the fatty acid distribution in Table 3 and TAG composition in Table 5, the previously reported finding of CPO containing only 1,3-dioleoyl-2-linoleoyl glycerol (OLO) by Chandra and Nair was oversimplified.

Quantification of regioisomers of type II TAG in Table 6 was performed based on critical ratio 2 [28]. The regioisomer composition was calculated from the observed $[AA]^+/[AB]^+$ ratio of $[DAG]^+$ fragments, compared to the tabulated values of Holčapek *et al*. [30], using the equation given by Byrdwell [28], based on a simplification of the precedent of Jakab *et al*. [31].

The minor components of the CPO, tocopherols and sterols were analyzed and their concentrations are reported in Table 7 along with the previously reported values. The γ-tocopherol is the major isomer present in CPO. Previous studies including the most recent confirmed the γ-tocopherol as the dominant isomer present, however, the individual and total tocopherol concentrations differed [11, 13, 16].

The thermal stability as indicated by thermal decomposition temperature was determined by TGA, which records the weight loss with respect to an increase in temperature, providing the onset of decomposition temperature. The thermal decomposition temperatures of CPO and other oils of commerce are provided in Table 8. The CPO thermal

Table 6 Percentage of 'ABA' regioisomers of type II TAG based on observed $[AA]^+/[AB]^+$ ratios compared to literature values [30] using regioisomer quantification equation [28]

TAG	% ABA (%)	TAG	% ABA (%)
ElElL	0.0	OOG_{OOA}	0.0
LElL	100.0	POP	100.0
ElElO	0.0	LLB_{LLA}	0.0
ElPEl	57.9	OOS	15.9
LLM_{LLP}	0.0	OOE_{OOA}	100.0
LLO	0.0	SSL	0.0
MLM_{PPL}	87.4	$LLLg_{LLA}$	0.0
OElO	91.2	OOA	0.0
LLP	0.0	PPS_{PPO}	0.0
LLG_{LLO}	0.0	$LLCe_{LLA}$	0.0
OLO	100.0	$OO\text{-}21{:}0_{OOA}$	0.0
$OOPo_{OOP}$	0.0	SOS	100.0
LLS	16.4	OOB_{OOA}	0.0
OMO_{OOP}	51.8	$OO\text{-}23{:}0_{OOA}$	0.0
PLP	100.0	$OOLg_{OOA}$	9.3
LLA	0.0	$OO\text{-}25{:}0_{OOA}$	0.0
OOP	0.0	$OOCe_{OOA}$	0.0
$LL\text{-}21{:}0_{LLA}$	0.0	$OOMo_{OOA}$	0.0

$Subscript_{TAG}$ indicates no standard critical ratio available, substitute TAG used

decomposition temperature is 353 °C, which did not change for the oil purified by the silica column that removed the polar compounds. The CPO thermal decomposition temperature is superior to other oils, indicating higher thermal stability, a desirable characteristic for frying and other high temperature applications.

The oxidative stability was measured by OIT using DSC. The OIT measures the amount of time the oil resists oxidation at a given temperature, with longer times being better. The OIT of CPO was 30 min and after purification through a column was reduced to 18 min. The column removed the tocopherols which provide antioxidant activity, resulting in decreased oxidative stability. The OIT of CPO and other commercial oils are presented in Table 8.

The factors contributing to the inherent oxidative stability of the oil come from the fatty acid composition and the antioxidants [32]. The oxidative stability of various fatty acids decreases with the amount of unsaturation, with saturates being the most stable, polyunsaturates the least stable, and monounsaturates being in between. Of the polyunsaturates, the higher the bis-allylic content, the lower the oxidative stability [32]. In general, the oxidative stability also depends upon the total tocopherol concentration and the isomer ratio. The higher the concentration of total tocopherols, the higher the oxidative stability. Of the tocopherol isomers, γ-tocopherol shows the best antioxidant activity [33].

To compare and explain the OIT of various oils to the CPO, the types of fatty acids, saturates, monounsaturates and polyunsaturates, along with total tocopherol and γ-tocopherol concentrations are presented in Table 9. The CPO contains a high concentration of polyunsaturated fatty acids, including conjugated El. However, CPO contains moderate amounts of tocopherols, with γ-tocopherol being the major contributor, resulting in reasonable oxidative stability. The OIT of CPO is comparable to canola oil, which

Table 7 The minor components of CPO, tocopherols and sterols

Component	Present study (ppm)	Matthäus and Ozcan [11] (ppm)	Yilmaz and Gokmen [13] (ppm)
Total tocopherols	525.2	240.2	428.6[a]
δ-Tocopherol	64.2	15.1	96.9
γ-Tocopherol	400.0	197.2	298.7[b]
α-Tocopherol	61.0	4.7	71.6
Campesterol	159.0	–	–
Stigmasterol	7.2	–	–
β-Sitosterol	3610.0	–	–

