

Evaluation of green extraction methods on the chemical and nutritional aspects of roselle seed (*Hibiscus sabdariffa* L.) oil[☆]

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Abstract – Roselle is one of the valuable plants grown in some regions of Egypt, which is used to make juices or as natural food color additive. Roselle seeds are waste, which can be used as a source of non-traditional oil, nutritious or functional compound. The evaluation of green extraction methods including supercritical CO₂ (SC-CO₂), screw, hydraulic press comparing to traditional method on oil yield, total phenolics, antioxidant activity (DPPH) and oxidative stability of roselle seeds oil were investigated. Fatty acid composition and tocopherol of the oil were also determined. The results showed that the roselle seeds oil extracted by solvent gave the highest oil content and extraction rate (17.98 and 98.34%, respectively) and the lowest peroxide and acid value. SC-CO₂ gave the higher content of alpha, gamma, and delta tocopherol comparing to the other extraction methods. Fatty acid showed that linoleic acid, an essential fatty acid, was dominant followed by oleic and palmitic acids. These fatty acids were higher amounts in oil extracted by SC-CO₂, followed by cold-press comparing to solvent extraction. The maximum stability (20 h), higher TPC (22.18 mg GAE/g) and antioxidant activity (DPPH, 65.15) were observed in oil extracted by SC-CO₂ followed by hydraulic-press, solvent extraction and finally screw-press. The results revealed that SC-CO₂ method is more efficient in determination of total tocopherol, oxidative stability, TPC and radical scavenging activity than the other extraction methods. Cold press extraction methods gave higher oil yield than SC-CO₂ and more economically than other methods.

Keywords: roselle seed oil / green extraction methods / oxidative stability / non-traditional oil / antioxidant activity

Résumé – Évaluation de méthodes d'extraction vertes sur les caractéristiques chimiques et nutritionnelles de l'huile de graines d'*Hibiscus (Hibiscus sabdariffa* L.). L'*hibiscus* constitue l'une des plantes d'intérêt cultivées dans certaines régions d'Égypte, utilisée pour fabriquer des jus ou comme source de colorant alimentaire. Les graines d'*hibiscus* étant inutilisées dans ces filières, elles peuvent être employées comme source d'huile non traditionnelle, de composés nutritionnels ou fonctionnels. Les méthodes d'extraction vertes, parmi lesquelles l'extraction au CO₂ supercritique (SC-CO₂), la presse à vis et la presse hydraulique, ont été comparées à la méthode traditionnelle en termes de rendement en huile, de teneurs en composés phénoliques totaux, d'activité anti-oxydante (DPPH) et de stabilité à l'oxydation de l'huile de graines d'*hibiscus*. Les résultats montrent que l'huile de graines d'*hibiscus* extraite par solvant donne les plus grands rendement et taux d'extraction (17,98 et 98,34 %, respectivement), et les plus faibles indices d'acide et de peroxyde. L'extraction au SC-CO₂ conduit aux teneurs les plus élevées en tocophérols alpha, gamma et delta par rapport aux autres méthodes d'extraction. En termes de profil en acides gras, l'acide linoléique, un acide gras essentiel, se révèle prédominant, suivi par les acides oléique et palmitique. Ces acides gras présentent des teneurs plus élevées dans l'huile extraite par SC-CO₂, puis dans l'huile produite par pression à froid, par rapport à l'extraction par solvant. La stabilité maximale (20 h), la plus haute teneur en TPC (22,18 ± 1,13 mg EAG/g), et l'activité anti-oxydante la plus élevée (DPPH, 65,15 ± 2,08 %) sont observées dans l'huile extraite par SC-CO₂ suivie par la presse hydraulique, l'extraction par solvant et enfin la presse à vis. Les résultats révèlent ainsi que l'extraction au SC-CO₂

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constitue la méthode la plus performante quant aux teneurs en tocophérols totaux, la stabilité oxydative, la teneur en TPC et la neutralisation des radicaux par rapport aux autres méthodes d'extraction. Les méthodes d'extraction à froid conduisent quant à elles à des rendements en huile plus élevés que l'extraction SC-CO₂ et s'avèrent plus économiques que les autres méthodes.

