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Fatty acid composition and antioxidant activity of oils from two cultivars of Cantaloupe extracted by supercritical fluid extraction

By **Maznah Ismail,**[a b*] **Abdalbasit Mariod,**[ac] **Gururaj Bagalkotkar**[a] and **Hoe Sy Ling**[b]

[a] Laboratory of Molecular BioMedicine, Institute of Bioscience, Universitiy Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[b] Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
[c] Department of Food Science & Technology, Sudan University of Science &Technology, P. O. Box 71, Khartoum North, Sudan
(* Corresponding author: maznahis@putra.upm.edu.my & maznah@medic.upm.edu.my)

RESUMEN

Actividad antioxidante y composición de ácidos grasos de aceites de semilla de melón de dos variedades extraídos mediante extracción con fluido supercrítico

El efecto del fraccionamiento mediante extracción con fluido supercrítico de tres fracciones (fracción 1ª, 2ª y 3ª) sobre la composición de ácidos grasos y actividad antioxidante de aceites de dos variedades de melón fué investigado. Aceites de melón de los cultivares Rock (RMO) y Golden Langkawi GLO) fueron extraídos usando SFE y los principales ácidos grasos en cada cultivar fueron ácido linoleico, oleico, palmítico y esteárico. Los ácidos grasos saturados (SFA) disminuyeron desde 15.78 a 14.14% en la 1ª fracción de RMO y los ácidos grasos monoinsaturados (MUFA) disminuyeron desde 18.30 a 16.56% en la 2ª fracción de RMO, mientras que los ácidos grasos poliinsaturados (PUFA) aumentaron de 65.9 a 69.30% en la 3ª fracción de RMO. Por otra parte, SFA disminuyo de 16.35 a 13.91% en la primera fracción de GLO y MUFA disminuyo de 17.50 a 15.57% en la 2ª fracción de GLO, mientras que PUFA aumento de 66.15 a 70.52% en la 3ª fracción de GLO. Las diferentes fracciones de los dos aceites mostraron una alta actividad antioxidante al reducir la oxidación del β-caroteno en el ensayo de decoloración de beta-caroteno (BCB) y en la eliminación del radical 1,1-difenil-2-picrilhidrazilo (DPPH).

PALABRAS CLAVE: Ácido graso – Actividad antioxidante – 1,1-difenil-2-picrilhidrazylo – Ensayo de decoloración de beta-caroteno – Extracción con fluido supercrítico – Melón

SUMMARY

Fatty acid composition and antioxidant activity of oils from two cultivars of Cantaloupe extracted by supercritical fluid extraction

The effect of supercritical fluid extraction **(**SFE) fractionation of three oil fractions (1st, 2nd, 3rd fraction) on the fatty acid composition and antioxidant activity of oils from two cultivars of cantaloupe were investigated. Rock melon oil (RMO) and Golden Langkawi oil (GLO) were extracted using SFE and the major fatty acids for both cultivars were linoleic, oleic, palmitic, and stearic acid. The SFA decreased from 15.78 to 14.14% in RMO 1st fraction, and MUFA decreased from 18.30 to 16.56% in RMO 2nd fraction, while PUFA increased from 65.9 to 69.30% in RMO 3rd fraction. On the other hand SFA decreased from 16.35 to 13.91% in GLO 1st fraction, and MUFA decreased from 17.50 to 15.57% in GLO 2nd fraction, while PUFA increased from 66.15 to 70.52% in GLO 3rd fraction. The different fractions of the two oils showed high antioxidant activity in reducing the oxidation of β-carotene in beta-carotene bleaching assay (BCB) and the quenching of 1,1-diphenyl-2-picrylhydrazyl (DPPH).

KEY-WORDS: Antioxidant activity – Beta-carotene bleaching assay – Cantaloupe – 1,1-diphenyl-2-picrylhydrazyl – Fatty acid – Supercritical fluid extraction.

