Effect of Extraction Techniques on the Physicochemical Properties, Fatty Acids Composition, and Stability of Purslane Seeds (*Portulaca oleracea*) Oil Compared to Soybean Seeds (*Glycine max*) and Sardine Fish (*Sardinella maderensis*) Oils

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Abstract: This study aimed to determine the physicochemical properties, fatty acids composition and thermal properties of purslane seeds oil extracted by solvent and cold-press techniques compared to soybean seeds and sardine fish oils. The obtained data indicated that extraction of purslane, soybean seeds and sardine fish oils by solvents produced significantly ($p \le 0.05$) higher oil contents as compared to the cold-press ones. Physicochemical properties of the three oils were not affected by the extraction methods and showed good and acceptable qualities. Furthermore, no significant differences were investigated in the fatty acids composition of the oils from the two extraction techniques. Linoleic acid ($C_{18:2}$) was the main unsaturated fatty acid in purslane and soybean seeds oils while, eicosapentaenoic acid ($C_{20:5}$) was the principal unsaturated fatty acid in sardine fish oil. On the other hand, results of thermal analysis by DSC and TGA showed differences and unusual behaviors for the three examined oils. The Thermal stability results revealed complete decomposition of linoleic and linolenic acids in the three oils after exposure for 2 h at 150 °C. Recommendations, the purslane seeds oil is a good source of omega- 6 and omega-3 fatty acids. Although the solvent extraction method gave high extraction yield, the oils extracted by the cold-press would retain the genuine flavor, aroma, and nutrients. Thus cold-extraction technique is recommended to be used in oil production industry as an low cost and effective method.

Keywords: Fatty acids composition; physicochemical; thermal properties; purslane; soybean; sardine fish oil

INTRODUCTION

The beneficial role of long chain omega-3 fatty acids to human health has increased the consumer demand for functional foods and dietary supplements enriched with this omega type of fatty acids (Gheysen *et al.*, 2019). The main omega-3 fatty acids associated with health beneficial properties are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), commonly found in seafood and microalgae, and α linolenic acid (ALA), most commonly found in plants (Perona *et al.*, 2018). Their health benefits appear to be associated with several different aspects, including their structure, their interactions with proteins that alter gene expression, and their unique metabolic fates (Briggs *et al.*, 2017).

Nowadays, the recommended daily intake of omega-3 fatty acids (250-500 mg/day) is obtained from dietary supplements containing fish oils or, consumption of seafood which are rich in EPA and DHA content, whereas a relatively minor proportion of daily intake is account for plant-based dietary sources (Andre *et al.*, 2019). Therefore, finding alternative sources of these fatty acids is urgently needed, especially since terrestrial plants are sources of ALA, which is likewise linked to positive effects and may be metabolized by the body to EPA and DHA (Perona *et al.*, 2018).

Various plants have been indicated as rich sources of omega-3 fatty acids, some of which are currently used in the food and pharmaceutical industry (Dubois *et al.*, 2007). Vegetable oils such as linseed are rich in ALA (52–55%; Tavarini *et al.*, 2019) and monounsaturated fatty acids (MUFA), whereas chia and purslane contain ALA (61.3% and 32.4%,

respectively) and linoleic acid (LA) in concentrations that account to omega-6/omega-3 ratios with values lower than 4, indicating a high nutritional value (Dubois *et al.*, 2007).

Purslane is a widely distributed wild edible species that is usually eaten raw as salad or cooked in numerous dishes of the larger Mediterranean region (Petropoulos et al., 2016). Its medicinal and therapeutic characteristics are well known since centuries ago and several scripts describe its cultivation for medicinal purposes (Gonnella et al., 2010). Purslane seed oils are very nutritious since they are rich in PUFA consisting mainly of LA, ALA and oleic acid (OA) as well as phenolic compounds and phenolic lipids (Gunenc et al., 2019; Kavosi et al., 2018). However, taking into account the market needs for alternative omega-3 fatty acids sources and the finite world production of fish oils justify the utilization of terrestrial sources of PUFA. Purslane could be a possible candidate species for this purpose because of its edible leaves and stems, while there is no documented widespread usage of the seeds and seed oils.

The extraction techniques utilized to extract high aggregate valued compounds from natural materials are crucial the product quality (Mezzomo *et al.*, 2010). Since the accurate determination of physicochemical properties and fatty acid composition in oils extracted by different extraction techniques can be used to evaluate their efficiency (Abdolshahi *et al.*, 2015). The aim of the present study was to evaluate the effect of extraction techniques on the purslane seed oils yield and physicochemical properties as well as thermal stability compared to soybean seeds and sardine fish oils, well-known worldwide for their favorable

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MATERIALS AND METHODS

Materials

Raw materials

Purslane seeds (*Portulaca oleracea*), soybean seeds (*Glycine max*) and sardine fish (*Sardinella maderensis*) were all purchased from local market, Ismailia Governorate, Egypt.