Mots clés : huile de graines d'hibiscus / méthodes d'extraction vertes / stabilité oxydative / huile non traditionnelle / activité antioxydante

1 Introduction

Roselle (*Hibiscus sabdariffa* L.) seeds are the waste that is left behind during processing of roselle, also known as hibiscus, for juices or other roselle-related products. Disposal of wastes is highly undesirable both economically and environmentally. Roselle seeds are also a good source of dietary fibers and proteins that have great promise as a feed resource for livestock and value-added nutritional foods like defatted seed meal, protein concentrates from defatted seed, seed cake etc. (Atta and Imaizumi, 2002; Dhar *et al.*, 2015).

Functional lipid components that are likely present in roselle seed oil include phytosterols and tocopherols which have therapeutic functions in preventing cardiovascular disease and types of cancer. (Nyam *et al.*, 2009; Hassanien *et al.*, 2014). Roselle seeds contain high levels of phytosterol, which have been associated with lowering total cholesterol and anti-carcinogenic effects in both animal and human studies. β -sitosterol, a phytosterol presence in roselle seeds, has secured an important place in the realm of health supplements with extensive scientific support for its prophylactic and therapeutic use for various physical ailments like arteriosclerosis, benign prostate cancer, and colon cancer (Nair *et al.*, 2006; Yokota *et al.*, 2006; Abdel-Razek *et al.*, 2011; Hassanien *et al.*, 2014; Rudzińska *et al.*, 2016).

Roselle seed contains about 20% oil and is considered to have high nutritional value and health-promoting properties due to the presence of natural balance between saturated: monounsaturated: polyunsaturated fatty acids (Abdel-Razek *et al.*, 2017; Al-Okbi *et al.*, 2017). Ismail *et al.* (2008) and Naeem *et al.* (2017) found that roselle seeds contain reasonable amounts of protein, crude fiber, and oil, as well as carbohydrate (146, 212, 146 and 356 g kg⁻¹ by weight respectively).

Generally, the methods of extraction of seed oils are an effective factor in the properties of oils. Solvent extraction, for example, is insufficient in selectivity and needs excessive heat, which could cause the degradation of the desired components (Pokorny *et al.*, 2001; Aniolowska *et al.*, 2016; Rudzińska *et al.*, 2018). The cold press (hydraulic and screw-press) extraction is the method for oil extraction. It involves no heat and/or chemicals and this is preferred by consumers concerned about natural and safe food (Kiralan *et al.*, 2014). However, this method affords low yields (Soto *et al.*, 2007), and the residual meal contains 10 to 12% oil content, which can eventually limit its uses in industries processing food (Zuniga *et al.*, 2003). Supercritical fluid extraction (SFE) is currently a technique among others used to extract plant oils and offers some favorable features over the traditional techniques that have been used in the oil extraction (Akanda *et al.*, 2012).

The aim of this work is studying the effect of green extraction method (supercritical CO₂ and cold-press methods [hydraulic and screw press]) on major and minor components of roselle seed oil (RSO) comparing to traditional one (solvent extraction). In addition, comparing the physico-chemical and nutritional characteristics of RSO extracted by green and traditional methods with regard to the oil quality properties, antioxidant activity, and the oxidative stability of the extracted oil.

2 Materials and methods

2.1 Materials

Roselle (*Hibiscus sabdariffa* L.) seeds were purchased from the local market in June 2018 from Aswan, Egypt. All reagents and solvents used in this study were analytical grade.

2.2 Methods

2.2.1 Roselle seeds pretreatment

The roselle seeds (RS's) were kept at 60 °C for one hour in oven, to facilitate the extraction from seeds (Elabd *et al.*, 2017).

2.2.2 Extraction method

2.2.2.1 Supercritical CO₂ extraction

The extraction process was carried out on laboratory scale high-pressure extraction plant (HPEP, NOVA-Swiss, Effretikon, Switzerland) according to the method described by Cvjetko *et al.* (2012). The ground sample of 300 g was placed into the extractor vessel. To fill up the extraction vessel, diatomaceous earth (kieselguhr) was used as inert material. The extracted oil was collected into a previously weighed glass vials. A balance (precision of ± 0.0001 g) was used to weigh the extracted oil at regular time intervals. A mass flow rate of CO₂, expressed under normal conditions, was 0.194 kg h⁻¹, low enough to ensure the saturation of supercritical CO₂ (SC-CO₂) with the solute. The investigated values of pressure varied from 20 to 30 MPa, temperature varied from 40 and 60 °C during the extraction time up to 4 h. Separation conditions were 1.5 MPa and 25 °C. The obtained RSO was placed into glass vials, sealed and stored at -18 °C until analysis.

