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Optimization of Super Palm Olein Stability using Natural Antioxidants from Jojoba Seed Waste

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Abstract

In the present investigation natural phenolic antioxidants were extracted from Jojoba seed waste. Samples of defatted jojoba seed meal (JSM), detoxified meal residue (DMR) and jojoba seed shell (JSH) were subjected to extraction by using various solvents: 70/30 (vol/vol) ethanol/water, 70/30 (vol/vol) methanol/water and 60/40 (vol/vol) acetone/water. Total phenolics content (TPC) is measured according to the Folin-Ciocalteu method. In addition, solvent extracts were tested for their antioxidant activity (AA) in vitro by the DPPH radical scavenging and the β-carotene linoleic acid bleaching methods. The highest amount of total phenolic compounds (TPC) is observed with 60% acetone extract of JSH (163.81±6.88 mg GAE g^{-1} extract DW). On the other hand, the 70% Methanolic extract of JSH indicates the highest activity in scavenging DPPH free radicals. DMR extracts had low amount of phenolics. Furthermore, the ethanolic extract of JSM and JSH at concentration 200ppm showed good antioxidant capacity for stabilization of super palm olein (SPO) than BHT as determined by the rancimat procedure. Therefore, these extracts could be a good alternative for synthetic antioxidant to protect the vegetable oils such as SPO which contain different levels of unsaturated fatty acids.

***Keywords**: Jojoba seed waste, rancimat test, DPPH.*

1. Introduction

Palm olein is the most produced and consumed refined oil in the world and is used for cooking, food formulations and fast food manufacturing (Osawa and Gonçalves, 2012). Super palm olein (SPO) is produced by double fractionation of palm olein. It contains more unsaturated fatty acids like oleic acid and linoleic acid, an essential fatty acid (Pantzaris, 1995). During storage, photosensitized oxidation and autoxidation are mainly responsible for the oxidation of oils. Besides the degradations caused in the quality properties of texture, flavor, color and nutritional value, lipids oxidation leads to the production of several secondary products that have high toxicological potential and mutagenic capacity (Krishnaiah *et al.,* 2010). However, oxidative stability of oils is the main factor that influences their acceptability.

Nowadays, an increase of research works about natural antioxidants plant-based extracts to obtain bioactive compounds that can be used to inhibit the oxidation of foods. These natural compounds have lower toxicity than synthetic antioxidant such as butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) (Somayeh *et al.,* 2012). Plant-derived products contain a wide range of phenolic compounds such as phenolic acids and flavonoids that mainly contributed to their antioxidant activi-

ties (Shahidi and Naczk, 2004). Phenolic compounds can donate hydrogen atoms to free radicals or react with lipid peroxyl radicals and consequently, inhibit the formation of oxidation products.

The oil seed industry yields two products: oil, chiefly for human consumption and meal (cake), which is actually used as animal feed.

Jojoba meal, as a by-product of jojoba seeds, is a promising feedstuff after being detoxified (Motawe, 2006). According to Abd El- Rahman, *et al*., (2006) defatted jojoba meal contained 31.89±1.12% crude protein, simmondsin 3.33±0.02%, and total phenolic compounds 2.67±0.02%. Some authors consider simmondsins to be toxic, probably after metabolism by gut microorganisms (Booth *et al*. 1974 and Verbiscar *et al*.,1980). In contrast, The USA Food and Drug Administration approved simmondsin as safe for human use and animal feed (Oksman-Caldentey and Inze, 2004).

Ethanolic extract from jojoba was found to inhibit oxidative stress induced by fumonisins (Abdel-Wahhab, *et al.,* 2010). In addition to the presence of phenolic compounds, phytosterols, toccopherols and fatty acids. Al-Qizwini *et al*., (2014) associated the antioxidant property of jojoba with the presence of simmondsin and its products: simmondsin-3′-ferulate, 4,5-didemethylsimmondsin and 4-demethylsimmondsin-2′-ferulate. Abdel-Mageed *et al.* (2014) have reported the isolation of antioxidants and lipoexgenase inhibitors in jojoba seed. The researchers isolated 10 flavonoids and four lignans. They reported that flavonoid aglycosides showed stronger antioxidant and lipoxygenase inhibitory effects than their glycoside counterparts. In a similar study, the essential oil extracted from jojoba displayed an inhibition percentage of 7.81% based on a DPPH radical scavenging assay (Abdul-Hafeez, *et al.* 2014). Fumonisins are mycotoxins that interfere with ceramide synthase, leading to the inhibition of synthesis of biological molecules and incurring lipid peroxidation in rat hepatocytes (Bryła *et al.*, 2013).

The shell (hulls) of oilseeds are fibrous and have low oil content. Oilseed hulls are among the food industry waste products that have been reported to contain beneficial phenolic compounds, Including sunflower hulls (Mohamed and Taha, 2005), soybean hulls (Corredor *et al.,* 2008), rice hulls (Asamarai *et al.,* 1996), peanut hulls (Duh and Yen, 1997), sesame coat (Chang *et al.,* (2002), flaxseed hulls (Singer and Wagdy, 2011), jatropha and jojoba hulls (Wagdy and Taha, 2012).

This study aimed to optimize the antioxidative potential of phenolic antioxidants extracts from jojoba seed waste (meal and shell) on the oxidative stability of super palm olein (SPO). Further the effect of the meal detoxification process on the antioxidative compound was also investigated.

2. Materials and Methods:

2.1. Materials

Palm olein free from additives was obtained from Ajwaa Factory for Food Industries, Attaka, Suez, Egypt. Jojoba seeds were donated from Faculty of Agriculture Farm of Minia University.

Chemicals

Diphenyl-picrylhydrazyl (DPPH), β–carotene, linoleic acid, Tween, and Folin-Ciocalteu reagent were obtained from Sigma chemical co. All other chemicals and reagents were of analytical grade and obtained from El-Gamhouria Trading Chemicals and Drugs Company (Egypt).

