

## OIL EXTRACTION AND FATTY ACID CHARACTERIZATION OF SWEET PEPPERS SEEDS *CAPSICUM ANNUM* (L.) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) AND ITS USE IN BEEF BURGER PATTIES PRESERVATION

Mohammed Zarah Eskander<sup>1</sup>, Khalid Hassak Abdel Hassan<sup>1</sup>, Ali Sh. Alshara<sup>1</sup>, Eman M. Taheer<sup>2</sup> and Zeinab I. Ali<sup>2</sup>\*

**Address(es):**

<sup>1</sup> Food Sciences Department, Faculty of Agriculture, University of Basrah, Iraq.

<sup>2</sup> Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Egypt.

\*Corresponding author: [zeinab\\_ali@cu.edu.eg](mailto:zeinab_ali@cu.edu.eg) / [dr.zeinab2010@gmail.com](mailto:dr.zeinab2010@gmail.com)

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**ABSTRACT**

The present study was conducted to estimate the physicochemical characteristics, fatty acids, phenolic compositions and antioxidant activity of the extracted sweet pepper seed oil (SPSO) using the Gas Chromatography-Mass Spectrometry (GC-MS) technique. The obtained oil yield was 14.7%. Outcomes of the extracted oil revealed the following physical and chemical properties: Iodine number 141.64 mg/100g, saponification number 197.51 mg KOH/g, peroxide number 3.63 meq/ Kg oil, free fatty acid 0.48, refractive index 1.467, specific gravity 0.924 g/cm<sup>3</sup> and viscosity 63.24 cp. Applying GC-MS technique, cis-Palmitoleic acid was the predominating fatty acid, followed by Myristic acid and Pentadecylic acid while Heptanoic acid was the lowest one. Additionally, esters of some fatty acids and bioactive chemical compounds such as Phenol, 2,2'-methylene bis [6-(1,1-di methyl ethyl)-4-methyl] were determined. The antioxidant activity of SPSO at different concentrations 0.625, 1.25, and 2.50 % was estimated during the cold storage of beef burger patties at 4°C, and results revealed that the highest inhibition of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) root was 74% at a concentration of 20 mg/ml. Whereas, a significant decrease (p≤ 0.05) in the peroxide value 12.27 mEq/ Kg was observed at 2.5 % SPSO, presenting the highest antioxidant activity through inhibiting the peroxide formation in burger samples by the end of the tenth day of storage at 4°C. In conclusion, SPSO is suggested as a powerful natural food preservative for application in the food industry. because of its bioactive antioxidant components.

**Keywords:** Sweet pepper seed oil, (SPSO), Fatty acid, Antioxidants, Beef burger patty, Capsicum

**INTRODUCTION**

Pepper is a crop in the eggplant family that has spread to many countries around the world. Sweet pepper is widely grown in Asia, North America, Africa, and countries of the Mediterranean basin (Jeong *et al.*, 2011). The Agro-food industry generates massive amounts of liquid and solid waste as a result of the food transformation and consumption chain. Pepper seed is a byproduct of the pepper processing industry. Every year, large amounts of pepper seeds are produced, and these byproducts are typically discarded as solid waste (Li *et al.*, 2018). This caused a major problem and aggravated the industry's burden on waste treatment, because it is expensive to transport, handle and dispose this waste (Silva *et al.*, 2013). These solid waste pepper seed resources have yet to be fully utilized. Nonetheless, pepper seeds, like pepper fruits, are a promising source of nutritional constituents and bioactive compounds such as capsaicinoids and phenolics that can be used for their biological potential (Sung *et al.*, 2015). Capsicum spp. is a remarkable source of several antioxidant compounds, including capsaicinoids (Topuz and Ozdemir, 2007).

Even though, lipids and fatty acids constitute a minor constitute of the edible portion of peppers, they play an important metabolic and structural role (Martinez *et al.*, 2006). Lipid content is an important quality parameter as several bioactive molecules responsible for pericarp (pulp) color are liposoluble. Furthermore, the nutritional quality of lipids is determined by their fatty acid composition (Zaki *et al.*, 2013).

The toxic and carcinogenic impacts of synthetic antioxidants have recently increased demand for natural antioxidants. Since it contains so many antioxidants compounds, *Capsicum annum* L. is well-known natural antioxidant. Fresh sweet peppers have exceptionally high ascorbic acid, a 100 g serving to supply 100% of the current Dietary Reference Intake (DRI) of 60 mg/day as well as moderate to high levels of neutral phenolics or flavonoids, namely quercetin, luteolin and capsaicinoids (Deepa *et al.*, 2006). Given the annual increase in sweet pepper production, the new application of using seed waste as an antioxidant material is away to fill this gap and maximize this resource use (Sim and Sil, 2008).