2.2.2.2 Hydraulic-press extraction

Roselle seed oil (RSO) was extracted by hydraulic cold pressing machine at room temperature using pressure at 10 MPa. Then, extracted oil was filtered using Whatman filter

No. 1, afterward was centrifuged at 3500 rpm for 20 min in order to separate those components settled during storage.

2.2.2.3 Screw-press extraction

A continuous process (screw-press) was used to extract oil from RS's. The extraction temperature was about 45 °C, at a rotational speed of 30 rpm. After pressing the oils were collected, and stored at -18 °C until analysis.

2.2.2.4 Solvent extraction

The oil extraction from ground RS's was performed in Fats and Oils Department National Research Centre, Cairo, Egypt according to the Soxhlet extraction method. The n-hexane was used as extraction solvent at a ratio of 1:10, a sample to solvent (Bhatnagar and Krishna 2013).

2.2.3 Determination of physicochemical properties

2.2.3.1 Oil content (%) and extraction rate

The percentage of oil content in RS's was calculated according to the method described by AOCS, Firestone (2009), using the following equation:

$$\% \text{ of extracted oil} = [W_O/W_S] * 100, \quad (1)$$

where: W_O is a weight of obtained oil in g, W_S is a weight of seeds in g.

2.2.3.2 The oil recovery efficiency (ORE)

The oil recovery efficiency (ORE) is the ratio of actual oil produced by certain method such as SC-CO₂ or hydraulic-press or screw-press to the oil contained in the RS recovered from solvent extraction (n-hexane) by Soxhlet method according to the following equation (Donough *et al.*, 2013):

$$\text{ORE}(\%) = [P_1/P_0] * 100, \quad (2)$$

where: P_1 is the weight of oil extracted by certain method; P_0 is the weight of oil recovered by solvent extraction method (Soxhlet).

2.2.3.3 Physicochemical properties

Physicochemical analyses of RSO samples such as refractive index, peroxide value, free fatty acid %, saponification value, specific gravity, and iodine value were accomplished based on Official Methods of AOCS, Firestone (2009).

2.2.3.4 Fatty acid composition (GC-MS)

Methyl esters of fatty acids (FAME) were prepared according to Official Methods and Recommended Practices of the AOCS, Firestone (2009). Diluted FAME was separated on an HP 5890 series II (Hewlett Packard, HP, Palo Alto, USA) equipped with an Innovax capillary column (30 m, 0.20 mm) and flame ionization detector (FID). Hydrogen was used as the carrier gas at a flow rate of 1.5 mL min⁻¹. The column temperature was isotherm 210 °C. Detector and injector temperatures were set at 240 °C. Fatty acids were identified by comparison of the retention times with authentic standards

and the results were reported as weight percentages after integration and calculation using Chem. Station (Agilent Technologies).

2.2.3.5 Calculated oxidizability value (Cox)

The Cox value of the oils was calculated from the values of the fatty acid percent, according to Fatemi and Hammond (1980) using the following equation:

$$\text{Cox value} = [1 * (C_{18:1}) + 10.3 * (C_{18:2}) + 21.6 * (C_{18:3})]/100. \quad (3)$$

2.2.3.6 Determination of RSO tocopherols

RSO Samples were saponified according to Lee *et al.* (2012). HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto-sampler and a diode-array detector. The analytical column was a LiChrospher 5 RP Select B (250 × 4.0 mm; 5 μm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and methanol (solvent B). The flow rate was kept at 1.0 mL/min for a total run time of 30 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 50 μL and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 μm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards.

2.2.3.7 Total phenolic content (TPC)

Folin-Ciocalteu method was used for determination TPC according to the reported method by Gutfinger (1981). RSO (2.5 g) was dissolved in 5 mL hexane and extraction was carried out by methanol/water solution (80:20, v/v). The aqueous phase was collected by centrifugation at 3500 rpm for 5 min, followed by vacuum drying at room temperature. The dried sample was dehydrated in 5 mL of the methanol solution, mixed with 2.5 mL of Folin reagent and 10 mL of sodium carbonate solution in 50 mL volumetric flask, and was adjusted to volume with deionized water. The absorbance was measured at 765 nm after 30 min. Gallic acid was used for calibration and the results were expressed as mg gallic acid equivalent (GAE) per 100 g of oil samples.