Preparation of sample

Jojoba seeds were cleaned and milled by using laboratory mixer and passed through a 1000 µ sieves. Jojoba meal (JSM) was prepared by extraction of jojoba seeds with hexane using the soxhlet extraction method for 8 hours according to the procedure described by Visavadiya *et al*. (2009). Jojoba hulls were obtained manually from a sample of jojoba seeds and also milled. The ground hulls were defatted with n- hexane in a Soxhlet apparatus and saved for further work. After extraction, the solvent were evaporated under reduced pressure using a rotary evaporator model (HS-2005V-N).

A sample of jojoba meal was therefore detoxified by using 70% aqueous isopropanol (DMR) in a ratios of 4:1 for 4h according to the procedure of Medina and Trejo-Gonzalez (1990).

2.2. Preparation of extracts

Three solvent systems were used as extraction media: 70/30 (vol/vol) ethanol/water, 70/30 (vol/vol) methanol/water and 60/40 (vol/vol) acetone/water. Each sample (20 g) was extracted with 200 ml of the aqueous solvent during 12 h of continuous stirring. After filtration, the residues were re-extracted twice under the same conditions. The extracts were combined, evaporated to dryness under vacuum at 45ºC, using rotary evaporator model (HS-2005V-N). The yield of the extracts was determined by weighing after further removing traces of water in a vacuum oven set at 35ºC overnight according to Kähkönen *et al*., (1999).

2.3. Chemical analysis

Moisture, ash, crude fiber and crude protein of JSM, DMR and JSH samples were evaluated by using official analytical method AOAC (2000). Nitrogen free extract (NFE) was calculated by difference.

Oil content was measured according to AOCS methods (2000) Ac 2-41. The AOCS methods (2000) were also used to determine Color, refractive index, melting point via slip point method, cloud point, Free fatty acids% as oleic acid, peroxide value (meq O2/kg oil) and iodine value of super palm olein.

2.4. Gas liquid chromatography analysis of fatty acids

The fatty acids methyl esters were prepared using benzene: methanol: concentrated sulfuric acid (10:86:4) and methylation was carried out for one hour at 80-90°C according to Stahl, (1967). The composition of fatty acids were achieved by Gas Liquid Chromatography analysis using PYE Unicam model PV 4550 Gas chromatography fitted with flame ionization detector, the column (1.5 m x 4 mm) packed with diatomite C (100-120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8°C/min from 70°C to 190°C then isothermally at this temperature for 20 min and nitrogen flow rate was 30ml/min. Detector, injection temperatures, hydrogen and

air flow rates and chart speed were 300°C, 250°C, 33 ml/min, 330 ml/min and 2 cm/min respectively. The presented fatty acids were identified according to an authentic sample of fatty acids chromatographed under the same conditions. Methyl heptadecanoate was used as an internal standard.

2.5. Total phenolic content (TPC) determination

TPC of JSM, DMR and JSH were determined using the method by Gutfinger (1981). The Jojoba seed meal and hulls extracts (1 mL,1 mg/mL) were mixed with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na2CO3, and centrifuged at 13400 ×*g* for 5 min. The absorbance of upper phase was measured using a spectrophotometer (T 80 uv/vis spectrometer PG Instruments LTD) at 750 nm after 30 min incubation at room temperature. Results were expressed as milligram per gram gallic acid equivalent per gram extract (DW). Each test was repeated three times and the results were averaged.

2.6. Preliminary tests of AA (In vitro-assay)

a) 2, 2-Diphenyl-1-picryl hydrazyl test (DPPH)

The radical scavenging ability of the extract was determined according to the method of Braca *et al.* (2002). A total of 4.5 ml of 0.002% alcoholic solution of DPPH was added to 0.5 ml of different concentrations (125, 250, 500, 1000, and 2000 µg/ml) of samples and standard solutions separately, in order to have final concentrations of products of 25–200 µg/ml. The samples were kept at room temperature in the dark and after 30 min the absorbance of the resulting solution was measured at 517 nm. The absorbance of the samples, control, and blank was measured in comparison with methanol. Synthetic antioxidant, butylated hydroxytoluene (BHT), which is a recognized powerful hydrogen donor, was used. The antiradical activity (AA%) was determined using the following formula:

$$AA\% = \{(Abs_{control} - Abs_{sample}) \times 100/Abs_{control}\}$$

b) β-Carotene/linoleic acid bleaching system

The ability of the extract to prevent the bleaching of β –carotene was assessed as described by Keyvan *et al.*, (2007). In brief, 0.2 mg β -carotene in 1 mL chloroform, 20 mg of linoleic acid and 200 mg of Tween 20 were transferred into a round-bottom flask. Once the chloroform had been removed at 40° C in a vacuum evaporator, 50 mL distilled H_2O was added and the resulting mixture was stirred vigorously. Aliquots (4 mL) of this emulsion were transferred into test tubes, to which were then added 0.2 mL aliquots of test samples in ethanol. Butylated hydroxytoluene (BHT) was used for comparative purposes. A control containing 0.2 mL of ethanol and 4 mL of above emulsion was also prepared. The test tubes were covered with aluminium foil and placed in a water bath at 50 °C. Absorbances for all samples at 470 nm were recorded at zero time (Abs = 0). The remaining samples were placed in a water bath at 50°C for 2 h. Thereafter, the absorbance of each sample was measured at 470 nm (Abs120). All determinations were

carried out in triplicate. Antioxidant activity (AA) was expressed as percentage inhibition relative to the control using the equation

$$\text{AA (\% Inhibitation of } \beta\text{-carotene bleaching)} = [1 - (\frac{Abs^{0}_{sample} - Abs^{120}_{sample}}{Abs^{0}_{control} - Abs^{120}_{control}}) \times 100]$$

2.7. Determination of the susceptibility of SPO to oxidation with the Rancimat method. The antioxidant activity of the natural phenolic extract from jojoba seed was examined in lipidic form (In vivo) by the rancimat method. The extract that presented the highest capacity in the in vitro method employed before was also evaluated lipidic system (double fractionated palm olein). For that stability tests were performed in duplicate using Metrohm Rancimat 679 (Metrohm Ltd., Herisau, Switzerland) as described by Taskins *et al.* (1999). 5.0 ± 0.05g of each oil was accurately weighed into each of the six reaction vessels, and the following procedure was carried out. The Metrohm Rancimat was switched on until the temperature of the oil batch reached 110°C. Then 60 cm^3 of distilled water was placed into each of the six conductivity cells, and the air flow rate was set at 20 L h^{-1}. The temperature was checked to ensure that it had a constant value. The air supply was connected to the tubes containing the oil samples, and the chart recorder was started. The determination continued automatically until the conductivity reached the maximum value and the induction period was read. The protection factors calculated following the formula: protection factor = induction time of oil with antioxidant/induction time of oil without antioxidant (Yanishlieva and Marinova, 2001).