[a] Adjusted from mg/L to mg/kg [13]

[b] Includes both beta and gamma tocopherols

Table 8 Thermal and oxidative stabilities of CPO compared to commercial oils

	Crude CPO	Purified CPO	Canola	Regular soybean	High oleic soybean
Decomposition temperature (°C)[a]	352.80	354.27	314.97	344.27	317.95
Oxidative induction time[b] (min)	30.30	18.10	34.01	63.21	260.67

[a] Determined by TGA

[b] Determined by DSC, average of triplicate

Table 9 Fatty acid and tocopherol contents of CPO and common oils of commerce

	CPO	Canola [35]	Regular soybean [35]	High oleic soybean [35, 36]
Saturated	12.0	7.37	15.3	10.5
Monounsaturated	48.7	63.3	22.7	75.0
Polyunsaturated	39.3	28.1	57.3	10.5
Total tocopherols	525.2	458	937	937
γ-Tocopherols	400	273	643	643

The fatty acids are expressed in percentages and the tocopherols in parts per million

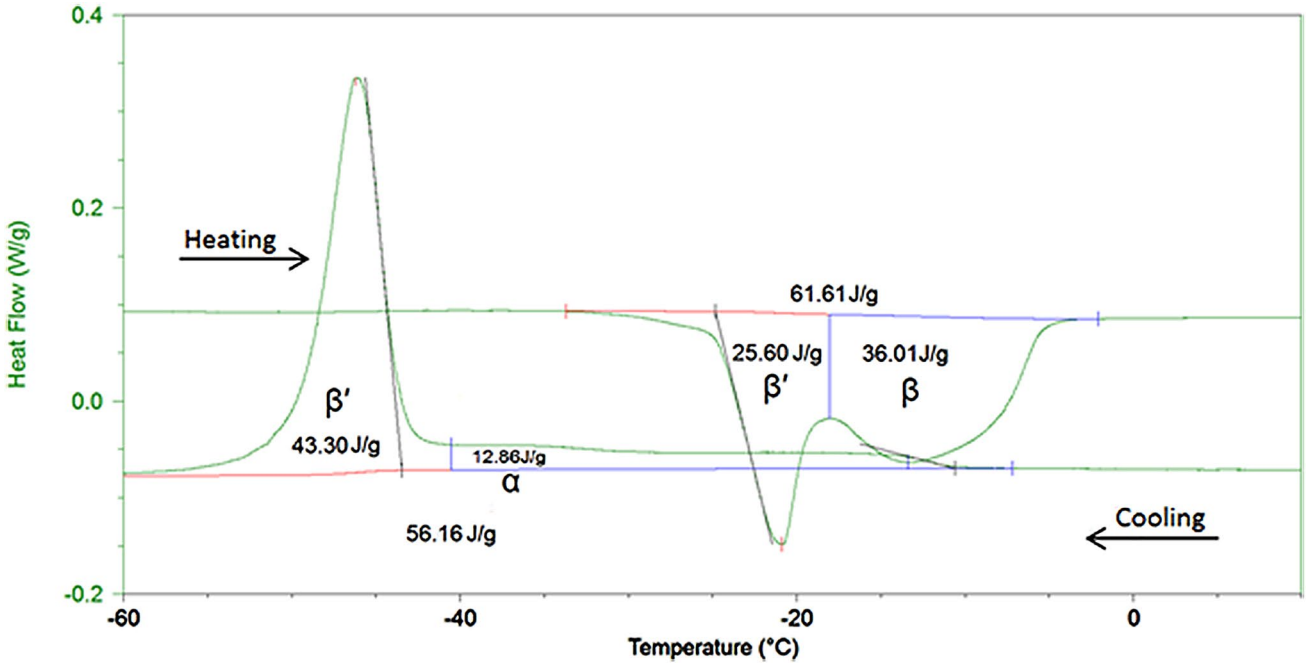

Fig. 2 Thermal behavior of the cherry oil by DSC

Table 10 Peak temperatures (°C) of crystallization and melting of CPO with corresponding enthalpy (J/g) of the CPO as determined by DSC

	Crystallization (°C)	Melting (°C)	Enthalpy crystallization (J/g)	Enthalpy melting (J/g)
α	−10.8[a]	–	12.9	–
β′	−46.2	−20.9	43.3	25.6
β	–	−13.4	–	36.0

[a] Onset of crystallization

can be explained by fatty acid composition and tocopherol concentrations. The higher OIT of soybean oil and high oleic soybean oil compared to CPO can be explained by the larger concentrations of total and γ-tocopherols along with monounsaturates present in high oleic soybean oil.