1. INTRODUCTION

The cantaloupe, from the family cucurbitaceae, refers to two different varieties, *Cucumis melo cantalupensis* known locally as Golden langkawi and *Cucumis melo reticulates* which is known locally as rock melon (Integrated Taxonomic System, 2007). Several authors (Al-Khalifa, 1996; Kamel *et al.*, 1985; Badlfu, 1991) have reported studies about some melon seeds and compared the physicochemical characteristics of their oils with those from conventional sources. Melon (*Cucumis melo*) seeds, besides possessing medicinal qualities (Bellakhdar *et al.* 1991), are also a rich source of protein (53.90%) and oil (37.67%) (Mariod *et al.* 2008; Rashwan *et al.* 1993). The proximate composition of the seeds or seed kernels of the *Cucumis melo* from different origin and varieties has been reported (Teotia and Ramakrishna 1984; Kaur *et al.* 1988; Tekin and Velioglu, 1993). as well as the fatty acid composition of the seed oil from Egypt (El-Magoli *et al.* 1979), India (Hemavatahy 1992), Sudan (Mariod *et al.* 2008) and Vietnam (Imbs and Pham, 1995). Maria *et al.* (2001) showed that the oil of *Cucumis melo* contains myristic acid (0.3%), stearic acid (6.1%), palmitic acid (8.5%), oleic acid (31%) and linoleic acid (51%). They showed that the *Cucumis melo* seed oil which was obtained by Soxhlet extraction contained 15.3% saturated fatty acid and 82.8% unsaturated fatty acid, which

further consisted of 26.2% as monounsaturated fatty acid and 56.6% as polyunsaturated fatty acid. Pumpkin seed extracts of different polarity and phenolic content are able both to quench DPPH free radicals and to inhibit lipid peroxidation catalyzed by lipoxygenase (Xanthopoulou *et al*., (2009). Budrat and Shotipruk (2009) presented a study which demonstrated that bitter melon is an important source of phenolic compounds which possess strong antioxidant activity.

Oils that are extracted by using environmental hazardous organic solvents such as *n*-hexane or petroleum ether might contain residual solvent due to the incomplete solvent removal. Supercritical fluid extraction (SFE) with supercritical carbon dioxide (SC-CO_2) has received considerable attention which can be an alternative method for the extraction of oils from foods and natural products (Gomes *et al.* 2007; Lu *et al.* 2007). It is because carbon dioxide is both non-toxic and non-explosive and its use can reduce the consumption of organic solvents; which is especially useful for the production of natural products used in foods and pharmaceuticals (Leo *et al.* 2005). The oils obtained by SC-CO_2 extraction are of outstanding quality and the yields are comparable with those by organic solvent extraction methods (Friedrich and List, 1982; Gómez *et al.* 1996). In fact, SFE extracts are generally recognized as safe (GRAS) to be used in food products (Gerard & May, 2002). Therefore, SFE may serve as a very promising technology in food and pharmaceutical processing (King, 2000).

Several methods have been recommended for the evaluation of antioxidant properties of plant materials and some methods in current use were compared (Gordon, 1990; Ou *et al*., 2002). 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH*) assay is a well known method for the evaluation of free radical-scavenging activity. The method is polarity-independent, very rapid, simple and reproducible (Koleva *et al.* 2002). Fatty acid decomposition is one of the main causes of food spoilage and hence inhibition of fatty acid oxidation is an important issue in the food industry. Food preservatives or antioxidants are mainly used as inhibitors of the oxidation of fatty acids. Therefore, the inhibition of linoleic acid oxidation can be measured in the presence of β-carotene that is used as a marker (Dapkevicius *et al.* 1998). Linoleic acid oxidation produces conjugated dienes and other volatile products that attack β-carotene and bleach its characteristic color (pale yellow in aqueous emulsion). In general, both free radical-scavenging and inhibition of linoleic acid oxidation are desired in the food industry.

Although the *C. cantalupensis* and *C. reticulates* melons are recently produced and consumed in Malaysia, there are however no studies on fatty acid composition, antioxidant properties and the health benefits of oils from these two species. Therefore, the objective of the work was the characterization of three oil fractions from successive extractions using SFE in terms of fatty acid composition and antioxidant activity by means of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay and β-carotene bleaching test.

2. MATERIALS AND METHODS

2.1. Materials

Golden Langkawi and rock melon were obtained from the MAHA (Malaysian Agriculture, Horticulture and Agrotourism) fair organized by Malaysia Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia.

The chemicals used were of analytical reagent grade that include 1,1-diphenyl-2-picrylhydrazyl (DPPH − 90% purity), (+)-α-tocopherol, -carotene (Type I synthetic, 95%) and linoleic acid (99%), were obtained from Sigma–Aldrich Co., St. Louis, MO, USA), toluene (Merck, Darmstadt, Germany), *n*-hexane and chloroform (Fisher Scientific, Loughborough, Leicestershire, UK), carbon dioxide (99.8%, Malaysian Oxygen Berhad, Petaling Jaya, Selangor, Malaysia), Tween 20 and absolute ethanol (Fisher Scientific, Loughborough, Leicestershire, UK).