2.7. Statistical analysis

Statistical analysis involved use of the Statistical analysis systems (SAS, 1987) software package. Significance difference between the means was determined by Duncan's multiple range tests.

3. Results and Discussion

3.1. Proximate chemical composition of jojoba seed waste

The chemical composition of jojoba seed meal (JSM), detoxified meal (DMR) and jojoba seed shell (JSH) are given in Table (1). The results indicate that the chemical composition (%) of JSM is 8.06±0.57 of moisture, 0.86±0.11 of oil, 23.46±0.74 of crude protein, 53.4% of nitrogen free extract (NFE), 10.88±1.22 of crude fiber and 3.34±0.40 of ash. Such results are in agreement with those of Mohamed, (2007) and Elsanhoty *et al.,* (2017). However, meal sample residue after detoxification (DMR), had 5.71±0.53% of moisture, 4.03±0.70% of ash, 11.70±0.91% of crude fiber, 26.19±1.05% of protein, 0.79±0.13% of oil and 51.58% of nitrogen free extract (NFE). On the other hand, the chemical composition of jojoba seed shell (JSH) were 7.76±0.33% of moisture, 2.78±0.19% of ash, 17.74±0.42% of crude fiber, 4.13±0.54% of protein, 1.18±0.46% of oil and 66.42% of nitrogen free extract (NFE). These values are in the range reported by Medina and Trejo-Gonzalez (1990) and Abobatta *et al*., (2015).

Table 1. Proximate chemical composition (g/ 100g Dry weight) of jojoba seed meal (JSM), detoxified meal residue (DMR) and jojoba seed shell (JSH)

Proximate composition (%)	JSM	DMR	JSH
Moisture	8.06±0.57	5.71±0.53	7.76±0.33
Ash	3. 34±0.40	4.03±0.70	2.78±0.19
Crude fiber	10.88±0.22	11.70±0.19	17.74±0.24
Protein	23.46±0.74	26.19±0.35	4.13±0.54
Oil	0.86±0.11	0.79±0.13	1.18±0.26
NFE**	53.40	51.58	66.42

*Results are expressed as means ±SD (n=3)

** calculated by difference.

3.2. Extraction yield (g/100g) of seed meal and shell with different solvent solutions

The extraction yields of the jojoba seed meal (JSM), detoxified meal residue (DMR) and the seed shell (JSH) obtained by using the different extraction solvent solutions are presented in Table 2. The extraction yield of the shell ranged from 9.35 to 13.81 g/100g, whereas, the extraction yield of the seed meal extract was in the range from 19.38 to 22.61 g/100g. The extraction yield of the shell (JSH) was significantly ($p<0.05$) lower than that seed meal extract (JSM) in solvent solutions. Similar result have been obtained for other plant species such as pecan (De la Rosa *et al*., 2010), hazelnut (Contini *et al*., (2008) and Jatropha curcas (Huang *et al*., 2020). Each solvent possesses various degree of polarity that resulted in different extraction strengths as described previously (Wagdy and Taha, 2012). Moreover, the highest extraction yield was achieved for the 60% acetone extract of JSM (22.61 g/100g).

It was also observable that the extraction yield from DMR was the lowest (3.69±0.70-5.33±0.29 g/100g) because of the effect of the detoxification process in the isopropyl solvents solution. The solvent types with different polarities could influence the yield produced. These results in agreement with Siahaan *et al*., (2020).

Table 2. Extraction yield with different solvent solutions (g/100g)

Extraction solvent	JSM (yield g/100g)	DMR (yield g/100g)	JSH (yield g/100g)
Acetone (60%)	22.61±0.57^{a}	5.33±0.29^{c}	13.81±0.78^{b}
Methanol (70%)	20.80±0.25^{a}	3.69±0.70^{c}	11.58±0.26^{b}
Ethanol (70%)	19.38±0.92^{a}	4.14±0.45^{c}	9.35±0.28^{b}

*Results are expressed as means ±SD (n=3)

*Means within a row followed by the same upper case letter are not significantly different at 5% level.

3.2. Total phenolic content (TPC)

Since the solubility of TPC in general is governed by their chemical nature which may vary from simple to very highly polymerized substances, and also because the solubility of phenolic compounds is affected by the polarity of solvent used, it was advisable to first examine the suitability of the type of solvent for optimum extraction of phenolic compounds from jojoba seed waste samples (JSM, DMR and JSH). TPC (mg gallic acid equivalent per g extract dry weight) of each extract was shown in Table 3. The extracts which displayed the lowest and highest content of TPC are the extract of DMR (Ethanol 70%) 45.93 mg GAE g^{-1} extract (DW) and JSH (Acetone 60%) 163.81 mg GAE g^{-1} extract (DW), respectively. Highest phenolic extraction was achieved with acetone (60%) reaching 66.75, 138.80 and 163.81 mg GAE g^{-1} extract (DW) for DMR, JSM and JSH, respectively. Acetone probably extracted more TPC because it is more polar than the other solvents. This finding is in agreement with the results of Kim *et al.* (2007), Taha *et al*. (2011) and Wagdy and taha, (2012). However, no significant differences were found between the 70% methanol extract and the 70% ethanol extract of JSM and DMR. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

Table 3. Total phenolic compound mg GAE g^{-1} extract (DW)

Extraction solvent	JSM	DMR	JSH
Acetone (60%)	$138.80\pm4.58^{b}_{x}$	$66.75\pm1.68^{c}_{x}$	$163.81\pm6.88^{a}_{x}$
Methanol (70%)	$104.16\pm5.30^{b}_{y}$	$49.32\pm2.03^{c}_{y}$	$122.34\pm3.35^{a}_{z}$
Ethanol (70%)	$107.39\pm2.88^{b}_{y}$	$45.93\pm1.82^{c}_{y}$	$131.08\pm2.71^{a}_{y}$

*Results are expressed as means ±SD (n=3)
*Means within a row followed by the same upper case letter (a-c) are not significantly different at 5% level.
* Means within a column followed by the same lower case letter (x-z) are not significantly different at 5% level.