2.2.3.8 Radicals scavenging activity

The radical scavenging ability of the extracts was determined using the stable DPPH free radical (2,2-diphenyl-1-picrylhydrazyl) according to Wong *et al.* (2016). The methanol solution prepared previously (0.3 mL) was mixed with 2.7 mL of a freshly prepared DPPH solution (6 × 10⁻⁵ M in 95% methanol). The mixture was shaken vigorously and left at room temperature for 60 min in the dark until stable absorbance values were obtained. The control contained methanol in place of the sample. The change in the

Table 1. Effect of green and traditional extraction methods on physico-chemical properties of RSO.

Characteristics	Extraction methods			
	Supercritical (CO ₂)	Hydraulic-press	Screw-press	Solvent
Oil content (%)	8.75 ± 1.04 ^c	9.35 ± 1.13 ^c	12.17 ± 0.89 ^b	18.98 ± 1.54 ^a
Free Fatty Acid (as % oleic acid)	0.30 ± 0.05 ^c	0.61 ± 0.11 ^{bc}	0.71 ± 0.15 ^b	2.41 ± 0.37 ^a
Iodine Value (g/100 g)	97.76 ± 0.35 ^a	94.50 ± 1.24 ^b	93.62 ± 0.95 ^b	93.41 ± 1.35 ^b
Refractive Index	1.4504 ± 0.0001 ^b	1.4494 ± 0.0003 ^b	1.4500 ± 0.0002 ^b	1.4562 ± 0.0001 ^a
Specific Gravity(g/cm ³)	0.895 ± 0.004 ^c	0.927 ± 0.01 ^b	0.911 ± 0.007 ^{bc}	0.951 ± 0.02 ^a
Peroxide value (mEq./kg)	1.01 ± 0.01 ^d	1.59 ± 0.27 ^c	2.14 ± 0.51 ^b	4.57 ± 0.46 ^a
Saponification value (mg/g)	191.04 ± 1.13 ^b	193.65 ± 2.50 ^a	193.91 ± 1.59 ^a	190.87 ± 3.47 ^b

The values were expressed as mean ± standard deviation of three determinations.

Values in the rows with different letters (a–d) are significantly different ($p < 0.05$). The same letters at the same column are not significant ($p < 0.05$).

absorbance of the extracts was measured at 517 nm using a spectrophotometer. The percentage inhibition of the DPPH radicals was calculated using the equation below.

$$\text{DPPH scavenging activity (\%)} = [(Ac - As) / Ac] \times 100, \quad (4)$$

where Ac and As is the absorbance of the blank (control) and samples, respectively.

3.3.9 Oxidative stability index (OSI)

For determination of oxidative stability of oils, AOCS Official Method Cd 12b–92 (AOCS, 1997) was used. The test was performed on an automated Metrohm Rancimat model 743 (Herisau, Switzerland) at 110 ± 0.1 °C and an airflow of 20 L h^{-1} to determine the induction period (IP) of the oil samples.

2.3 Statistical analysis

All measurements were replicated three times. Statistical analyses were conducted using SPSS program version 20 for windows. Significant differences among samples were analyzed by using ANOVA, Duncan-*post hoc* test at a level of $p \leq 0.05$.

3 Results and discussion

3.1 Physicochemical properties of roselle seed oil (RSO)

The results of oil contents, extraction rate, refractive index, specific gravity, free fatty acid, peroxide value, iodine value and a saponification value of RSO extracted by different methods are presented in Table 1. The results obtained showed that RS is considered as a valuable source of oil content (%) ranged from 8.75 to 18.98%. According to the results which are shown in Table 1, it was found that the solvent extraction gave the highest oil yield while screw-press gave the higher oil recovery efficiency (ORE) (Fig. 1). This result agreed with Al-Okbi *et al.* (2017), which found that the oil content obtained by solvent extraction was 20%.

Determination of free fatty acid (FFA) is usually considered to be one of the main parameters to reflect the

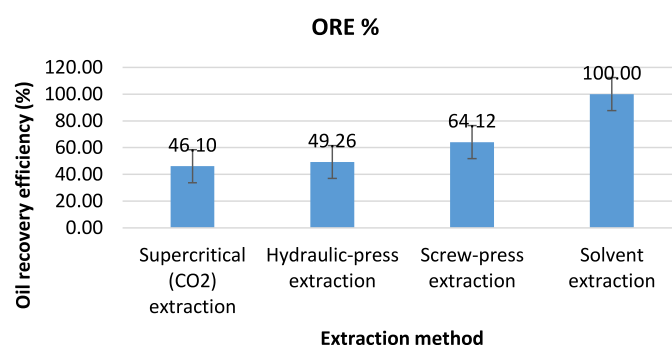


Fig. 1. Oil recovery efficiency (ORE) of RSO extracted by different methods.

quality and the degree of purity of the oil. (Tasan *et al.*, 2011). RSO extracted by solvent gave the highest FFA (2.41%). This result agrees with Mohamed *et al.* (2007), who found that the acidity of RSO extracted by solvent was 2.24%. While the FFA in other extracted methods amounted to 0.3, 0.61 and 0.71% for supercritical-CO₂ (SC-CO₂), hydraulic and screw-press respectively. Moreno *et al.* (2003) reported that lower FFA indicated that the quality of oil was better.