Chemicals and reagents

All chemicals and reagents used for analysis were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Methods

Oil extraction methods

Oil extraction by solvents

Oils were extracted from the raw materials by solvents based on the method described by Ridzwan *et al.* (2014). Ground materials were mixed with solvents mixture consisting of chloroform and methanol (2:1 v/v). For optimization of extraction conditions, the oils were extracted by the two solvents, chloroform and methanol in 1:2, 1:3, 1:4, 1:5 and 1:6 ratios (sample-solvent) for 2, 4, 6 and 8 hours extraction times at room temperature ($25\pm2^{\circ}$ C). The extracts were filtered and the solvent was evaporated using a rotary evaporator (BüCHI-ROTAVAPOR-R) at 40°C. Then, the extraction yields were calculated.

Oil extraction by mechanical pressing

The extraction by mechanical pressing was carried out with a hydraulic cold-pressing machine (Kern Kraft, Germany) on the samples at room temperature and pressure (10 MPa) for 10 min according to the method of Uquiche *et al.* (2008). The extracted oils were filtered then centrifuged at 3500 rpm for 20 min to separate the components that were settled. The oil content was calculated.

Determination of physicochemical properties of the extracted oils

Specific gravity, refractive index, acid (mg KOH/ g oil), saponification, iodine and peroxide values (meq O_2 / kg oil) were performed based on the official methods (AOAC, 2019).

Fatty acids composition determination

Direct fatty acid methyl ester (FAME) synthesis was performed according to O'fallon *et al.* (2007). Oils (40 µl) were placed into a 16 × 125 mm screw-cap Pyrex culture tube to which ml of Methanol (MeOH; HPLC grade), 0.7 ml of 10 N KOH in water, and 5.3 ml of MeOH were added. To appropriate permeating, dissolving, and hydrolyzing the sample, tubes were incubated at 55 °C in a water bath for 1.5 h with shaking vigorously for 5 sec every 20 min. After cooling the tubes below ambient temperature, 0.58 ml of 24 N H₂SO₄ in water was added. The tubes were mixed by inversion and the precipitate was incubated again in a water bath at 55 °C for 1.5 h with shaking for 5 s every 20 min. After FAME synthesis, the tube was cooled in a cold tap water bath. Hexane (3 ml) was added and the tubes were mixed using a multi tube vortex for 5 min. The tubes were centrifuged and the hexane layer, containing the FAME, was placed into a GC vial. The vial was capped and placed at -20 °C until GC analysis.

Fatty acids composition of the obtained FAME was assayed by capillary GC on a SP-2560, 100 m \times 0.25 mm \times 0.20 µm capillary column (Supelco) installed on a Hewlett Packard 5890 gas chromatograph (available at agriculture research center). The initial temperature was 140°C, held for 5 min, then increased to 240°C at a rate of 4°C min⁻¹, and held for 20 min. Helium was used as the carrier gas at a flow rate of 0.5 ml. min⁻¹, and the column head pressure was 280 kPa. Both the injector and the detector were set at 260°C. The split ratio was 30:1. Fatty acid methyl standards were used to identify the samples composition from each detected fatty acid by comparing their retention times.

Vitamin E determination

In a round bottom flask 0.03 mg sample, 2 ml of KOH solution (50% w/v), 10 ml glycerol and 25 ml methanol were added and mixed well. Then the mixture was refluxed for 45 min on a boiling water bath, cooled and transferred into separating funnel and extracted with 50 ml ether for 5 min. The ether layer was separated and then filtered through anhydrous sodium sulphate. After that the ether layer was evaporated to dryness and then dissolved in methanol. About 5 ml of each standard (In a 100 ml volumetric flask, accurately 25 mg vitamin E were weighted and mixed with 50 ml of methanol, then the volume was made to the mark with methanol). The sample and blank solutions were taken in three 25 ml volumetric flasks. To each flask, 2 ml of 0.1% 2, 2 bilyridil solution (in methanol) and 1 ml of 0.1% ferric chloride solution (in water) were added and mixed well. Then each mixture was diluted to 25 ml with methanol and the absorbance was measured at 525 nm against the blank (Ashok and Kumar, 2011).

Vitamin D determination

For standard preparation, accurately 25 mg vitamin D was weighed in a 25 ml volumetric flask then dissolved, mixed well and made up to the mark with the solvent (chloroform and methanol; 1:9 v/v). For sample preparation and procedure, accurately 4.24 g from each sample were weighed in a 25 ml volumetric flask with solvent (chloroform and methanol ratio 1:9), then dissolved, mixed well and made up to the mark. The absorbance was recorded at 264 nm against the blank (Ashok and Kumar, 2011).

Thermo Gravimetric Analysis (TGA)

A thermo-balance (TGA-50, PerkinElmer Co., Akron, Ohio, USA) (available at agriculture research center). was used to investigate the thermal behavior of samples as a function of temperature. The oils (5 mg) were heated at temperatures ranged from 40 to 600°C with a rate of 10°C min⁻¹ increasing in temperature under nitrogen gas (50 ml min⁻¹). TG and its derivative plot (DTG) were used to identify the T_{onset} which indicates the initiation of decomposition in oils. The horizontal baseline of TG curve was extrapolated at 1% decomposition, and its intercept with tangent gave T_{onset} (Ghosh *et al.*, 2019). The thermal decomposition of the oils was followed in a step-wise regime to track the associated changes in their mass and degree of unsaturation-dependent stability.