3.3. In vitro AA assay of natural phenolic extracts from jojoba seed waste

Antioxidant properties of botanical extracts should be evaluated in a variety of model systems using several indices because the effectiveness of such antioxidant material is largely dependent upon the chemical and physical properties of the system to which they are added on a single analytical protocol adopted to monitor lipid oxidation may not be sufficient to make a valid judgment.

As stressed by Huang *et al.* (2005), no single method is adequate for evaluating the antioxidant capacity of foods, since different methods can yield widely diverging results. Various methods, based on different mechanisms, must be used. Here, we have applied DPPH and β-carotene/linoleic acid bleaching assays to each extracts.

3.3.1. 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) test

DPPH radical is a synthetic organic radical. The principle that antioxidants that react with DPPH will donate hydrogen atoms so that DPPH is reduced to DiphenylPicryl Hydrazine (DPPH-H) which is stable and color decay occurs from purple to yellow (Molyneux, 2004). The color change shows the activity of free radical reduction which can be measured at a wavelength of 517 nm.

Using DPPH• radical, the free radical scavenging ability of the JSM, DMR and JSH was evaluated considering that DPPH• radical is commonly used for the assessment of antioxidant activity in vitro and is foreign to biological systems. With increasing concentration or degree of hydroxylation of phenolic compounds, DPPH radical scavenging activity (RSA) also increased and is defined as antioxidant activity (AA) (Zhou and Yu 2004). The whole system is done in a very low concentration due to the high sensitivity of free radicals in the presence of hydrogen donors (Iqbal *et al*. 2006).

DPPH radical-scavenging abilities of the methanolic extract from JSM, DMR and JSH along with the reference standard BHT are shown in Figure 1. The results suggest that components within JSM, DMR and JSH are capable of scavenging free radicals via electron- or hydrogen-donating mechanisms and thus should be able to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices (e.g. biological membranes). The extract demonstrated a concentration-dependent scavenging activity by quenching DPPH radicals. Consequently, DPPH radical scavenging activities of JSM, DMR and JSH increased with increased content. It has been proven that antioxidant activity of plant extracts is mainly ascribed to the concentration of the phenolic compounds present in the plants (Heim *et al*., 2002). The radical scavenging activity of BHT was significantly ($p<0.05$) higher than the experimental samples at all concentration. The results coincided with those of Chang *et al*., (2002). Moreover, scavenging activities of seed shell methanolic extract (JSH) at 200 µg/ml was significantly higher ($P < 0.05$) than those of all the natural phenolic extracts. The DPPH scavenging activity (RSA) of methanolic extract was in the following order BHT> JSH> JSM> DMR.

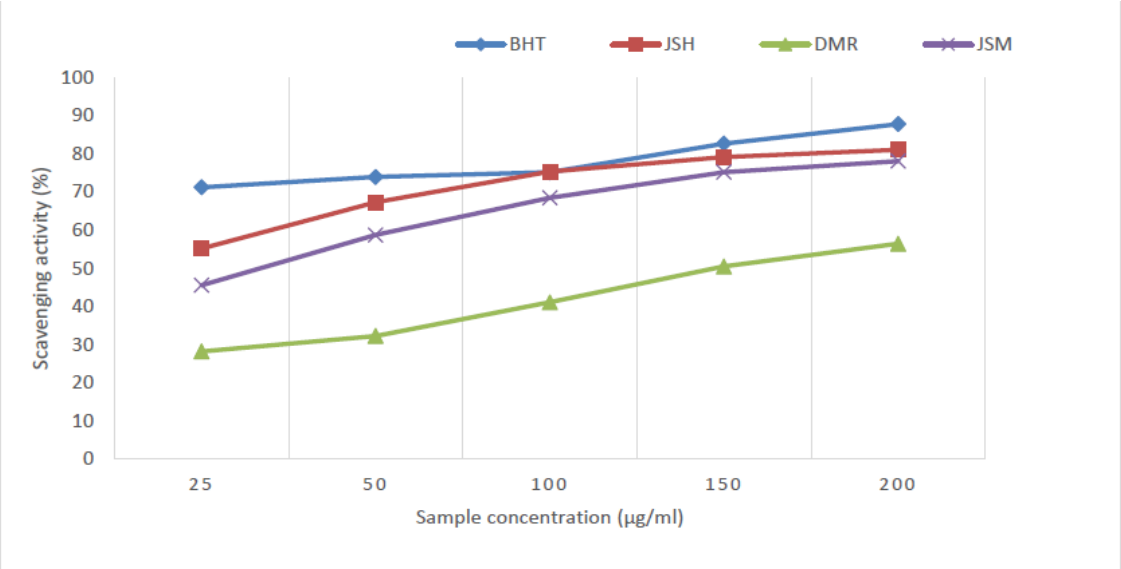

Figure 1. Effect of methanolic extract of JSM, JSH and DMR compared to BHT on AA as determined by the DPPH Scavenging method

The effect of different extractions solvent solutions on the AA of JSM, DMR and JSH as determined at 200 µg/ml by the DPPH are displayed in Table 4. Results demonstrated that 70% methanolic and 70% ethanolic extracts of JSH had the best DPPH free radical Scavenging activity 81.00% and 80.41%, respectively. On the other hand, 60% acetone extract of DMR had the lowest DPPH free radical scavenging activity. This was due to that the method used for simmondsins and other related compound elimination reduced also the phenolic content and the AA of extract. Results are in agreeing with Medina and trejo-gonzalez (1990).