Hence, the current study was conducted to investigate the yield of seed oil extracted from *Capsicum annum* L. and its fatty acid composition as well as the physicochemical properties. Additionally, the antioxidant activity was

assessed using radical scavenging (DPPH) antioxidant assay coupled with an experimental trial on the processed meat (beef burger patties) model.

**MATERIALS AND METHODS**

**Sweet Pepper seeds**

Sweet pepper seeds were purchased from Basrah, IRAQ. The seeds were thoroughly cleaned, rinsed, and dried for three days at 30 °C until became completely dry. The dried capsicum seeds were separately ground in a coffee grinder for one minute to assess the proximate composition and seed oil characteristics, and then stored at -18 °C for further analysis. All reagents used in this study were of analytical grade and obtained from Sigma-Aldrich (USA).

**Chemical composition of Sweet Pepper seeds**

Determination of the physicochemical properties of Sweet Pepper seeds including the moisture content, protein, fat and ash contents was done according to the official methods (AOAC, 2000) 925.09, 2001.1, 932.06, and 985.29 receptively.

**Oil extraction**

Lipids were extracted from the seeds as described before by (Bligh and Dyer, 1959). Briefly, five grams of ground pepper seeds were placed in an Erlenmeyer flask then a mixture of 25 ml chloroform, 50 ml methanol, and 20 ml of deionized water (1:2:0.8, v/v/v) were poured into seeds powder. The mixture was then shaken for 10 min. Following the completion of the lipid extraction, the mixture was promptly filtered through cheesecloth to eliminate any remaining seeds and prevent further lipid extraction. The mixture was moved to a glass separating funnel to allow the organic and aqueous layers to be separated, and then the rotary evaporator was used to evaporate the chloroform layer, leaving behind the extracted lipids. The extracted lipids' weight was then measured.

**Analysis of SPSO using Gas Chromatography-Mass Spectrometer (GC-MS)**

The gas chromatograph analysis of the extracted SPSO using a Shimadzu QP2010 quadrupole(GC-MS) instrument equipped with a carbowax (30 m × 0.25 mm ID; 0.25µm film thickness) capillary column (intercut DB5MS . japan). One microliter of the sample was injected into the capillary column and Helium was used as a carrier gas. The column temperature was programmed initially at 50°C for 1min and increased at a rate of 5°C per min till obtain a final temperature of 280°C. Compounds identification was based on mass spectral comparisons with the National Institute of Standards and Technology (NIST) Library 2008 (De Souza et al., 2013)

**Physicochemical characterization of the extracted oil**

Official methods were used to determine the free fatty acid content using AOCS method Ca5a – 40 (AOAC, 1997). Saponification number Cd 3-25 (AOCS,1990), iodine value Cd 1-25 ( AOCS,1997) and peroxide values Cd 8b-90 (AOCS, 2005).The refractive index of oil samples was determined using a Carl-Zeiss model G Abbe Refractometer with temperature adjustment (Alamu et al., 2008).

**DPPH radical scavenging assay**

The ability of the seed oil to scavenge DPPH free radicals was assessed using the standard method (Xiao et al., 2015). Aliquots 2 ml of various concentrations(5-25mg.ml<sup>-1</sup>) of pepper seed were added to2 ml of 0.004% methanolic solution of DPPH. After an incubation period of 30 min in darkness at room temperature 25C° , the color reduction of the DPPH substrate was measured by a UV-vis spectrophotometer at the wavelength of 517 nm. The control sample contained the same amount of methanol and DPPH solution. Ascorbic acid was used as a positive control .

The degree of DPPH radical-scavenging was calculated as follows

$$\text{DPPH inhibition activity(\%)} = [A0 - A1/A0] \times 100$$

A0= Absorbance of control (blank, without extract)

A1= Absorbance of sample

**Preparation of beef burger patties**

Fresh boneless beef was obtained and chopped into smaller pieces, then minced with 10% fat and 0.75% salt. The samples were divided into treated and untreated groups (control). Different concentrations of pepper seed oil were added to the treated groups 0.625, 1.25, and 2.50%, then burgers were prepared using a manual-burger machine 65 g weight and 10 cm diameter. Ideally, beef burger patties are usually stored frozen at – 18C°, but our experiment designed to assess the antioxidant activity of SPOS in bad storage condition, that’s why the samples were stored in refrigerator at 4°C for 10 days .