Differential Scanning Calorimetry (DSC) analysis

The oxidation induction times (OIT) test (min) of oil oxidation under accelerated heating was measured by DSC (DSC Pyris 6, PerkinElmer Co., Akron, Ohio, USA) (available at agriculture research center). The oil sample (5 mg) was weighed in an aluminum pan then kept in the measuring chamber. An empty aluminum pan was used as a reference. The oils were first examined under a dynamic heating regime ranging from 40 to 600°C and the onset temperature (T_{onset}) of oxidative degradation was obtained from DSC curve as an inflection point. At Tonset of 10°C, the oils were then isothermally evaluated.by passing purified oxygen 99.8% (50 ml min⁻¹) and nitrogen (50 ml min⁻¹) through sample enclosure. The thermal ramping was configured as (1) heat from 40°C to T_{onset} at a rate of 10°C min⁻¹, (2) hold for 2 min at T_{onset} and before switching to oxygen, and (3) hold for 120 min at T_{onset} . The OIT was defined as the point where an extrapolated baseline intersected with a tangent line of DSC endotherm (Ghosh et al., 2019).

Oxidative stability of the extracted oils

Oil samples were stored for 6 weeks into two sets of 50 ml brown glass bottles. One of them was stored in a refrigerator $(5 \pm 1^{\circ}C)$ and the other was stored at the room temperature $(22 \pm 1^{\circ}C)$. On the first day, 5 ml from each bottle were drawn and examined for oxidative stability by determination of peroxide value (Kaleem *et al.*, 2015).

Thermal stability determination

The oil samples packed in closed brown glass bottles were heated at 50, 100 and 150°C in adjusted oven for 2 h in a continuous manner. Samples were cooled prior for fatty acids composition testing by GC.

Statistical analyses

Analysis of data was carried out using SPSS statistical software version 21 (SPSS Inc., Chicago, IL). In order to identify the significant differences between the oil samples, analysis of variance (ANOVA) followed by the Duncan's multiple range procedure was used at $p \le 0.05$ (Duncan, 1955). All experiments were performed in triplicate, and results were expressed as mean \pm standard deviation (SD).

RESULTS AND DISCUSSIONS

Extraction yield of oils

The effect of extraction time and sample: solvent ratios on the oil extraction yield of purslane and soybean seeds and sardine fish is shown in Table (1). It is clear that the yield percentage of purslane and soybean oils increased with the increase in extraction time and sample: solvent ratios until they reached 9.92 and 18.60%, respectively using ratio of 1:6 for 8 hours.

For the sardine oil, the rate of extraction increased by increasing the sample: solvent ratio until it reached 6.65% using a 1:5 ratio for 8 h then tended to decrease.

Solvent and cold-press extraction methods are the most commonly used methods for extracting oils from their sources. Table (2) shows the extraction vield (%) of purslane and soybean seeds and sardine fish oils extracted using solvent (chloroform and methanol, 2:1) compared to cold-press extraction method. While the solvent extraction method for purslane, soybean seeds and sardine fish produced the highest oil content of 9.92 and 18.60% [for the ratio 1:6 sample: solvent] after 8 h] and 6.65% [for the ratio of 1:5 sample: solvent) after 8 h] of extraction, respectively, the coldpress method gave 9.45, 17.42 and 6.44%, respectively of oil content. It is worth mentioning that sample: solvent ratio that gave the best yield varied from 1:5, chloroform: methanol with sardine fish 1:6 with soybean seeds and purslane seeds as previously shown (Table 1). These results are consistent with those of literature which indicated high efficiency of solvent method for oils extraction (Delfan-Hosseini et al., 2017). Three years latter Desta et al. (2020) found that the purslane seeds had 11.25% oil extracted by petroleum ether.

Although the solvent extraction method gave high extraction yield, the oils extracted by cold-press extraction method would retain their genuine flavor, aroma, and nutrients. Furthermore, the cold-pressed oils were much safer as they are devoid of solvent residues (Lutterodt et al., 2011). The higher levels of natural antioxidants in the cold-pressed oils help to improve the shelf-life stability and safety without adding additional synthetic antioxidants (Lutterodt et al., 2010). According to Ionescu et al. (2014), mechanical extraction or pressing methods are frequently utilized in the oil industry as being most cost-effective methods; Therefore, customized screw presses with specifications optimized for very small seeds such as purslane seed should be considered to increase the efficiency of oil extraction (Ionescu et al., 2015).

Physicochemical properties of the extracted oils

Physicochemical properties of the extracted oils including moisture content, refractive index, specific gravity, acid, peroxide, iodine and saponification values of the three different oils extracted by solvents and cold-press extraction methods are illustrated in Table (3). It can be noticed that the extraction methods had no significant effect on these properties of the studied three oils.