2.2. Methods

Preparation of dried cantaloupe seeds

Cantaloupe seeds collected from the fruits were cleaned and dried to constant weight at 50°C in a drying oven (FD 115, Fisher Scientific, Loughborough, Leicestershire, UK). The final moisture content of the seeds was 4.43 ± 0.07%. The dried seeds were ground using a blender (Waring, Torrington, CT, USA) and kept at 4°C prior to oil extraction.

Supercritical fluid extraction (SFE) with carbon dioxide

Cantaloupe seeds were extracted using a Supercritical Carbon Dioxide Extractor (Thar 1000 F, Thar Technologies, Inc., Pittsburgh, PA, USA) at a pressure (bars)/temperature (C) of 600/40 with three successive extractions for 1h each (1st, 2nd or 3rd fraction) according to the method described by Bruhl and Matthaus (1999). Briefly, one hundred grams of cantaloupe seed powder was placed into a 1 L extraction vessel. The extractor was sealed, oven temperature was set at 40 °C and pressurized to 600 bars. Pressure within the extraction vessel was built up with a constant carbon dioxide flow rate of 25 g/min and regulated by an automated back pressure regulator. The collection flask was kept at ambient pressure and temperature of 60°C. The SFE extraction was initiated after the desirable temperature and pressure were achieved. The extracted oil from the extraction vessel was collected every hour for 3 h in order to obtain different fractions and the yield of the oil

was measured. After the extraction was completed, the extraction vessel was depressurized and the oil was collected. The cooled flask containing the oil was weighed and the oil content was calculated.

Cantaloupe seed oil extracted by Soxhlet method

The cantaloupe seed oil was extracted by Soxhlet following the AOAC method (1990). Briefly, about 10g of dried sample were placed into a dried extraction thimble, with porosity permitting a rapid flow of petroleum ether. The sample in the thimble was covered with wool. The pre-dried boiling flask was weighed. Petroleum ether was placed in the boiling flask. The sample was extracted in a Soxhlet extractor for 6 h at a rate of 2 to 3 drops per second by the heated solvent in the boiling flask. The boiling flask with extracted fat was dried in an air oven at 100°C for 30 min, cooled in a desiccator, and weighed.

*Determination of oil content and fatty acid composition of cantaloupe seed oil by gas chromatography***.**

The oil content was determined using the AOCS method Am 2-93 (1993), while fatty acid composition in cantaloupe seed oil was determined using gas chromatography according to the method described by Baye and Becker (2004). Seed oil (100 mg) was weighed into 20 ml test tubes and dissolved in 10 ml hexane. Then, 100 µl of 2N potassium hydroxide in methanol (11.2 g in 100 ml) was added into the test tube, vortexed for 30 seconds and centrifuged (Hettich Rotofix, Ramsey, MN, USA). The clear supernatant (2 ml) was transferred to an auto sampler vial and injected into gas chromatography (Agilent GC 68, Santa Clara, CA, USA) for analysis. The column used was DB-23, diameter 250 µm, length 60 m, film thickness 0.25 µm, and the void time was 3.017 min. The peak areas were computed by integration software and percentages of fatty acid methyl esters (FAME) were obtained as weight percent by direct internal normalization with the Agilent Technologies ChemStation software. All analyses were performed in triplicate.

Antioxidant activity (AOA) measurements

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity test. The antioxidant activity of cantaloupe oils was measured according to the method described by Ramadan, et al. (2006). BHT was used as the standard lipophilic antioxidant in this test. In brief, 0.1 mL of toluenic sample solution at different concentrations was added with 0.39 mL fresh toluenic DPPH solution (0.1 mM). Then, the mixture was shaken vigorously and left in the dark for 60 min. Finally, the absorbance of the mixture was measured against pure toluene (blank) at 515 nm by using a UV–Visible spectrophotometer (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). The absorbance of the DPPH radical without antioxidant, i.e. the control was measured. The data is commonly reported as IC_{50}, which is the concentration of antioxidant required for 50% scavenging of DPPH radicals in the specified time period. All determinations were made in triplicate