**Determination of Peroxide value**

The Peroxide value (POV) of the beef burger prepared was measured according to the standard method of the Association of Official Analytical Chemists (AOAC, 2000) at three time-points of refrigerated storage 0, 5 and 10 day.

**Statistical analysis**

The study's data were subjected to a Completely Random Design (C.R.D.) with factorial experiment. The data were statistically analyzed using the statistical program SPSS version 21. The means were compared using the less significant difference (L.S.D.) at the 0.05 level.

**Table 2** Physicochemical properties of the extracted seed oil from *Capsicum annum*.

Parameters	Oil yield (%)	Refractive index (25C°)	Density (25C°)	Free fatty acid% (oleic acid)	Saponification number (mg KOH/g of oil)	Peroxide value meq O <sub>2</sub> /kg oil	viscosity 20C <sup>0</sup> (cp)	Iodine value (gI <sub>2</sub> /100g oil)
Value	17.4	1.762	0.924	0.48	197.51	3. 63	63.24	141.64

**Fatty acids profiling using the GC-MS method**

(Table 3) showed the fatty acids and active chemical compounds extracted from sweet pepper seed oil using the GC-MS method. It was observed that numerous fatty acids were present in various ratios. Interestingly,cis-palmitoleic acid(also known as cis9-Hexadecenoicacid) constituted the majority of these fatty acids 29.88 % . Additionally, this fatty acid makes up roughly 13% of the fat in Duriograveolens fruit's fat content (De Souza et al., 2018).

Moreover, Myristic acid represented 11.94% of the area in the MS-GC profile(Figure 1). It is a long-chain saturated fatty acid 14:0. It can be found in a variety of vegetable oils, including palm oil, coconut oil(Verruck et al., 2019). Pentadecylic acid represented1.37% of MS-GC ratio, which is not normally synthesized by animals, but it is found in trace amounts in dairy products (Korma

**RESULTS AND DISCUSSION**

**Physicochemical characteristics of sweet pepper seeds.**

The approximate composition of the examined sweet pepper seeds revealed that carbohydrates were the most abundant component 44.2%, followed by protein 29.1% , fat 15.1 % and Ash 4.4% (Table 1). Similarly, (Chouaibi et al., 2019) showed that red pepper seeds contain 43.6, 18.31, 28.33, 3.05, and 6.63%of carbohydrates, fat, protein, ash and moisture respectively. On contrary, (Jarret et al., 2013) stated that the fat content of five different types of pepper seed oil ranged from 18.26 to 28.08%. This could be because the chemical composition of pepper seeds varies greatly, particularly in their oil content, based on plant type, climatic conditions, harvesting time, maturity phase, and humidity (Kadri et al., 2015). Pepper seeds are a good source of oil, and thus a good source of nutrition, although more research is needed to ascertain the functional qualities of pepper seed oil (Yilmaz et al., 2015).

**Table1** Chemical composition of sweet pepper seeds.

Component	Water	Protein	Fat	Ash	Carbohydrate
Value (%)	7.2	29.1	15.1	4.4	44.2

**Physicochemical characteristics of extracted sweet pepper seed oil.**

The physicochemical properties of SPSO showed that the iodine value was 141.64 mg/100g (Table2). The iodine number represents the degree of unsaturation of the fatty acids involved in triglyceride formation (Nzikou et al., 2010), while the saponification number was 197.51 mg/g. Interestingly, the degree of saponification was within the permissible range for cooking oil, indicating the pepper seed oil's purity (Siyانبola et al., 2013). Additionally, the results showed a lower peroxide value 3.63Meq/kg compared to palm oil 4.0 Meq/kg and sunflower seed oil 4.2 Meq/kg.Thisresult indicated the high resistance of pepper seed oil to oxidation that could be attributed to the presence of natural antioxidants in sweet pepper seed oil, such as phenolic compounds and tocopherol (Eze, 2012) .

Determination of free fatty acid contents is considered an important indicator of the rancidity of different fats and oils (Ruttarattanamongkol et al., 2014), the obtained results revealed that the sweet pepper seed oil containing 0.48% free fatty acid (Table2). The obtained result was similar to that of (Raimi et al., 2014) who revealed that the fatty acid contents of *C. annum* (Bell pepper) and *C. frutescens* were 0.395 and 0.564%, respectively.While *C. annum* (Bird eye chili) and *C. Frutescens* (Bird pepper) had the highest percentages of free fatty acids 0.68 and1.353 % , respectively.