For purslane seeds oil, the moisture contents were 2.93 and 2.86% for solvent and cold-press extraction oil, respectively. The refractive index (RI) is a parameter that relates to molecular weight, fatty acid chain length, degree of unsaturation and degree of conjugation (Gunstone, 2002). The refractive indices of the oil samples were 1.480 for solvent extracted oil and 1.481 for cold-press extracted oil. The specific gravity of oils for both extraction methods was 0.935. Free fatty acids (FFAs) levels are used to determine the

degree of hydrolysis of oils and their acceptability for usage in the food industry (Amos-Tautua and Onigbinde, 2013). The quantities of free fatty acids measured by the acid value for purslane seeds oil were 1.88 and 1.83 mg KOH/g oil for solvent and cold-press methods, respectively. The peroxide values for purslane seeds oil obtained by solvent and cold-press methods were 0.383 and 0.353 meq O₂/kg oil, respectively which were remarkably low indicating a low oxidation rate of the samples during the extraction process (Delfan-Hosseini *et al.*, 2017). The degree of unsaturation of purslane oil assayed by the iodine value was 138.53 g $I_2/100$ g oil for extraction methods. Saponification value is used as a measure of the fatty acids molecular weights and in checking the adulteration of edible oils. The saponification values of purslane seeds oil extracted by solvents and cold-press methods were 180.92 and 188.40 mg KOH/ g oil, respectively.

 Table (1): Effect of extraction time and sample: solvent ratios on oil extraction yield (%) of purslane, soybean seeds and sardine fish

Extraction	Extraction	Sample: solvent ratio	Oil extraction yield (%)			
solvent	time (h)	Sample. Solvent l'atto	Purslane seeds	Soybean seeds	Sardine fish	
СМ	2	1:2	0.41±0.03	2.30±0.31	1.43±0.13	
СМ	2	1:3	1.75 ± 0.01	4.24±0.28	2.65 ± 0.06	
СМ	2	1:4	3.14±0.03	5.41±0.39	3.30±0.13	
СМ	2	1:5	4.08±0.19	5.61±0.31	4.41±0.12	
СМ	2	1:6	5.87 ± 0.08	6.41±0.88	4.16±0.02	
СМ	4	1:2	0.94 ± 0.02	3.71±0.40	2.30±0.13	
СМ	4	1:3	2.32±0.03	7.07±0.72	3.13±±0.25	
СМ	4	1:4	4.29±0.06	7.92±0.32	4.20±0.05	
СМ	4	1:5	7.47 ± 0.07	8.65±0.50	5.43±0.80	
СМ	4	1:6	8.41±0.10	10.60±0.35	5.32±0.95	
СМ	6	1:2	1.26±0.03	5.71±0.40	2.85±0.15	
СМ	6	1:3	4.85±0.03	10.70±0.99	3.91±0.07	
СМ	6	1:4	5.31±0.05	10.70±0.25	5.50±0.13	
СМ	6	1:5	8.25±0.09	10.40±0.46	6.45±0.10	
СМ	6	1:6	9.43±0.07	15.60±0.34	6.33±0.10	
СМ	8	1:2	1.55 ± 0.02	6.89±0.11	3.18±0.07	
СМ	8	1:3	4.82±0.11	11.40±0.70	4.38±0.15	
СМ	8	1:4	7.02 ± 0.07	13.40±0.78	5.85±0.13	
СМ	8	1:5	8.89±0.02	13.30±0.36	6.65±0.26	
СМ	8	1:6	9.92±0.10	18.60±0.72	6.53±0.12	
Analyses of varian	ce					
Main effects			Interaction			
Extraction time (T)	***	T x R	***		
Sample: solvent ra	tio (R)	***	T x S	***		
Oil source (S)		***	R x S	***		
\mathbf{R}^2		0.9948	TxRxS	***		
CV%		5.26				

CM: chloroform: methanol (2:1)

Data represented as mean \pm SD_(n=3)

CV: coefficient of variation. \mathbb{R}^2 : determination coefficient. ^{ns} not significant; * significant at p < 0.05; ** significant at p < 0.01; *** significant at p < 0.001.

 Table (2): Extraction yield (%) of purslane, soybean seeds and sardine fish oils extracted by solvent and cold-press methods

Extraction method			
Extraction method	Purslane seeds	Soybean seeds	Sardine fish
Solvent (Chloroform: Methanol, 2:1)	9.92 ^A _b ±0.10	$18.60^{A}_{a} \pm 0.72$	6.65 ^A c±0.26
Cold-press	9.45 ^B b±0.10	$17.42^{B}_{a}\pm 0.37$	6.44 ^A c±0.03

Data represented as mean \pm SD (n=3)

Means with different capital character in the same column (extraction method) or small character in the same row (oil source) are significantly different at $p \le 0.05$.

Physicochemical properties of soybean seeds oils extracted by solvent and cold-press extraction methods are displayed in Table (3). The moisture contents were 1.93% for solvent extracted oil and 1.90% for coldpress extracted oil. The refractive index was 1.470 for both solvent and cold-press extracted oils. These results are quite similar to those reported for soybean oil (1.466) by Amos-Tautua and Onigbinde (2013). Regarding the specific gravity, the value was 0.921 for both solvent and cold-press extracted oils. The acid values were 0.49 for solvent extracted oil and 0.47 mg KOH/ g oil for cold-press extracted oils. The peroxide values of the oil obtained by solvent and cold-press extraction methods were 0.253 and 0.263 meqO₂/ kg oil, respectively indicating relatively good quality of these oils. Assaved iodine values reflect the degree of unsaturation were 130.5 g I/ 100 g oil for solvent extraction and 132.82 g I/ 100 g oil for cold-press extraction. Jokić et al. (2013) reported that the soybean oil obtained by supercritical carbon dioxide is rich in PUFA. Also, the authors found that the most abundant unsaturated fatty acid in soybean oil was linoleic acid. The saponification values of soybean oil ranged from 187.65 for solvent extraction to 190.27 mg KOH/ g oil for cold-press extracted oils.