The presence of multiple melting and solidification of crystal forms in fats and oils has been witnessed for more than a century. This phenomenon is called polymorphism and it arises due to different packing arrangements of the long hydrocarbon fatty acid chains with similar lattice energy. The broad classifications of polymorphs include α, β′ and β, and can be identified using characteristic wide angle XRD lines. The α-phase subcell is hexagonally packed with no specific chain–chain interaction, leading to an unstable transient nature. In the β′-phase the acyl chains are packed in an orthorhombic perpendicular subcell (O⊥) with specific chain–chain interactions, and have characteristic wide angle XRD lines at ~4.3, 4.1 and 3.8 Å. In the β-phase the chains pack in a triclinic parallel (T//) structure with the zig-zag planes parallel to each other with specific chain–chain interactions characterized by multiple wide-angle XRD spacings with an intense line at ~4.6 Å [34].

The polymorphic behavior of CPO as characterized by DSC is shown in Fig. 2 and has not been reported previously. During cooling, the unstable α-phase crystallized first, with the onset of crystallization temperature at −11 °C.

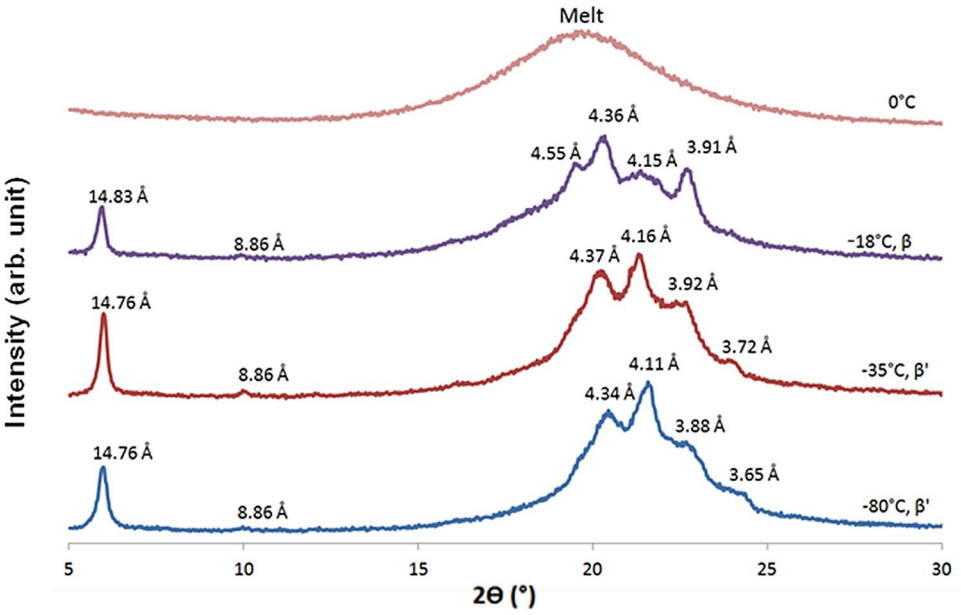

Fig. 3 Wide angle XRD was obtained by cooling the sample from room temperature to −80 °C with scans taken at various temperatures

This unstable α-phase transforms into β′, without melting, at −46 °C. Then, upon heating the β′-phase melts at −21 °C and transforms into the most stable β-phase, which melts at −13 °C into a liquid. The crystallization and melting temperatures, along with the enthalpies of various phases, are presented in Table 10. The polymorphs are confirmed by wide-angle XRD patterns typical for β′ and β and are shown in Fig. 3. The complex wide angle diffraction lines for both β′ and β are due to the multi-TAG components present in the CPO. The long spacings in the low angle region correspond to the acyl chain packing of the molecules on the long axis. The 14.8-Å spacing present in β and β′-phases is a third order of 45-Å long spacing, corresponding to a two chain length structure typical of many TAG.

In conclusion, the CPO composition was fully characterized by fatty acid and TAG compositions, and minor components. The presence of the nutritionally important fatty acid El in CPO was confirmed. The CPO had moderate oxidative stability and high thermal stability. The polymorphism of CPO revealed an α-polymorph and confirmed β′- and β-polymorphs. Based on these results, CPO can be useful in dietary applications such as cooking and in cosmetics.

Acknowledgments This research was partially funded by the University of Minnesota's Undergraduate Research Opportunity Program. We acknowledge Dr. Ramesh Avula for the shipment of sour cherry pits from Cherry Central of Michigan.

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