The refractive index of the extracted sweet pepper seed oil was 1.467 (Table 2), and this result complied with (Chouaibi et al., 2019). Oil refractive index is affected by the length of the fatty acid chain, molecular weight, and degree of unsaturation (Gohari et al., 2011). Moreover, the specific gravity and viscosity were 0.924 g/cm<sup>3</sup> and 63.24 cpu, respectively. The viscosity of the sweet pepper seed oil considered higher compared to the other vegetable oils (olive, rapeseed, soybean, sunflower and grape seed oils), this might be related to the method of oil extraction and the length of chain fatty acids (Chouaibi et al., 2012).

The oil yield was 7. 14%,in contrast to the findings of Chouaibi et al. (2019) which stated that the oil yield varied depending on the method of extraction; extraction with organic solvents resulted in higher yield 18.39% of red pepper seed oil, while cold extraction yielded a lower yield14.6%. All physicochemical properties of extracted sweet pepper seed oil in the current study were agreed with the Codex Alimentarius guidance for vegetable oils (Fao/Who/Codex Alimentarius Commission, 2019).

et al., 2022). It’s also approved by the Food and Drug Administration (FDA) as a flavoring agent. Heptanoic acid (Enanthicacid); a saturated fatty acid consisting of a chain of 7 carbon atoms was present in a small percentage. While, Heptanoic acid is found naturally in alcoholic oils, beer, and rum, as well as coffee and black tea. This acid can also be found in fermented cabbage, some types of moldy cheese, meat, and whale oil (George, 2010), yet it might contribute to the rancid smell of some oils. It is slightly soluble in water, but highly soluble in ethanol and ether.

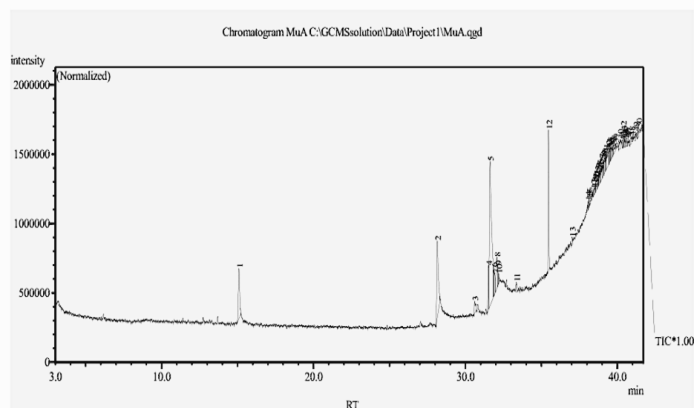


Figure 1 GC-MS total ion chromatogram of Fatty acid composition of the extracted seed oil from Capsicum annum.

Table 3 The chemical profile of main components in the Capsicum annum (Seed oil of sweet pepper)

Peak	RT	% Area	Molecular Formula	Compound name	Molecular weight
1	15.093	6.39	C12H14O4	Diethyl Phthalate	222.24
2	28.144	11.94	C15H30O2	Myristic acid	242.40
3	30.601	1.42	C22H44O2	1-Heneicosyl formate	340.584
4	31.52	3.91	C10H16O2	Endo- bicycle [3.3.1] Endo- bicycle [3.3.1] nonan-3-carboxylic acid	168.23
5	31.619	29.88	C18H34O2	cis- Palmitoleic acid	282.5
6	31.947	2.31	C8H10O4	2- Propenoic acid -1,2-ethanediyl ester	170
7	31.86	0.89	C12H23NO2	1-Hexyl-2-nitrocyclohexane	356.5
8	32.063	3.83	C22H44O4	Octadecanoic acid, 2-(2-hydroxy ethoxy) ethyl ester	460.7
9	32.127	1.37	C15H30O2	Pentadecanoic acid	242.40
10	32.197	0.44	C6H13N5O4	Nitro -L- Arginine	
11	33.359	0.65	C9H16O2	beta.-Phenylpropionic acid	183.29
12	35.469	8.83	C23H32O2	Phenol, 2,2'- methylene bis [6-(1,1-di methyl ethyl)-4-methyl]	1049.4
13	38.474	0.42	C7H14O2	Heptanoic acid	250.38
14	39.567	0.90	C24H54SeSi	Hexa-t-butylselenatrisiletane	506
16	40.427	0.42	C6H7N5	2-Myristynoyl-glycinamide	149.15
17	41.200	0.48	C38H74	1,37-Octatriacontadiene	994530.