Regarding the sardine fish oil, the moisture contents were 0.60% for solvent extraction and 0.58% for cold-press extraction. For refractive index, the obtained values were the same (1.470) for both extraction methods. The specific gravities were 0.927 and 0.929 for solvent and cold-press extraction, respectively. The acid values were 1.82 mg KOH/g for solvent extraction and 1.72 mg KOH/g for cold-press extraction. The peroxide values for the sardine oil samples were 0.173 meqO₂/ kg for solvent extraction and 0.156 meqO₂/ kg for cold-press extraction which consistent with the result obtained by Ferdosh et al. (2015) and fell within the range of FAO/WHO and ES (Musa et al., 2012). The iodine values of the sardine oil samples were 155.44 g I/ 100 g for solvent and 158.19 g I/ 100g for cold-press extraction. The sardine fish oil have saponification values for solvent and cold-press extraction methods of 193.54 and 195.88 mg KOH/ g oil, respectively.

Based on the results of the previous analysis, it can be said that the characteristics of the three extracted oils by the two methods of extraction, solvent and cold-press showed high and acceptable quality characteristics with purslane seeds oil having relatively a higher acid value than those of soybean or sardine oils regardless of the extraction method.

 Table (3): Physicochemical properties of purslane, soybean seeds and sardine fish oils extracted by solvent and coldpress extraction methods

Property	Purslane seeds oil		Soybean	seeds oil	Sardine fish oil		
	Solvent	Cold-press	Solvent	Cold-press	Solvent	Cold-press	
Moisture content (%)	2.93 ^a ±0.09	2.86 ^a ±0.06	1.93 ^a ±0.06	1.90 ^a ±0.17	0.60 ^a ±0.15	0.58 ^a ±0.10	
Refractive index	$1.480^{a} \pm 0.0004$	1.481 ^a ±0.0005	1.470 ^a ±0.0005	1.470 ^a ±0.0001	1.470 ^a ±0.0009	1.470 ^a ±0.0005	
Specific gravity	$0.935^{a} \pm 0.015$	0.935 ^a ±0.015	0.921 ^a ±0.003	0.921 ^a ±0.004	$0.927^{a} \pm 0.005$	0.929 ^a ±0.004	
Acid value (mg KOH/g oil)	1.88 ^a ±0.016	1.83 ^b ±0.016	0.49 ^a ±0.002	0.47 ^a ±0.008	1.82 ^a ±0.022	$1.72^{b}\pm 0.042$	
Peroxide value (meq O ₂ / kg oil)	0.383 ^a ±0.009	$0.353^{b}\pm 0.006$	0.253 ^a ±0.004	$0.236^{b} \pm 0.005$	0.173 ^a ±0.005	$0.156^{b} \pm 0.006$	
Iodine value (g I ₂ / 100g oil)	138.53 ^a ±0.79	138.53 ^a ±0.97	130.50 ^b ±0.10	132.82 ^a ±0.29	155.44 ^b ±0.52	158.19 ^a ±1.46	
Saponification value (mg KOH/ g oil)	180.92 ^b ±1.43	188.40 ^a ±1.01	187.65 ^b ±0.23	190.27 ^a ±0.81	193.54 ^a ±1.40	195.88 ^a ±0.81	

Data represented as mean \pm SD (n=3)

Means with different character between each two columns (extraction method) for the same oil source are significantly different at $p \le 0.05$.

Fatty acids composition of the extracted oils

Table (4) reveals the fatty acids composition (%) of purslane and soybean seeds and sardine fish oils extracted by the two oil extraction methods. It can be noticed that there are no differences in the fatty acids composition of purslane, soybean and sardine oils obtained with the two extraction procedures. Regarding the purslane oil, the main unsaturated fatty acids were oleic 20.18 and 20.23%, linoleic 42.73 and 42.82% and linolenic 33.42 and 33.60% acids for solvent and coldpress extraction methods. Kavosi *et al.* (2018) also detected higher amounts of linoleic than α -linolenic acid in oils obtained with solvent extraction regardless

of the solvent used. Moreover, Delfan-Hosseini *et al.* (2017) evaluated three procedures for purslane seed oil extraction, namely solvent, cold-press and microwave assisted cold-press extraction and observed a higher amount of linolenic acid than α -linolenic acid. These differences in the literature indicate that the extraction method has a significant effect on the fatty acids profile so optimization of extraction protocols is needed to obtain oils of the desired quality (Stroescu *et al.*, 2013).

For soybean oil oleic, 13.95 and 13.93%, linoleic 61.73 and 61.19% and linolenic 12.95 and 12.93% acids were the most abundant. These results are in agreement with those obtained by Jokić *et al.* (2013).

For sardine oil, palmitolic 2.37 and 2.23%, oleic 13.17 and 13.14%, linoleic 24.92 and 24.74%, linolenic 9.2 and 9.23% and Eicosapentaenoic 33.91 and 33.90% acids were the principal unsaturated fatty acids in sardine fish oil.