The sensitive methods are required to detect slight changes in unsaturation (Alexandri *et al.*, 2017) such as measurement of iodine value (IV) and refractive index (RI) has been done in this study (Tab. 1). The quality of the oils is dependent on their chemical compositions, like the percentage of the degree of unsaturation. The IV of RSO extracted by SC-CO₂ was $97.76 \text{ g } 100 \text{ g}^{-1}$ and for oil extracted by hydraulic-press, it was $94.5 \text{ g } 100 \text{ g}^{-1}$. RSO extracted by screw-press and traditional method (solvent) gave nearly the same value of IV (93.5 and $93.4 \text{ g } 100 \text{ g}^{-1}$). From the results, it was noticeable the reduction in the IV in case of extraction oil by screw-press and solvent, this variation of unsaturation values may be due to the different extraction methods.

The RI of RSO was 1.4504, 1.4494, 1.4500 and 1.4562 for oil extracted by SC-CO₂, hydraulic and screw-press as well as traditional method respectively. It was noticeable that there is no marked change in RI using different extraction methods. These results agreed with Mohamed *et al.* (2007), who found that RI of RSO was 1.4770. The RI of oil could be a function of

Table 2. Fatty acid composition of oil samples extracted by different methods.

Fatty acids	Peak area (%)			
	Supercritical (CO ₂) extraction	Hydraulic-press extraction	Screw-press extraction	Solvent extraction
Myristic acid	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.13 ± 0.01
Palmitic acid	20.52 ± 0.87	19.95 ± 0.24	19.93 ± 0.71	19.50 ± 0.90
Pamitoleic acid	0.41 ± 0.07	0.53 ± 0.05	0.47 ± 0.03	0.43 ± 0.01
Margaric acid	0.09 ± 0.01	0.10 ± 0.04	0.13 ± 0.01	0.13 ± 0.02
Stearic acid	5.39 ± 0.61	7.45 ± 0.72	7.12 ± 0.64	6.78 ± 0.34
Oleic acid	29.38 ± 1.13	27.65 ± 1.01	27.44 ± 1.34	28.07 ± 1.07
Linoleic acid	39.91 ± 1.65	37.44 ± 1.59	36.95 ± 1.35	36.53 ± 1.81
Linoleic acids (conjugated)	0.07 ± 0.01	1.44 ± 0.27	1.52 ± 0.22	1.41 ± 0.08
Linolenic acid	1.20 ± 0.17	2.18 ± 0.10	2.30 ± 0.31	2.31 ± 0.11
Arachidic acid	0.40 ± 0.12	0.37 ± 0.02	0.85 ± 0.07	0.81 ± 0.14
Behenic acid	0.10 ± 0.00	0.21 ± 0.03	0.33 ± 0.07	0.30 ± 0.07
Lignoceric acid	ND	ND	ND	0.13 ± 0.01
Others	2.80	2.55	2.82	3.47
Σ SFA	26.63	28.20	28.50	26.97
Σ MUFA	29.79	28.18	27.91	28.50
Σ PUFA	41.18	41.06	40.77	40.25
Σ UFA	70.97	69.24	68.68	68.75
Σ UFA/ Σ SFA	2.66	2.45	2.4	2.54
Cox value	4.67	4.25	4.73	4.69

ND=not detected; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; Σ UFA /ΣSFA: unsaturated fatty acids to saturated fatty acids ratio.

its unsaturation and the higher the content of double bond, the greater the effect of reducing the refraction angle (Hasenhuettl, 2000)

Concerning specific gravity (g cm^3), it was noticed that no remarkable changes in it by using different extraction methods.

The peroxide value (PV) is a useful indicator of the extent of oxidation of lipids, fats, and oils (Mohamed *et al.*, 2018). The PV shows the degree of peroxidation and measures the primary oxidation products in the substance (Kouba and Mourot, 2011). The lowest value of PV was found in oil extracted by SC-CO₂ (1.0 mEq. O₂ kg⁻¹), while the highest PV in case of oil, extracted by solvent (4.57 mEq. O₂ kg⁻¹) (Tab. 1).