The β-carotene–linoleic acid assay. The antioxidant activity (AOA) of the different fractions was evaluated using the β-carotene–linoleic acid assay following the method of Amarowicz *et al*. (2003). In brief, a solution of β-carotene was prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform. Two milliliters of this solution were pipetted into a 100 ml round-bottom flask. After chloroform was removed under vacuum, using a rotary evaporator at 40 °C, 40 mg of purified linoleic acid, 400 mg of Tween 40 as an emulsifier, and 100 ml of aerated distilled water were added to the flask with vigorous shaking. Aliquots (4.8 ml) of this emulsion were transferred into a series of tubes containing 200 µl of the extract (200 ppm in methanol). The total volume of the systems was adjusted to 5 ml with methanol. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm with a Shimadzu spectrophotometer (Shimadzu Co., Ltd., Kyoto, Japan). Sub-sequent absorbance readings were recorded by keeping the samples in a water bath at 50 °C. Blank samples, devoid of β-carotene, were prepared for background subtraction.

2.3. Statistical analyses

Statistical analyses were conducted using SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) version 12.0 for Windows. Analysis of variance (ANOVA) and Pearson's correlation coefficients were performed to compare the data. All determinations were made at least in triplicate and all were averaged. The confidence limits used in this study were based on 95% ($P < 0.$

3. RESULTS AND DISCUSSION

3.1. The total oil content of *C. cantalupensis* and *C. reticulates* seeds

In a previous study (Chan and Ismail 2009), our group showed that using the extraction pressure 600 bars and temperature of 40°C gave a high yield of oil extraction for *Hibiscus cannabinus* using SFE. Depending on that result we used 600/40 extraction parameters for the extraction of the cantaloupe oil. Table 1 shows the yield of the two varieties of cantaloupe seed oil through 600/40 SFE extraction parameters in comparison with the conventional Soxhlet method. In general, the total oil content obtained from SFE for *C. reticulates* was 30.4% and for *C. cantalupensis* was 22.7%. On the other hand, the total oil content obtained from Soxhlet extraction

was found to be significantly higher ($P < 0.05$). *C. reticulates* was 33.5% and *C. cantalupensis* was 29.8%. These results are in agreement with those obtained by Teotia & Ramakrishna (1984) and Maria *et al*. (2001), who reported 33.0% and 32.3%, respectively, for melon seed using Soxhlet extraction. It can be observed that the total oil content obtained by Soxhlet extraction was 3.08% higher for *C. reticulates* and 7.14% higher for *C. cantalupensis* than the amount obtained by SFE, but CO_2 reverts to a gas at room temperature and pressure and has minor toxicity. In addition to that, SFE extraction is safer because there is no leftover contaminant in the extract because the carbon dioxide is allowed to escape in the form of a gas. It was therefore easy to conclude that SFE with CO_2 would offer clear advantages over organic solvents for oil analysis.

The total oil content of C. cantalupensis obtained from SFE 1st fraction was 15.2%, 2nd hour was 5.7% and the 3rd fraction was 1.8% of the dried seeds. Whereas the amount of oil obtained from *C. reticulates* 1st, 2nd and 3rd fraction was 22.0%, 6.0%, and 2.4%, respectively (Table 1). From the SFE fractionation of two cantaloupe species, results showed that the amount of oil obtained for both species decreased with time because most of the oil (65% and 75%) was already extracted from both seeds in the first hour of SFE extraction. So the oil content from the 2nd and 3rd fractions was less than the first hour of SFE fraction.

3.2. Fatty acid composition

The fatty acid profiles of seed oil from the *C. reticulates* (RMO) were analyzed by GC, as summarized in Table 2. The major fatty acids present in the SFE fractions from *C. reticulates* was linoleic acid (65%), followed by oleic acid (16.3%), palmitic acid (8.1%), stearic acid (5.5%), arachidic acid (2.9%), eicosanoic acid (1.1%), linolelaidic acid (0.25%), α-linolenic acid (0.13%), and γ-linolenic acid (0.06%). Whereas no significant changes in the amounts of oleic, stearic and palmitic acids from 1st fraction, 2nd fraction and 3rd fraction were found, this is not in accordance with the findings of Yu *et al.* (1994) , who studied the solubilities of fatty acids, fatty acid ester, triglycerides, and fats and oils in supercritical carbon dioxide, where they reported that linoleic acid decreased while oleic, stearic and palmitic acid increased over extraction time.