Also, some saturated fatty acids were identified, though in less quantity, including palmitic 3.31 and

3.34% and behenic 0.37% acids in purslane oil. The only saturated fatty acid detected in soybean oil was palmitic 1.45% for cold-press extraction and 1.57% for solvent extraction. Myristic and palmitic acids were the main saturated fatty acids detected in sardine fish oil for the solvent and cold-press extraction methods (Table 4).

 Table (4): Fatty acids composition (%) of purslane and soybean seeds and sardine fish oils extracted by solvent and cold-press methods

	Purslane seeds oil		Soybean seeds oil		Sardine fish oil	
Fatty acids	Solvent	Cold- press	Solvent	Cold- press	Solvent	Cold- press
C14:0	-	-	-	-	3.14 ^a	3.13 ^a
C16:0	3.31 ^a	3.34 ^a	1.45 ^a	1.57 ^a	12.82 ^a	12.80^{a}
C16:1	-	-	-	-	2.37 ^a	2.23 ^a
C18:0	-	-	-	-	-	-
C18:1	20.18^{a}	20.23 ^a	13.95 ^a	13.93 ^a	13.17 ^a	13.14 ^a
C18:2	42.73 ^a	42.82 ^a	61.73 ^a	61.19 ^a	24.92 ^a	24.74 ^a
C18:3	33.42 ^a	33.60 ^a	12.95 ^a	12.93 ^a	9.21 ^a	9.23 ^a
C20:5	-	-	-	-	33.91 ^a	33.90 ^a
C22:0	0.37 ^a	0.37^{a}	-	-	-	-
Saturated fatty acids (SFAs)	3.68 ^a	3.71 ^a	1.45 ^a	1.57^{a}	15.96 ^a	15.93 ^a
Unsaturated fatty acids (UFAs)	96.33ª	96.65ª	88.63 ^a	88.05 ^a	83.58 ^a	83.24 ^a
Polyunsaturated fatty acids (PUFAs)	76.15 ^a	76.42 ^a	74.66 ^a	74.18 ^a	68.29 ^a	67.87 ^a
Monounsaturated fatty acids (MUFAs)	20.18 ^a	20.23 ^a	13.95 ^a	13.93 ^a	15.54 ^a	15.37 ^a
PUFAs/SFAs	20.69 ^a	20.59 ^a	51.49 ^a	47.25 ^a	4.27 ^a	4.26 ^a
Omega-6/omega-3	1.28 ^a	1.27 ^a	4.76 ^a	4.73 ^a	0.58 ^a	0.57 ^a

Means with different character between each two columns (extraction method) for the same oil source are significantly different at $p \le 0.05$.

The obtained data indicated that individual fractions of oil samples had very slight differences but generally, total saturated and unsaturated fatty acid contents in the three oil samples had no significant differences (p>0.05). These findings are in agreement with the results of iodine value. The total unsaturated fatty acids in purslane seeds oil were 96.33 and 96.65%, soybean oil 88.63 and 88.05% while sardine fish oil 83.58 and 83.24% for solvent and cold-press extraction methods, respectively. Unsaturated fatty acids exhibited good nutritional and physiological properties that would help in preventing cancer and coronary heart diseases (Oomah et al., 2000). Polyunsaturated fatty acids (PUFAs) were the major component of total unsaturated fatty acids in the extracted oils by different methods, mainly because of the higher amount of linoleic and linolenic acids in oils obtained from the three raw materials; in purslane seeds oil 76.15 and 76.42%, sovbean oil 74.66 and 74.18% while sardine fish oil 68.29 and 67.87% for the solvent and cold-press extraction methods, respectively.

Regarding the monounsaturated fatty acids (MUFA) which could lower the "bad" cholesterol (low density lipoproteins or LDL) and retain "good" cholesterol (high density lipoproteins or HDL) (Ramadan *et al.*, 2010). Purslane seeds oil 20.18 and 20.23%, soybean oil 13.95 and 13.93% and sardine fish

oil 15.54 and 15.37% for solvent and cold-press extraction methods, respectively. The high MUFA content makes the investigated oils especially purslane a potential functional component to be used in food industry.

The ratios of omega-6/omega-3 fatty acids for purslane seeds oil 1.28 and 1.27; sardine fish oil 0.58 and 0.57 were lower than soybean seeds oil 4.76 and 4.73 for solvent and cold-press techniques respectively. The low omega-6/omega-3 fatty acids ratios indicate high nutritional value (Dubois *et al.*, 2007). Seed oils obtained with cold-press method showed lower n6/n3 ratio due to the higher content in α -linolenic acid comparing to solvent extraction method.

Vitamins E and D contents in the investigated oil samples

Oils and fats represent the important sources of vitamin E in human diet. Vitamin E content of plant oils varied depending on varieties, genotypes, growing and storage conditions (Trela and Szymańska, 2019). Oil extraction methods significantly influenced the vitamin E content of the extracted oils. Solvent extraction provided the highest concentrations of vitamin E due to the efficiency of the organic solvent in permeating protein bodies, penetrating cell walls, and extracting all fats and dissolved soluble compounds.