Table 4. Effect of different solvent solutions on the antioxidant activity (AA) of JSM, DMR and JSH as determined by the DPPH assay

Extraction solvent	JSM	DMR	JSH
Acetone (60%)	$75.18\pm0.37^{b}_{y}$	$51.30\pm0.71^{c}_{z}$	$77.90\pm0.52^{a}_{y}$
Methanol (70%)	$78.07\pm0.48^{b}_{x}$	$56.38\pm0.16^{c}_{y}$	$81.00\pm0.34^{a}_{x}$
Ethanol (70%)	$78.52\pm0.32^{b}_{x}$	$58.11\pm0.74^{c}_{x}$	$80.41\pm0.62^{a}_{x}$

*Results are expressed as means ±SD (n=3)
*Means within a row followed by the same upper case letter (a-c) are not significantly different at 5% level.
* Means within a column followed by the same lower case letter (x-z) are not significantly different at 5% level.

3.3.2. β-Carotene bleaching system

Synthetic free radical scavenging (DPPH) models are valuable tools to indicate the potential antioxidant activity of plant extracts, however, these systems do not use a food or biologically relevant oxidizable substrate, so no direct information on an extract's protective action can be determined (Dorman *et al.*, 2003). Therefore, it was considered important to assess the extracts in a β-carotene/linoleic acid lipid:water emulsion assay despite its reported limitations. In this assay, the oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of hydrogen atom from diallylic methylene groups of linoleic acid. The free radical then will oxidize the highly

unsaturated β-carotene. The presence of antioxidants in the extract will minimize the oxidation of β-carotene by hydroperoxides. Hydroperoxides formed in this system will be decomposed by the antioxidants from the extracts. Thus, the degradation rate of β-carotene depends on the AA of the extracts. An extract capable of inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant.

The inhibition of bleaching of β-carotene in oil in water in JSM, DMR and JSH emulsion system was screened at 200 µg/ ml extract (Table 5). It was clear that the presence of phenolic antioxidants in these extract reduced the oxidation of β-carotene. The percentage of inhibition of bleaching of β-carotene was significantly varied from 85.04 to 93.88 for JSM, 61.02 to 67.96 for DMR and 88.92 to 95.78 for JSH. However, the ethanolic extract of JSH showed the highest inhibition of bleaching of β-carotene followed by the ethanolic extract of JSM (95.78±1.03 and 93.88±0.13 %), respectively. These extracts were better in their effect on reducing the oxidation of β-carotene than BHT. According to the β-carotene/linoleic acid bleaching data, the extracts are capable of scavenging free radicals in a complex heterogenous medium. This suggests that the ethanolic extract of JSH and JSM may have potential use as antioxidative preservatives in emulsion-type systems. What is more, DMR still have moderate antioxidant activity.

Table 5. Effect of different solvent solutions on the antioxidant activity of JSM, DMR and JSH as determined by β-carotene/linoleic acid

Sample	AA (%inhibition against β-carotene bleaching)
JSM (60% acetone extract)	85.04±1.51[e]
JSM (70% methanol extract)	89.61±0.75[d]
JSM (70% ethanol extract)	93.88±0.13[b]
DMR (60% acetone extract)	61.02±0.19[g]
DMR (70% methanol extract)	66.19±0.95[f]
DMR (70% ethanol extract)	67.96±0.44[f]
JSH (60% acetone extract)	88.92±0.49[d]
JSH (70% methanol extract)	91.71±0.60[c]
JSH (70% ethanol extract)	95.78±1.03[a]
BHT	90.76±0.52[c]

* Means within the column followed by the same upper case letter are not significantly different at 5% level.

3.4. Physicochemical properties of SPO

The physicochemical of SPO has been shown in Table 6. The iodine value is considered one of the most chemical constants for quality assurance of the edible oils and is good index for unsaturated extent of fatty acids in oils. As shown in Table 6, the iodine value of SPO was 60.55. The F.F.A (% as oleic acid) was 0.06%, whereas, the peroxide value was 3.16 meq O_2/kg. In addition, melting point was 13 ºC, cloud point was 7ºC and refractive index was 1.4597. The color R/Y was 2.6/35 and total amount of color was 61.0.These results are in harmony with those of Hashem *et al.,* (2018).

Table 6. Physico-chemical properties* of SPO

Physicochemical test	Value
Refractive index	1.4597
Peroxide value (meqo/kg)	3.16
Iodine value	60.55
F.F.A (% as oleic acid)	0.06
Melting point (ºC)	13.0
Cloud point (ºC)	7.0
Color R/Y	2.6/35
Total livobond color (R+10Y)	61.0

*Results are means of duplicate determinations

3.5. Fatty acid profile of SPO

The fatty acid profile of SPO is presented in Table 7. Based on the GC analysis done, there were three major fatty acids. These fatty acids were oleic acid (46.88%) followed by palmitic acid (33.35%) and linoleic acid (13.87%). The others were Lauric acid (0.5%), heptadecanoic acid (0.08%), hexadecenoic acid (0.03), myristic acid (1.09%), palmitoleic acid (0.26%), stearic acid (3.68%), and linolenic acid (0.25%). In addition, the total amount of saturated fatty acids (SFA) was (38.71%) while, the total amount of unsaturated fatty acids (USFA) was (61.29%).The total amount of monounsaturated

fatty acids (MUSFA) was (47.17%) and, the total amount of polyunsaturated fatty acids (PUSFA) was (14.12%). The proportions between SFA and USFA, MUSFA and PUSFA, and PUSFA and SFA were 0.63, 3.34 and 0.36, respectively. These results are in accordance with Hashem, *et al*., (2018). The level of unsaturated fatty acids makes the SPO a suitable substrate for oxidation studies.