The saponification value (SV) of an oil or fat has an opposite correlation with molecular weight or chain length of its fatty acids (Abayeh *et al.*, 1998; Ortiz *et al.*, 2003). From the results in Table 1, it was found that the SV ranged from 190.04 until 193.91 mg/g. The RSO extracted by screw-press gave the highest SV (193.91 mg/g). Mathur (2016) found that the value of SV (200, 190, 196) resembles the normal values for vegetable oils. Nielsen (1989) and Kirk and Sawyer (1991) reported that high or low SV's of fats and oils are due to shorter or longer carbon chain lengths of the fatty acids.

3.2 Fatty acid composition

The fatty acid composition (FAC) determined by GC of RSO samples, illustrated in Table 2 and Figure 2, showed that the total content of unsaturated fatty acid (UFA) ranged from 68 to 70%, from 26 to 28% for saturated fatty acids (SFA) and from 27 to 29% for monounsaturated fatty acid (MUFA). The ratio of UFA/SFA and Cox value in RSO extracted by SC-CO₂, hydraulic, screw-press (green methods) and conventional

methods (solvent) were calculated to 2.66, 2.45, 2.4, 2.54 and 4.67, 4.25, 4.73, 4.69, respectively. Oleic and linoleic acids had the highest values of 29.38 and 39.91%, respectively for RSO extracted by SC-CO₂. The FAC shown the high content in UFA, especially linoleic acid (ranged from 36.53 to 39.91%), thus, is indicating the nutritional benefits of RSO. Linoleic acid has beneficial effects on blood lipids, lowering blood pressure and serum (Savage, 2001; Enujiugh and Akanbi, 2008). Among the UFA (Tab. 2), palmitoleic acid content was the lowest, nearly kept constant in RSO extracted by SC-CO₂, hydraulic, screw-press (green methods) and conventional methods (solvent), whereas palmitic acid was (SFA) 20.5% for oil extracted by SC-CO₂, 19.9% for oil extracted by cold-press and 19.5% for oil extracted by solvent methods (Tab. 2). Minor identified FA's lauric, myristic, arachidic, etc. These results were agreed with Al-Okbi *et al.* (2017). In addition, Al-Okbi *et al.* (2017) found that RSO was a unique balanced fatty acid ratio (S:M:P) refers to its possible direct human consumption.

3.3 Tocopherol content of the RSO

Tocopherols are the most important lipid-soluble natural antioxidants present in vegetable oils. The contents of total tocopherols in RSO extracted by green methods (SC-CO₂, hydraulic and screw-press) as well as solvent are given in Table 3 and Figure 3. The total tocopherol concentration increased in the case of RSO extracted by the SC-CO₂ method, followed by solvent extraction. While RSO extracted by hydraulic-press was higher total tocopherol than screw-press and the two extracted methods less than SC-CO₂ and solvent (Fig. 3). The optimum conditions yielded highly tocopherol concentration from RS were extracting by SC-CO₂, followed

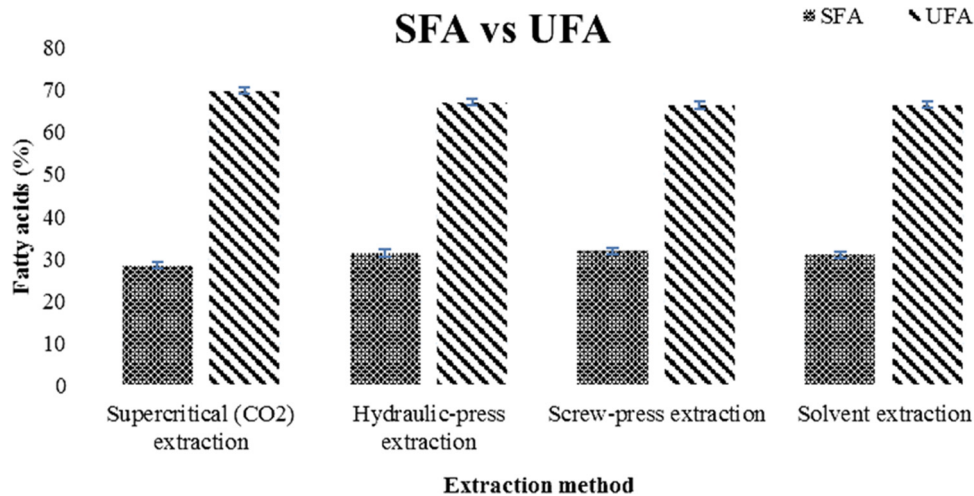


Fig. 2. SFA vs. UFA of RSO extracted by different methods.