From Table (5), it could be noticed that, vitamin E (tocopherols) recorded the highest content compared to vitamin D in the investigated oil samples. The soybean oil exhibited the highest content of vitamin E (70 mg/ 100 g) compared to purslane seeds oil (10.7 mg/ 100 g). On the other hand, vitamin E was not detected in the sardine fish oil. Similarly, soybean oil

exhibited the highest content of vitamin D as compared to the sardine fish oil, whereas vitamin D was not detected in the purslane seeds oil. Zaaboul and Liu (2022) reported that soybean oil had α tocopherol content of 29.80 mg/ 100g and total tocopherols content of 179.10 mg/ 100 g oil.

Table (5): Vitamin E and D contents of the investigated oil samples

Oil sample	Vitamin E (mg/ 100 g)	Vitamin D (as Cholecalciferol (D ₃), mg/ 100 g)		
Purslane oil	10.70	0.00		
Soybean oil	70.00	5.49		
Sardine fish oil	0.00	0.007		

Differential scanning calorimeter (DSC) and thermogravimetric analysis (TGA) of the investigated oil samples

Thermal analysis characterizes the physical and chemical properties of substances, depending on the temperature at a defined heating rate (dynamic measurement) or on the time at a constant temperature (static measurement). Among the techniques developed to continuously monitor physical or chemical changes, which occurr as the temperature of a sample is increased or decreased, thermogravimetry (TG), differential thermal analysis (DTA), and differential scanning calorimeter (DSC) are the principal methods for the thermo-analytical analysis. In addition, the DSC curve obtained from an oil sample is a unique of that oil and acts as a "fingerprint". DSC is in fact an excellent method for determining differences between oils, for example between refined and natural oils (Roos, 2003).

The DSC and TGA curves (Fig. 1 A and B) show the thermal behavior of the purslane seeds oil, whose decomposition begins at around 180 °C (Fig. 1 B). The DSC curve showed endothermic peaks at 200 and 380°C (Fig. 1 A). On the other hand, soybean seeds oil showed an endothermic peak at around 90°C, the peak revealed a reflection point at 200 °C. As the TG curve showed no weight loss at these temperatures, indicating loss of water (Fig. 1 B). These observations were also noticed by Freire et al. (1999), when studying olive oil. The analysis of the TG curve showed that the soybean oil has a single weight loss that started at 230°C and ended at 320°C. In the DSC curve, there was a very strong exothermic reaction that caused deformation of the DSC peak and the deformation in the TG curve (adding the oven heat and the heat generated by burning of the sample).



Figure (1): A) Differential scanning calorimeter (DSC) and B) Thermogravimetric analysis (TGA) curves for purslane, soybean and sardine fish oils

For the sardine fish oil, an endothermic peak at around 70°C has been found (Fig. 1 A). TG curve showed no weight loss up to 360°C, (this indicated the loss of water at these temperatures) (Fig. 1 B). The differences and the unusual behaviors of the examined crude oils may be attributed to the presence of various classes of lipids and natural substances (Embaby et al., 2022).

Oxidative stability of the extracted oils

Oils containing relatively high proportions of polyunsaturated fatty acids (PUFAs) may experience

stability problems. The breakdown products alcohols, aldehydes, ketones, and hydrocarbons, generally impact offensive off-flavors. These compounds may also interact with other food components and change their functional and nutritional properties (Siddiq *et al.*, 2005).

The peroxide value is used to measure lipid oxidation, and is suitable for measuring peroxide formation in the early stages of oxidation (Smith *et al.*, 2007). The peroxide values of the purslane, and soybean seeds and sardine fish oils samples during storage at 5 and 22°C for 6 weeks are shown in Figure (2).



Figure (2): Changes in the peroxide values of purslane and soybean seeds and sardine fish oils during storage at 5 and 22°C for 6 weeks

There were no significant differences (p>0.05) in the peroxide values for the purslane and sardine fish oil samples stored at 5 and 22°C from the beginning of storage up to 3 weeks. However, for soybean oil samples there were differences starting from the 7thday in purslane and sardine fish oil samples. Thereafter clear differences were observed with increasing storage time for the three oil samples. The steady increase in the peroxide value indicated the formation of hydroperoxides during oxidation of oil. It can be observed that at the end of the experiment, 6 weeks of storage, the peroxide values of the soybean oil were the highest (3.927 meqO₂/ kg oil at 5°C and 4.123 meqO₂/ kg oil at 22°C), followed by the purslane oil then the lowest was sardine fish oil.

The lower peroxide value in the purslane oil may be attributed to the oxidative protection of the α -tocopherol and ascorbic acid content (Uddin *et al.*, 2014). In addition, the total phenolics content plays an important role in the oxidative stability of purslane seeds oil (Jalali Mousavi *et al.*, 2015). The degradation of peroxides during the course of oxidation could also occur explaining the lower values of the sardine oil values.

Thermal stability of the extracted oils

The fatty acids composition (FAC) of oil can be an indicator of its stability, physical properties and nutritional value. Studies have shown that the thermal stability of oils during heating is related to the fatty acids composition, the presence of antioxidant in foods (Moulodi *et al.*, 2015).