Table 7. Fatty acid profile* of super palm olein (SPO)

Fatty acid	SPO (%)
Lauric acid C12:0	0.51
Myristic acid C14:0	1.09
palmitic acid C16:0	33.35
Palmitoleic C16:1	0.26
Heptadecanoic C17:0	0.08
Hexadecenoic C17:1	0.03
Stearic acid C18:0	3.68
Oleic acid C18:1	46.88
Linoleic acid C18:2	13.87
Linolenic acid C18:3	0.25
Saturated fatty acids	38.71
Unsaturated fatty acids	61.29
MUSFA	47.17
PUSFA	14.12
SFA/USFA	0.63
MUSFA/PUSFA	3.34
PUSFA/SFA	0.36

*Results are means of duplicate determinations

3.6. Induction period at 110ºC of SPO without or with the addition of the natural antioxidant extracted from jojoba seed waste

The Rancimat test is a technique based on the conductometric determination of volatile degradation products, which is currently used for the evaluation of the oxidative stability of oils and fats as well as to the study of antioxidant potentiality of new molecules. Longer induction times indicate higher resistance to oxidation or good efficiency of the added antioxidants. The effects of the ethanolic extract of JSM, JSH and DMR on IT and PF of SPO in comparison with the control and BHT are presented in Table 8. It was clearly observed that the added extract, at all concentration had prolonged the induction time (IT) of oxidation and the protection factors (PF). SPO + JSM_{200ppm} (IT = 21.77 h; PF = 1.20), SPO + JSH $_{200ppm}$ (IT = 22.43 h; PF =1.24), SPO+ DMR $_{200ppm}$ (IT =

19.38 h; PF= 1.07), SPO+ DMR_{600ppm} (IT= 21.82 h; PF= 1.21),SPO+ $DMR_{1000ppm}$ (IT= 23.29 h; PF= 1.29), compared to control (IT = 18.06 h; PF = 1.0), and SPO + BHT $_{200\ ppm}$ (IT = 20.94 h; PF= 1.16). Similar results have been reported by Womeni *et al*,, (2016). These observation might be due to the good thermal stability of the antioxidant detected in these extracts compared to BHT. The low stability of BHT at elevated temperature has been previously reported by Thorat *et al*., (2013).

Table 8. Induction time (IT, h) and protection factor (PF) of SPO samples* as affected by the addition of natural antioxidant extracted from jojoba seed waste

Sample	IP (h)	PF
Control	18.06	1.00
SPO+ BHT (200_{ppm})	20.94	1.16
SPO +JSM (200_{ppm})	21.77	1.20
SPO + JSH (200_{ppm})	22.43	1.24
SPO + DMR (200_{ppm})	19.38	1.07
SPO + DMR (600_{ppm})	21.82	1.21
SPO + DMR (1000_{ppm})	23.29	1.29

*Results are means of duplicate determinations

4. Conclusion

In the current study, extracts with different phenolics concentrations and antioxidant activities were obtained from jojoba waste (meal and shell). Two different in vitro methods were done to evaluate antioxidant activity of extracts (DPPH and β-Carotene/linoleic acid bleaching system), since the nature of these methods are different, different responses were observed among extracts. This results showed the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage. Radical scavenging activity (RSA) of these natural extract was compared to that of BHT at 25-200 µg/ml. It has been noticed that the RSA of BHT was greater than of all experimental samples that evaluated by the DPPH assay. It is noteworthy that 60% acetone extract of JSH seemingly has considerably higher TPC (163.81 mg GAE g^{-1} extract) than either 70% ethanol extract or 70% methanol extract but does not appear to have correspondingly high AA. The seed shell extract (JSH) obtained using 70% methanol exhibited the best AA among the other extracts. Phenolic compounds can explain this high antioxidant capacity.

In addition, the β-carotene bleaching inhibition of the ethanolic extract JSM and JSH recorded using the linoleic acid emulsion system showed very good antioxidant activity. Based on the test result of the AA of each extract, the 70% ethanol extract was chosen as the most active extract. Ethanol was selected as the most appropriate solvent for the extraction of phenolic compound for protective β-carotene/linoleic model system oxidation. Furthermore, super palm olein (SPO) was used in this study as in vivo an oxidation substrate. The oxidative stability were evaluation by determining the induc-

tion time of SPO supplemented with 200_{ppm} JSM and JSH on rancimat at 110°C, comparing to SPO containing 200_{ppm} BHT and SPO without antioxidant. DMR was assessed at the range of 200-1000ppm because it had moderate RSA. It is noticeable that the ethanolic extract of JSM and JSH at concentration of 200ppm can stabilize SPO. They were efficient than that of BHT in the rancimat test. Detoxified meal prepared for animal feed (DMR) had lower TPC concentration residue with moderate (% of inhibition activity AA) compared to JSM and JSH.

Based on the results of this study the ethanolic extract of JSM and JSH extracts could be used as potential natural antioxidant to protect oil rich-food to avoid the possible risks resulted from using the synthetic antioxidant to prevent food oxidation.

References

Abd El-Rahman, A. A., Abd El-Aleem, I. M., El-Deeb, A. E. and Hussein A. E. (2006). Elimination of toxic compounds and nutritional evaluation of jojoba meal proteins. Benha University.

Abdel-Mageed, W.M., Bayoumi, S., Salama, A.A.R., Salem-Bekhit, M.M., Abd-Alrahman, S.H. and Sayed, H.M. (2014). Antioxidant lipoxygenase inhibitors from the leaf extracts of S*immondsia-chinensis*. Asian Pac J Trop Med. 7:S521–6.

Abdel-Wahhab, M., Sharaf, H. and Abou-Salem. F. (2010). Jojoba extract counteracts oxidative stress in rats fed fumonisin-contaminated diet. ToxicolLett. 196:S328.

Abdul-Hafeez, E.Y., Karamova, N.S. and Ilinskaya, O.N. (2014). Antioxidant activity and total phenolic compound content of certain medicinal plants. Int J Biosci. 5(1):213–22.

Abobatta, W.F.R., El Ghadban, E. A. E. and Mahmud, G. F. (2015). Chemical studies on grown jojoba oils under Egyptian conditions. Glob. J. Agric. Food Safety Sci., Vol.2 (3): pp. 270 – 283.