Table 3. Effect of extraction methods on tocopherols content of RSO by HPLC.

Tocopherol fractions content (mg/100 g oil)	Extraction methods			
	Supercritical (CO ₂)	Hydraulic -press	Screw -press	Solvent
α-tocopherol	55.11 ^a	75.23 ^c	69.41 ^{bc}	65.57 ^b
β-tocopherol	ND*	ND	ND	ND
γ-tocopherol	127.24 ^b	133.64 ^b	116.49 ^a	168.03 ^c
δ-tocopherol	107.43 ^d	25.14 ^a	29.33 ^{bc}	33.75 ^c

*ND= not detected

Values in the rows with different letters (a–d) are significantly different ($p < 0.05$). The same letters at the same column are not significant ($p < 0.05$).

by solvent then hydraulic-press and finally screw-press. Concerning the tocopherol composition of RSO, it was found that the highest and lowest amount of α-tocopherol in case of hydraulic-press and SC-CO₂ extraction respectively (75.23 and 55.11 mg 100 g⁻¹). The highest amount of δ-tocopherol (107.43 mg 100 g⁻¹) was found in oil extracted by the SC-CO₂ method. δ-Tocopherol ranged from 25.14 to 33.75 mg 100 gm⁻¹ oil in other three extracted methods. Concerning γ-tocopherol, solvent method gave the highest level followed by hydraulic-press, SC-CO₂ and then screw-press amounted to 168.03, 133.64, 127.24 and 116.49 mg 100 gm⁻¹ oil respectively. γ-tocopherol could delay the oxidation of PUFA in oils. In addition, α- and γ-tocopherols have a special activity against free radicals and play an important role in the regulatory functions in the live cells (Chandra *et al.*, 2002; Ricciarelli *et al.*, 2002). Mohamed *et al.* (2007), they found that RSO extracted by solvent gave highest γ-tocopherol as antioxidant which agreed with our results.

3.4 Total phenolic content (TPC)

Results obtained in this study reveal that the level of total phenolic compounds (TPC) in RSO is considerable (Tab. 4).

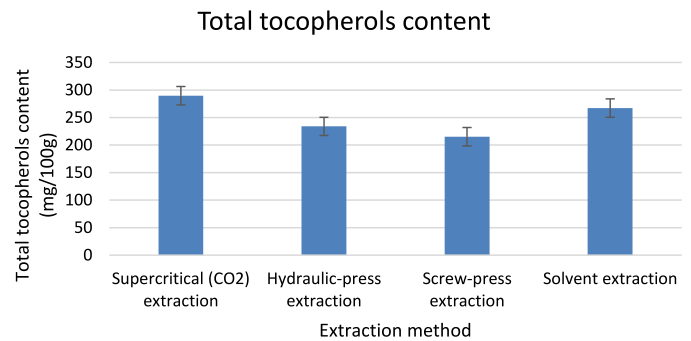


Fig. 3. Total tocopherols content of RSO extracted by different methods.

TPC of different RSO samples varied significantly ($p \leq 0.05$) ranging from 13.74 to 22.18 mg GAE g⁻¹. The RSO prepared using SC-CO₂ extraction showed the highest total phenolic content (22.18 mg GAE g⁻¹), while the lowest was observed in case of oil extracted by screw-press (13.74 mg GAE g⁻¹), with a significant difference ($p \leq 0.05$) when compared with other method extraction (Tab. 4). This study showed that RSO obtained using different extraction methods, had different total phenolic contents. There is a positive correlation between the total content of phenolic compounds and the antioxidant activity and over 95% of the antioxidant capacity is due to their phenolic components (Turkmen *et al.*, 2007; Wang *et al.*, 2017). Al-Okbi *et al.* (2017) found that RSO (extracted by screw press) was rich in bioactive constituents represented by total phenolics (56.31 mgGAEg⁻¹), and total tocopherol (99.86 mg/100 g) especially γ-tocopherol. Tsuda *et al.* (2003), found that a diet is rich in polyphenols could prevent obesity, or optimize the treatment of diabetes.