Figure (3) (a, b and c) shows the fatty acids stability in the purslane, soybean and sardine fish oils

during the thermal treatment at temperature ranging from 25 to 150°C for 2 h. For the purslane and soybean oil samples, the PUFAs decomposition increased by the increase in temperature at the specified time. It can be noticed that linoleic and linolenic acids showed complete decomposition after 2 h at 150 °C, while oleic acid decreased from 20.23% to 11.6% and from 13.93% to 8.99% for purslane and soybean oils, respectively (Fig. 3 a and b). These oils being a rich source of omega-3 fatty acids are relatively unstable to autoxidation, which limits their use in high-temperature applications or when shelf life extended is required (Akoh, 2017).

The five main unsaturated fatty acids detected in the sardine fish oil were palmitoleic, oleic, linoleic, linolenic and eicosapentaenoic acids. At 150 °C, the thermal treatment of both linoleic acid and linolenic acid revealed total degradation, whereas eicosapentaenoic, oleic and palmitoleic acids decreased from 33.90 to 7.44%, 13.14 to 7.44% and from 2.23 to 0.39%, respectively (Fig. 3c).

It is noteworthy that the relative content of linoleic and linolenic acids markedly decreased in earlier studies (Suleiman *et al.*, 2006). This may be due to that linoleic and linolenic acids are more susceptible to oxidation than oleic acid. Hădărugă *et al.* (2016) studied the thermal and oxidative stability of Atlantic salmon oil (*Salmo salar* L.). The results revealed that the main omega-3 fatty acids, EPA and DHA, significantly degraded, even at 50°C. Their relative concentrations decreased from 6.1% for EPA and 4.1% for DHA to 1.7 and 1.5% after storage at 150°C, respectively.



Figure (3): Unsaturated fatty acids composition for (a) purslane, (b) soybean and (c) sardine fish oils treated at 50°C, 100°C and 150°C for 2 hours

RECOMMENDATIONS

Extraction methods had a significant effect on the yield. The solvent method gave a higher extraction yield than the cold-press extraction method. The physicochemical parameters and fatty acids composition of the three oils studied were not affected by the extraction methods. The thermogravimetry analysis (TGA) and differential scanning calorimeter (DSC) revealed unusual behaviors for the examined crude oils that may be attributed to the presence of various classes of lipids and natural substances. Regarding the oxidative stability, there were no significant differences in the peroxide values for purslane, soybean and sardine fish oil samples stored at 5 and 22°C from the beginning up to 3 weeks. Thereafter clear differences were observed with increasing storage time for the three oil samples.

Consequently, purslane seeds oil has the potential to be a nutritional functional component due to its content of the valuable compounds such as omega-3 fatty acids that have a desirable effect on human health. So, it can be considered as new potential nutritious oil for usage in the food industry.

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تأثير طرق الاستخلاص على الخصائص الطبيعية الكيميائية، تركيب الأحماض الدهنية وثبات زيت بذور الرجلة مقارنة بزيت بذور فول الصويا وزيت سمك السردين

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هدفت هذه الدراسة إلى تقدير تركيب الأحماض الدهنية، الخصائص الطبيعية الكيميائية والحرارية لزيت بذور الرجلة المستخلص بطريقتي المذيبات والضغط على البارد مقارنة بزيت فول الصويا وزيت سمك السردين. أظهرت النتائج أن استخلاص زيوت بذور الرجلة، فول الصويا وسمك السردين بالمذيبات أعطى كمية زيت أعلى معنوياً مقارنة بطريقة الاستخلاص بالضغط على البارد. لم تتأثر الخصائص الطبيعية الكيميائية الثلاثة زيوت بطريقة الاستخلاص وأظهرت صفات جودة جيدة ومقبولة. علاوة على ذلك، لا توجد فروق معنوية في محتوى الأحماض الدهنية للزيوت المتحصل عليها بطريقتي الاستخلاص. كان حمض اللينوليك (C₁₈₋₂) هو الحمض الدهني السائد في زيوت بذور فول الصويا والرجلة، بينما كان حمض الايكوسابنتايونيك (C_{20:5}) هو الحمض الدهني غير المشبع السائد في زيوت بذور أخرى, أظهرت نتائج التحصل عليها بطريقتي الاستخلاص. كان حمض اللينوليك (C₁₈₋₂) هو الحمض الدهني السائد في زيوت بذور فول الصويا والرجلة، بينما كان حمض الايكوسابنتايونيك (C_{20:5}) هو الحمض الدهني غير المشبع السائد في زيت سمك السردين. من ناحية أخرى, أظهرت نتائج التحليل الحراري بواسطة المسح الحراري التفاضلي (DSC) وتركيا وتحليل الوزن الحراري الثلاثة بعد التعرض عادي للزيوت الثلاثة. أيضا أظهرت نتائج الثبات الحراري تحللا كاملا لأحماض الالينوليك والينولينيك في الريوت الثلاثة بعد التعرض الحرارة لمدة ساعتين على درجة حرارة ١٥٠^٥م. نوصى، علي الرغم من أن طريقة الاستخلاص بالمنيبات أعطت كمية زيت أعلى، فان الزيوت المتخلصة بطريقة الاستخلاص على المارد يتوقع احتفاظها بالنكهة والرائحة والمغذيات، وبالتالي فإن طريقة الاستخلاص عليه الاردين الحرار الزيوت المترفي زيت أعلى، منا