The fatty acid composition of RMO obtained from SFE 1st fraction was 15.78% of saturated fatty acid (SFA), 18.30% of monounsaturated fatty

Table 1
Percent oil content on a dry basis in seeds of two cantaloupe species obtained with soxhlet and SFE*

Sample	*C. reticulates*	*C. cantalupensis*
Soxhlet method	33.5± 0.4	29.8± 0.5
SFE 1st fraction	22.0± 0.2	15.2± 0.3
SFE 2nd fraction	6.0± 0.3	5.7± 0.2
SFE 3rd fraction	2.4± 0.3	1.8± 0.1
Total content oil by SFE	30.4	22.7

*All supercritical fluid extraction (SFE) fractions were performed at 600 bars and 40 °C. All determinations were carried out in triplicate and mean value ± SD reported.

Table 2
Fatty acid (FA) composition (%) of *C. reticulates* seed oil (RMO) in different SFE* fractions

FA (1st fraction)	(%)	FA(2nd fraction)	(%)	FA (3rd fraction)	(%)
Linoleic acid	64.98± 0.31	Linoleic acid	66.02±0.74	Linoleic acid	65.42±0.87
Oleic acid	16.13±0.85	Oleic acid	16.45±0.29	Oleic acid	16.56±0.12
Palmitic acid	8.13±0.53	Palmitic acid	8.09±0.31	Palmitic acid	8.33±0.42
Stearic acid	5.16±0.28	Stearic acid	5.59±0.56	Stearic acid	5.81±0.64
Arachidic acid	2.49±0.39	Arachidic acid	2.92±0.82	–	–
Linolelaidic acid	0.55±0.18	Linolelaidic acid	0.55±0.16	Linolelaidic acid	0.56±0.36
α-linolenic acid	0.21±0.62	α-linolenic acid	0.20±0.60	α-linolenic acid	3.32±0.22
γ-linolenic acid	0.18±0.40	γ-linolenic acid	0.18±0.47	–	–
Eicosanoic acid	2.17±0.22	–	–	–	–
ΣSFA	15.78		16.60		14.14
ΣMUFA	18.30		16.45		16.56
ΣPUFA	65.92		66.95		69.30
Ratio UFA/SFA	5.3		5.0		6.0

*All supercritical fluid extraction (SFE) fractions were performed at 600 bars and 40°C. All determinations were carried out in triplicate and mean value ±SD reported. All determinations were carried out in triplicate and mean value ±SD reported. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids.

acid (MUFA) and 65.92% of polyunsaturated fatty acid (PUFA). The 2nd fraction obtained from SFE contained 16.60% SFA, 16.45% MUFA and 66.95% PUFA, whereas the 3rd SFE fraction of RMO contained 14.14% SFA, 16.56% MUFA and 69.30% PUFA. It can also be noted that the percentage of PUFA was the highest in the three fractions. For RMO, the highest SFA was from the 2nd fraction, highest MUFA was from the 1st fraction and highest PUFA came from the 3rd SFE fraction. The number of different fatty acids obtained decreased with extraction time. For RMO 1st fraction, there were 9 types of fatty acids in fraction 1, in the 2nd fraction there were 8 types and for the 3rd fraction there were only 6 types of fatty acids present (Table 2). The ratios of unsaturated/saturated acid for RMO 1st, 2nd and 3rd fractions are 5.3, 5.0 and 6.0 respectively, which is very high because of the low content of saturated fatty acids and high content of unsaturated fatty acid (Table 2).

This change of composition may be due to the differences in solubility of fatty acid in supercritical CO_2 where soluble components can be extracted first.

The composition of the fatty acids by GC from the GLO is presented in Table 3. The major fatty acid present in the fractions from SFE for GLO was linoleic acid (64.7 %), followed by oleic acid (16.4%), palmitic acid (9.1 %), stearic acid (5.2%), arachidic acid (1.5%). It can be observed that for GLO, the content of linoleic acid only decreased slightly from the 2nd to the 3rd fraction and no significant differences were found between the 1st and 2nd fractions. There seems to be no significant differences in the contents of oleic, stearic and palmitic acids between the GLO fractions. This is not in accordance with the findings of Yu *et al.* (1994) because a fatty acid with shorter chain length and higher degree of unsaturation has higher solubility. So, the percentage of linoleic acid should decrease with time whereas the percentage of oleic, stearic and palmitic should increase with time.