Al-Qizwini, H., Ekbal, A. K., Mhaidat, N. M. and Maraqa, A. (2014). Antioxidant and antimicrobial activities of Jordanian *Simmondsia-chinensis* (link) ckschneid. EurSci J. 10:229–41.

AOCS (2000). Official methods and recommended practices of the american oil chemists' society.

Asamarai, A.M., Addis, P. B., Epley, R. J. and Krick, T. P. (1996). Wild rice hull antioxidants. J. Agric. Food Chem., 44: 126-130.

Association of analytical chemists (AOAC 2000). Official Methods of Analysis of Association of official Analytical Chemists, Association of Official Analytical Chemists. Arlington, Virginia, USA.

Booth, A.N., Elliger, C. A. and Waiss, A. N. (1974). Isolation of toxic factor from jojoba meal". J. Life Sci. 15:1115-1120.

Braca, A., Sortino, C., Politi, M., Morelli, I. and Mendez, J. (2002). Antioxidant activity of flavonoids from Licania licaniaeflora, J. Ethnopharmacol. 79, 379–381.

Bryła, M., Roszko, M., Szymczyk, K., Jędrzejczak, R., Obiedziński, M.W. and Sękul, J. (2013). Fumonisins in plant-origin food and fodder—a review. Food AdditContam Part A. 30(9):1626–40.

Chang, L.W., Yen, W. J., Huang, S.C. and Duh, P.D. (2002). Antioxidant activity of sesame coat. Food Chem., 78: 347-354.

Contini, M., Baccelloni, S., Massantini, R. and Anelli, G. (2008). Extraction of natural antioxidants from

hazelnut (Corylus avellana L.) shell and skin wastes by long maceration at room temperature. Food Chem. 110, 659–669. [CrossRef]

Corredor, D.Y., Sun, X. S., Salazar, J. M., Hohn, K. L. and Wang, D. (2008). Enzymatic hydrolysis of soybean hulls using dilute acid and modified steam-explosion pretreatments. J. Biobased Mater. Bioenergy, 2: 43-50.

De la Rosa, L.A., Alvarez-Parrilla, E. and Shahidi, F. (2010). Phenolic compounds and antioxidant activity of kernels and shells of Mexican pecan (Carya illinoinensis). J. Agric. Food Chem. 59, 152–162.

Dorman, H.J.D., Kosar, M., Kahlos, K., Holm, Y. and Hiltunen, R. (2003). Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties and cultivars. J. Agric. Food Chem. 51:4563–4569.

Duh, P.D. and Yen, G.C. (1997). Antioxidant efficacy of methanolic extracts of peanut hulls in soybean and peanut oils. J. Am. Oil Chem. Soc, 74: 745-748.

Elsanhoty, R. M., Al-Soqeer, A. and Ramadan, M. F. (2017). Effect of detoxification methods on the quality and safety of jojoba (Simmondsiachinensis) meal. J. Food Biochem. https://doi.org/10.1111/jfbc.12400.

Gutfinger, T. (1981). Polyphenols in olive oils. J Am Oil ChemSoc 58:966–968.

Hashem, H.A., Abd- Ellh, N. E., Abd-Eltawab, G. A. and Abdel-Razek, A. G. (2018). Industrial scale production of palm super olein using modified and innovative dry fractionation technique. Egypt. J. Chem. Vol. 61, No.1, pp. 1–11.

Heim, K.E., Taigliaferro, A.R. and Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J. Nutr. Biochem. 13, 572–584.

Huang, D., Band, O. and Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53, 1841–1856.

Huang, S.L., Wang, W. H., Zhong X.Y., Lin, C.-T., Lin, W. S., Chang, M.Y. and Lin, Y. S. (2020). Antioxidant properties of jatropha curcas l. Seed shell and kernel extracts. Appl. Sci. 10, 3279; doi: 10.3390/app10093279.

Iqbal, S., Bhanger, M.I., Akhtar, M., Anwar, F., Ahmed, K.R. and Anwer, T. (2006). Antioxidant properties of methanolic extracts from leaves of Rhazya stricta. J. Med. Food 9:270–275.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Raucha, J.P., Pihlaja, K. and Kujala, T.S. (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. 47, 3954–3962.

Keyvan, D.H. J., Damien, D., Into, L. and Raimo, H. (2007). Chemical composition and antioxidative activity of Moldavian balm (DracocephalummoldavicaL.) extracts. LWT Food Sci. Technol. 40, 1655–1663.

Kim, J. M., Chang, S. M., Kim, I. H. and Kim, Y. E. (2007). Design of optimal solvent for extraction of bioactive ingredients from mulberry leaves. Biochemical Engineering Journal, 37: 271-278.

Krishnaiah, D., Sarbatly, R. and Nithyanandam, R. (2010). A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 89(3):1-17. DOI:http://dx.doi.org/10.1016/j.fbp.2010.04 .008.

Medina, L.A. and Trejo-gonzalez, A. (1990). Detoxified and Debittered Jojoba Meal: Biological Evaluation and Physical-Chemical Char-

acterization. Cereal Chem. Vol. 67, No. 5: 476-479.

Mohamed, E.A. (2007). Nutritional, biochemical and histopathological studies on jojoba protein isolate. Brazilian Journal Food Technology, Campinas, v. 10, n. 3, p. 198-204.

Mohamed, G.F. and Taha, F.S. (2005). Extraction of sunflower hulls: Their antioxidant activity on lipids of cooked mackerel fish. Alex. J. Food Sci. Technol., 2: 11-23.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology. 26(2), 211-21.

Motawe, H.F.A. (2006). Chemical and biological evaluation ofjojoba seeds and jojoba meal (Simmondsia chinensis) in comparison with some other plant protein sources. J. Agric. Sci. Mansoura Univ., 31: 6945-6955.

Oksman-Caldentey, K. M. and Inze, D. (2004). Plant cell factories in the post-genomic era: new ways to produce designersecondary metabolites". TRENDS in Plant Science. 9:433 - 40.