3.5 DPPH radical scavenging activity

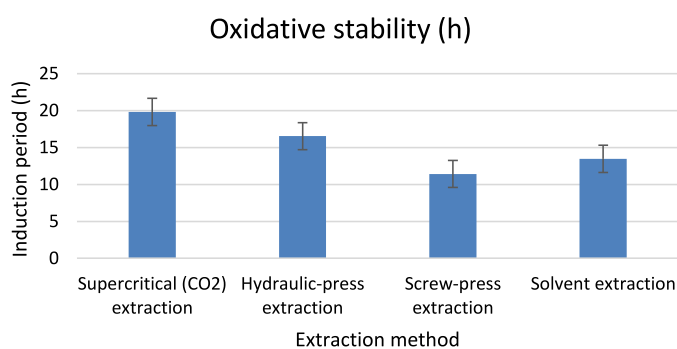
The effect of green methods extraction on DPPH free radical scavenging activity of RSO was investigated (Tab. 4).

Table 4. Total phenolic content, antioxidant activity and oxidative stability of RSO samples.

Parameter	Extraction methods			
	Supercritical (CO ₂)	Hydraulic-press	Screw-press	Solvent
Total phenolic content (mg of GAE/g)	22.18 ± 1.13 ^a	17.25 ± 1.07 ^b	13.74 ± 0.55 ^c	18.10 ± 1.43 ^b
DPPH radical-scavenging activity (%)	65.47 ± 2.08 ^a	57.52 ± 1.33 ^b	49.59 ± 0.74 ^c	55.67 ± 1.09 ^b
EC ₅₀ (mg/mL)	0.507 ^d	0.577 ^c	0.661 ^a	0.593 ^b

EC₅₀ value, the effective concentration at which the antioxidant activity was 50% and it was obtained by extrapolation from linear regression analysis; data are expressed as mean ± standard deviation of three determinations.

Values in the rows with different letters (a–d) are significantly different ($p < 0.05$). The same letters at the same column are not significant ($p < 0.05$).

**Fig. 4.** Oxidative stability of RSO extracted by different methods.

The results showed that SC-CO₂ extraction method exhibited the highest DPPH scavenging activity (65.47). Oil extracted by hydraulic-press gave the higher DPPH (57.52) than solvent extraction (55.67). In addition, the SC-CO₂ extraction method had the highest scavenging activity; therefore, the lowest EC₅₀. Statistical analysis revealed that the difference between the SC-CO₂ extraction method and the other extraction methods was significant ($p \leq 0.05$). There is a positive correlation between the total content of phenolic compounds and the antioxidant activity (Turkmen *et al.*, 2007; Wang *et al.*, 2017).

3.6 Oxidative stability index (OSI)

According to the results of oxidative stability, it was found that the induction period (IP) of RSO's ranged from 12 to 20 h (Fig. 4), as measured by Rancimat at 110 °C. RSO oil extracted by SC-CO₂ had the highest IP (20 h), followed by oil extracted with hydraulic-press (18 h), then oil extracted by solvent and by screw-press. Thus, the stability of oil extracted by SC-CO₂, maybe due to contained appreciable amounts of more stable FA (omega-9). As well as, higher content of total phenolic, total tocopherol and γ -tocopherol, which is potent antioxidant and the best ratio of UFA/SFA, compared to other investigated oils. The oxidative stability of the oil is an important quality and safety parameter for their potential commercial applications and utilization in food and other commercial products, and it depends mostly on the FA composition as well as the potency and content of antioxidant of these oils (Yang *et al.*, 2010). Al-Okbi *et al.* (2017), found that RSO extracted by screw press have IP (24.9 h at 100 °C).

4 Conclusion

The results show that the type of extraction methods is affecting the ratios and quality of major and minor components present in extracted RSO; subsequently, affecting the physico-chemical properties on produced oil. From the results, it was found that green extraction methods were more efficient than the traditional one (solvent extraction). The results revealed that the RSO extracted by SC-CO₂ method gave the highest content of natural antioxidants (total tocopherol, TPC) as well as oxidative stability (DPPH, IP) than the other extraction methods. The cold-press extraction methods (hydraulic and screw-press) gave highest α -tocopherol. Hydraulic-press extraction method gave the higher γ -tocopherol than SC-CO₂. Cold press extraction methods gave higher oil yield than SC-CO₂ and more economically than other methods. Cold-press involves no heat and/or chemicals and this is preferred by consumers concerned about natural and preserving the phytonutrients components of RSO that can be used as non-traditional edible oil.

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