The fatty acid composition of GLO obtained from SFE 1st fraction consisted of 16.35% SFA, 17.50% MUFA and 66.15% PUFA. The SFE 2nd fraction consisted of 17.61% SFA, 16.59% MUFA and 65.80% PUFA, while the 3rd SFE fraction of GLO contained 13.91% SFA, 15.57% MUFA and 70.52% PUFA. It can also be observed that the percentage of PUFA was highest in all fractions of SFE extraction for GLO. For GLO, the highest SFA was from the 2nd fraction, the highest MUFA was from the 1st fraction, and the highest PUFA was from the 3rd fraction of Extraction. The ratio of saturated fatty acids to unsaturated fatty acids was 5.1, 4.7 and 6.2 for 1st, 2nd, and 3rd GLO fractions, respectively.

For GLO 1st fraction, there were 16 types of fatty acids were present in fraction 1, in the 2nd fraction there were 8 types and for the 3rd fraction there were only 6 types of fatty acids (Table 3). Linoleic acid was the predominant fatty acid in the oil from both melon seeds followed by oleic, palmitic and stearic acids with concentrations of 64, 16, 8.5 and 6%, respectively. These results were in good agreement with that of Norulaini *et al.,* (2009) who studied the effects of supercritical carbon dioxide extraction parameters on virgin coconut oil and they reported that the composition of the fatty acid in the extracted oil varied, based on the extraction conditions. While Sanchez-Vicente *et al.,* (2009) found no change in terms of fatty acid distribution in all the extracted peach seed oils at the conditions of

Table 3
Fatty acid composition (%) of *C. cantalupensis* seed oil (GLO) in different SFE* fractions

FA (1st fraction)	%	FA (2nd fraction)	%	FA (3rd fraction)	%
Linoleic acid	64.77 ± 0.31	Linoleic acid	64.67 ± 0.61	Linoleic acid	61.41 ± 0.44
Oleic acid	16.42 ± 0.25	Oleic acid	16.59 ± 0.15	Oleic acid	15.57 ± 0.69
Palmitic acid	9.19 ± 0.64	Palmitic acid	9.51 ± 0.80	Palmitic acid	8.67 ± 0.72
Stearic acid	5.24 ± 0.19	Stearic acid	5.65 ± 0.76	Stearic acid	5.24 ± 0.23
Arachidic acid	1.57 ± 0.73	Arachidic acid	2.45 ± 0.28	–	–
Linolelaidic acid	0.70 ± 0.26	Linolelaidic acid	0.68 ± 0.39	Linolelaidic acid	0.63 ± 0.54
α-Linolenic acid	0.26 ± 0.28	α-Linolenic acid	0.25 ± 0.57	α-linolenic acid	8.49 ± 0.10
γ-Linolenic acid	0.42 ± 0.12	γ-Linolenic acid	0.20 ± 0.33	–	–
Eicosanoic acid	0.27 ± 0.43	–	–	–	–
Elaidic acid	0.21 ± 0.50	–	–	–	–
Myristic acid	0.19 ± 0.81			–	–
Palmitoleic acid_	0.18± 0.33	–	–	–	–
Pentadecyclic acid	0.18± 0.55	–	–	–	–
Dimethyl-2-tridecenoic acid	0.16± 0.70	–	–	–	–
Physeteric acid	0.14± 0.87	–	–	–	–
2-Heptadecylenic acid	0.10± 0.46	–	–	–	–
ΣSFA	16.35		17.61		13.91
ΣMUFA	17.50		16.59		15.57
ΣPUFA	66.15		65.80		70.52
Ratio UFA/SFA	5.1		4.7		6.2

*All supercritical fluid extraction (SFE) fractions were performed at 600 bars and 40°C. All determinations were carried out in triplicate and mean value ±SD reported. All determinations were carried out in triplicate and mean value ±SD reported. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, unsaturated fatty acids.

supercritical extraction. The fatty acid profile of GLO and RMO resembled other Cucurbitaceae species, which were reported in previous studies (Mariod *et al.* 2008; Kamel *et al.* 1985; Badlfu 1991; Al-Khalifa 1996). Maria *et al.* (2001) reported linoleic acid, oleic, palmitic and stearic acids as predominant fatty acids in *Cucumis melo Var. Saccharinus* oil. Hemavatahy (1992) and Imbs and Pham (1995) also observed linoleic acid as the principal fatty acid followed by oleic acid from the oil extracted from an unspecified variety of musk melon.