Osawa, C. C. and Gonçalves, L.A.G. (2012). Deep-fat frying of meat products in palm olein. Ciênc. Tecnol. Aliment. 32(4):804811. nDOI: http://dx.doi.org/10.1590/S0101.20612012005000109.

Osawa, T. (1994). Novel natural antioxidants for utilization infood and biological systems. Pp. 241–251 in I. Uritani, V.V. Garcia and E. M. Mendoza, eds. Post-harvest biochemistry of plant food-materials in the tropics. Japan Scientific Societies Press, Japan.

Pantzaris, T.P. (1995). Pocket book of palm oil uses. Palm oil Research Institute ofMalaysia. 158.

SAS. (1987). SAS/STAT Guide for personal computers, Version 6 ed., SAS Institute, CaryNC, USA.

Shahidi, F. and Naczk, M. (2004). Phenolics in food andnutraceuticals. Applications and Health Effects. Florida: CRC Press, Boca Raton.

Siahaan, A.P., Rohaeti, E., Muddathir, A.M. and Batubara, I. (2020). Antioxidant activity of jojoba (Simmondsia chinensis) seed residue extract. Journal of Biology & Biology Education. Biosaintifika 12 (3), 350-355.

Singer, F.A. and Wagdy, S. M. (2011). Preliminary study on the bioactivity of a phenolic extract from flaxseed hulls. J. Food Ind. Nut. Sci, 1: 17-30.

Somayeh, N., Ali, M., Abbas, D., Hossein, N. and Solmaz, A. (2012). Ontogenetic variation of total phenolics and antioxidant activity in roots, leaves and flowers of *Astragaluscompactus* Lam. (Fabaceae). BioImpacts.2:105-109.

Stahl, E. (1967). Thin layer chromatography. A laboratory Handbook, Ed. Springer verloag, Berlin, p.359. Heidelberg, New York.

Taha, F. S., Mohamed, G. F., Mohamed, S. H., Mohamed, S. S. and Kamil, M. M. (2011). Optimization of the extraction of total phenolic compounds from sunflower meal, and evaluation of the bioactivities of chosen extracts. American Journal of Food Technology, (published on line October 2011).

Taskins, J., Lalas, S., Gergis, V., Dourtoglou, V. and Spiliotis, V. (1999). Characterization of Moringa oleifera seed oil of Kenya. J. Agric. Food Chem.; 47: 4495-4499.

Thorat, I.D., Jagtap, D.D., Mohapatia, D., Joshi, D.C., Sutar, R. F. and

Kapdi, S.S. (2013). Antioxidants, their properties, uses in foods products and their legal implications, IJFS 2, 81–104, http://dx.doi.org/10.7455/IJFS/2.1.2013.a7.

Verbiscar, A.J., Banigan, T.F., Weber, C.W., Reid, B. L., Trei, J. E., Nelson, E. A., Raffauf, R. F. and Kosersky, D. (1980). Detoxification of jojoba meal. J. Agric. Food Chem. 28:571-578.

Visavadiya, N.P., Soni, B. and Dalwadi, N. (2009). Evaluation of antioxidant and anti-atherogenic properties of *Glycyrrhiza glabra* root using in vitro models.

Wagdy, S.M. and Taha, F.S. (2012). Primary assessment of the biological activity of jojoba hull extracts. Life Science Journal; 9: 244-253.

Womeni, H. M., Djikeng, F.T., Anjaneyulu, B., Karuna, M. S. L., Prasad, R. B. N. and Linder, M. (2016). Oxidative stabilization of RBD palm olein under forced storage conditions by old Cameroonian green tea leaves methanolic extract. NFS Journal, 3, 33-40.

Yanishlieva, N.V. and Marinova, E.M. (2001). Stabilization of edible oils with natural antioxidants, Eur. J. Lipid. Sci. Tech. 103, 752–767, http://dx.doi.org/10.1002/1438–9312(200111)103:11. (b 752: AID-EJLST52 N 3.0.CO; 2–0).

Zhou, K. and Yu, L. (2004). Effects of extraction solvent on the wheat bran antioxidant activity estimation. LWT Food Sci.Technol. 37:717–721.

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تحسين ثبات زيت سوبر أوليين النخيل بإستخدام مضادات الأكسدة الطبيعية المستخرجة من بقايا بذور الجوجوبا

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الملخص

تم استخراج مضادات الأكسدة الطبيعية من بقايا بذور الجوجوبا. وتعرضت عينات من كسب بذور الجوجوبا ومخلفات كسب مزالة السمية وقشور بذور الجوجوبا للأستخلاص باستخدام مذيبات مختلفة،٧٠% إيثانول و٧٠% ميثانول و٦٠% أسيتون. تم قياس إجمالى محتوى الفينولات وفقا لطريقة Folin-ciocalteu. بالإضافة إلى أختبار مستخلصات المذيبات معمليا لمعرفة نشاطها المضاد للأكسدة عن طريق اختبار DPPH ومستحلب البيتا كاروتين وحامض اللينوليك. ولوحظ أن أعلى كمية من الفينولات الكلية فى قشور بذور الجوجوبا المستخلصة ب ٦٠% أسيتون. على الجانب الآخر، مستخلص ٧٠% ميثانول من قشور بذور الجوجوبا أشار إلى أعلى نشاطا فى ال DPPH الكاسحة للجذور الحرة. كما تبين أن مستخلصات مخلفات الكسب المزالة السمية تحتوى على كمية منخفضة من الفينولات. علاوة على ذلك، أظهر مستخلص الإيثانول من كسب بذور الجوجوبا وقشور بذور الجوجوبا بتركيز ٢٠٠ جزء فى المليون قدرة جيدة مضادة للأكسدة من أجل ثبات سوبر أوليين النخيل عن ال BHT مقدرة بالرانسيمات. وبالتالى فإن هذه المستخلصات يمكن أن تكون بديلا جيدا لمضادات الأكسدة الصناعية لحماية الزيوت النباتية مثل سوبر أوليين النخيل التى تحتوى على مستويات مختلفة من الأحماض الدهنية الغير مشبعة.