3.3. DPPH radical scavenging activity

DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples. It is a stable free radical with a characteristic absorption at 517 nm and was used to study the radical-scavenging effects of extracts. As antioxidants donate hydrogen radicals to this radical, the absorption decreases. The effect of GLO and RMO fractions (1st, 2nd, and 3rd) on the reduction of DPPH radicals is shown in Figure 1. It was clear that the presence of antioxidants in the GLO and RMO fractions showed potent free radical scavenging activity on DPPH. Antioxidants, upon interaction with DPPH, either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character (Naik *et al.* 2003). The color changes from purple to yellow and its absorbance at wavelength 517 nm decreases. The DPPH values for different GLO and RMO fractions against the control (BHT) expressed as IC_{50} are shown in Fig. 1. Results are expressed in IC_{50} values (µg/ml), and the IC_{50} values (concentration of sample required to scavenge 50% free radical or to prevent lipid peroxidation by 50%) in GLO were found to be the least in the GLO 3rd fraction (6.5 ± 0.25), followed by the GLO 2nd fraction (7.1 ± 0.76), and the GLO 1st fraction (21.6 ± 0.66). In the case of RMO IC_{50} values were found to be the least in the 3rd fraction (8.5 ± 0.35), followed by the RMO 1st fraction (10.7 ± 0.26), and the RMO 2nd fraction (21.3 ± 0.46). The radical scavenging activities of the different fractions of RMO are in the order 3rd better than 1st and t the 2nd fraction gave the lowest antioxidant activity. In general, GLO showed significantly higher ($P < 0.05$) antioxidant activity than RMO except in the 1st fraction where RMO showed higher antioxidant activity than GLO. The antioxidant activity of the different fractions from GLO and RMO may be attributed to components other than fatty acids in the oils. This activity may have been partly contributed by some constituents other than fatty acids e.g. tocopherols and phenolic compounds (Tasioula-Margari and Okogeri, 2001; Lee *et al.,* 2002). For BHT, the DPPH tests showed that scavenging ability as IC_{50} was 0.29 µg/ml. It can be observed that the synthetic antioxidant BHT is a stronger antioxidant in comparison to GLO and RMO seed oil extracts.

3.4. Beta-carotene bleaching (BCB) antioxidant activity

In the BCB assay, the oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of a hydrogen atom from diallylic methylene groups of linoleic acid (Kumaran and Karunakaran, 2006). The free radical will then oxidize the highly unsaturated β-carotene. The presence of antioxidants in the fraction will minimize the oxidation of β-carotene by hydroperoxides. Hydroperoxides formed in this system will be neutralized by the antioxidants from the fractions. Thus, the degradation rate of β-carotene depends on the antioxidant activity of the fractions.

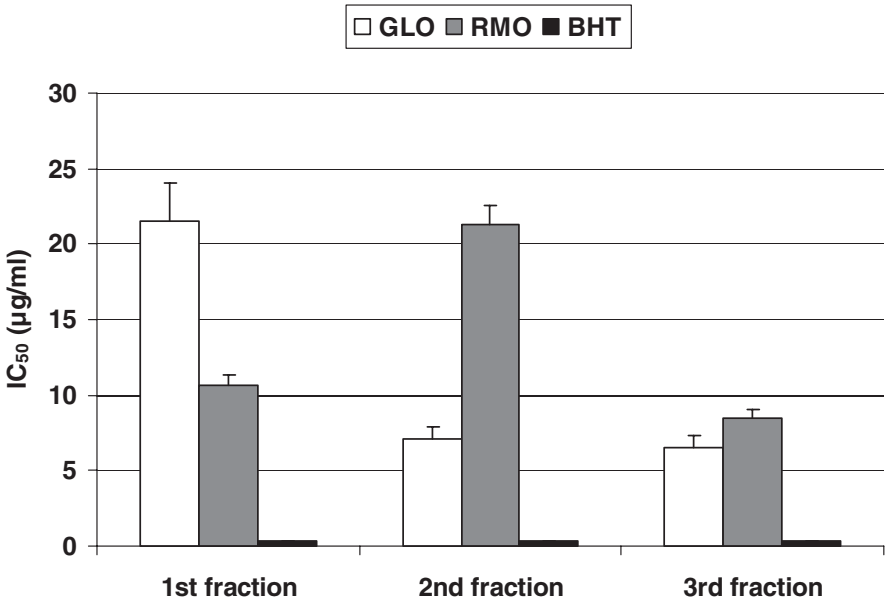

Figure 1
Antioxidant activity (IC_{50} µg/ml) of GLO and RMO fractions on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.

The antioxidant activity of the SFE fractions from GLO and RMO as measured by the β-carotene bleaching method are shown in Fig.2. The BCB mean activity of GLO 1st fraction was 13.2, for the 2nd fraction was 55.6 and for the 3rd fraction was 65.7. The BCB antioxidant test of GLO showed that the fractions were effective in the order: 3rd > 2nd > 1st (Fig. 2). The BCB mean activity of RMO for the 3rd fraction was 78.4, for the 2nd fraction it was 51.0 and for the 1st fraction it was 23.0. This means that the BCB antioxidant activity increased with time for both GLO and RMO. In the present study, RMO showed a higher ability to prevent the bleaching of β-carotene than that of GLO in the 1st and 3rd fractions and this antioxidant capacity can possibly be due to other oil components e.g. tocopherols, phytosterols and phenolic compounds, which were not investigated in this study. The BCB mean for BHT was 97.16±2.2. It can be observed that the synthetic antioxidant, BHT has a stronger antioxidant activity when compared to GLO and RMO oil fractions.

For comparison of DPPH and BCB antioxidant activity methods GLO showed significantly higher ($P < 0.05$) antioxidant activity than RMO (except in the 1st fraction) in the DPPH test while in contrast, RMO showed a higher ability to prevent the bleaching of β-carotene than that of GLO in the 1st and 3rd fractions and this is might be due to the different types of antioxidants that are assayed by the two methods.

4. CONCLUSIONS

Cantaloupe seed oils (RMO and GLO) serves as a new source of edible oil, the use of supercritical CO_2 in cantaloupe oil extraction resulted in antioxidant-rich oil with different amounts and types of fatty acids. The number of fatty acids in the two oils decreased with increased extraction time. Based on the obtained results, RMO and GLO may play potential roles as health-promoting agents with high antioxidant activity in human diets, as well as providing valuable natural antioxidants for the pharmaceutical industry. Further studies are needed to evaluate other biological activities of RMO and GLO and to identify and characterize the active components which are responsible for antioxidant activity in these oils, other than fatty acids.

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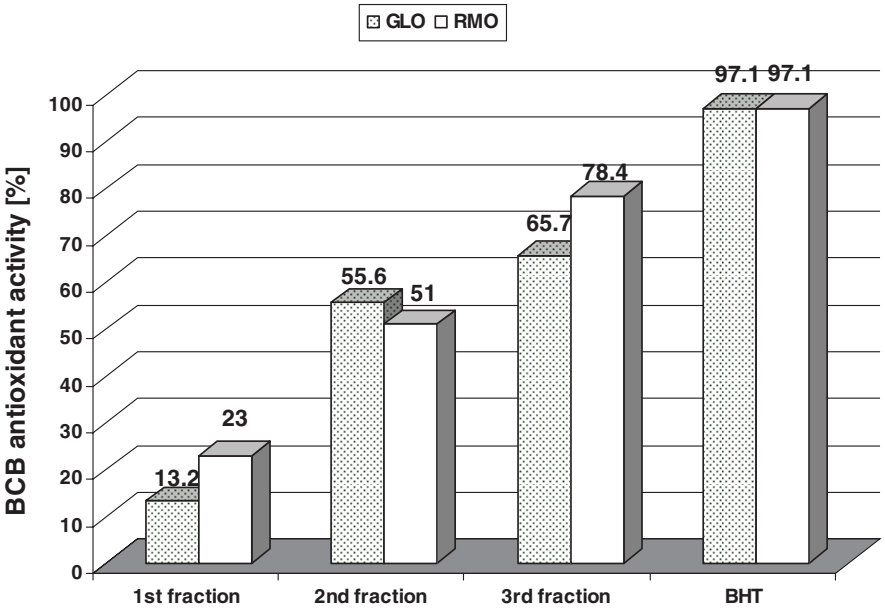

Figure 2
Antioxidant activity of supercritical fluid extraction (SFE) fractions from RMO and GLO in β-carotene-linoleate bleaching system (BCB**).**

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