RT\* (Retention time)

**Antioxidant activity (Radical-scavenging DPPH activity of Sweet Pepper seed oil)**

Figure (2) compared the effectiveness of sweet pepper seed oil at concentrations ranging from 5 to 20 mg/ml in inhibiting the free radicals to Ascorbic acid using DPPH. Interestingly, increasing the oil concentration increased the probability significantly ( $p \leq 0.05$ ). At a concentration of 20 mg/ml, the oil had a maximum effectiveness of 74% in comparison to ascorbic acid which had better effect of 96% at the same concentration. This effect could draw backs to presence of antioxidant compounds such as 9-Octadecanoic acid (Z)-2-hydroxy-1 - and Phenol, 2,2'-methylene, which could inhibit free radicals (Table 3). Similarly, (Alvarez-Parrilla et al., 2011) showed that phenolic compounds were the main contributors to the antioxidant activity of fresh and processed peppers. Also, (Fazel et al., 2008) confirmed that pepper seed oil contains antioxidant compounds; such as, phenols, tocopherols, and vitamin E. Using the extracted oil as a natural preservative in the meat industry.

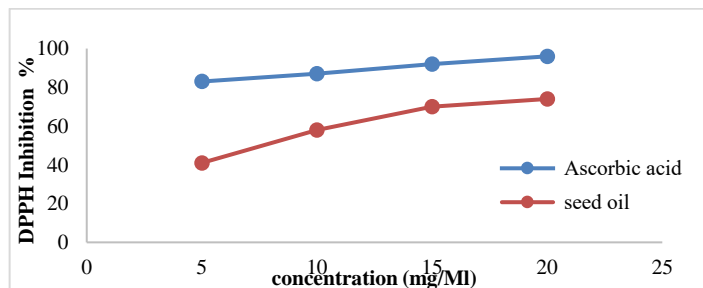


Figure 2 The DPPH radical scavenging activity of sweet pepper seed oils and Ascorbic acid

**Using the extracted oil as a natural preservative in the meat industry**

Because of the rapid decrease in quality parameters in addition the microbial growth, beef burger patties have a maximum shelf life of 3 days at 4°C (Parafati

In addition to the presence of fatty acids, esters of some of these acids were also observed (Figure 1), such as Octadecanoic acid, 2-(2-hydroxy ethoxy) ethyl ester which presented in a high percentage followed by Oleic acid, 9-hexadecenyl ester whose area in the MS-GC graph was 3.83% and 1.26%, respectively. Vegetable oil is a healthy source of fat in the diet and is commonly used as an alternative to animal fat sources that are high in saturated fat (Choi et al., 2010).

These results indicated that many compounds that have biological activity present in the extracted sweet pepper seed oil in different proportions, including those have anti-oxidant activity, such as 1- Heneicosyl formate (Gomathi et al., 2015). Other compounds were successfully separated such as Phenol, 2,2'-methylene bis [6-(1,1-di methyl ethyl)-4-methyl]. Also, Myristynoyl-glycinamide-2 compound (Saikarthik et al., 2017). Interestingly, many antimicrobial compounds have been detected in the extracted SPSO, such as beta-phenylpropionic acid and -1,37-octatriacontadiene, which previously determined by (Ravi et al., 2018). Presence of these numerous and diverse active compounds that possess antimicrobial and antioxidant activities in sweet seed oil extract potentiate its application as a natural food preservative.

et al., 2019). Oxidation process is one of the major causes of meat deteriorations, leading to negative changes in nutritive value, sensory and physicochemical properties of meat (Horbańczuk and Kurek, 2019). In order to estimate the antioxidant effect of the extracted sweet pepper seeds oil in a food model, beef burger patties were prepared with different concentrations of the oil and the peroxide values in the prepared beef burger patties were assessed during the refrigerated storage period of 0, 5, and 10 days at a temperature of 4°C. The results in (Table 4) reveals there was a significant decrease of the peroxide value accompanied by increasing the oil concentration with significant difference between the examined concentrations ( $p \leq 0.05$ ). The highest inhibitory activity that hinders the oxidation of burger fat was observed with 2.5% SPSO while peroxide value was 12.27 mEq/kg by the end of the tenth day of cold storage period. Interestingly, there was no significant difference between the used concentrations of the extracted oil and the commercial antioxidant butylated hydroxyl toluene (BHT). This might be attributed to presence of antioxidants compounds like phenols, tocopherols, and vitamin E that can stop or lessen the self-oxidation of dietary oils and fats (Fazel et al., 2008).

These findings were consistent with those of (Fazel et al., 2008), who revealed that crude oil extracted from tea seeds had antioxidant properties and could be used as a natural antioxidant substitute.

Table 4 Peroxide value of prepared burger samples

Treatment	Storage (day)		
	0	5	10
Control	5.36 <sup>a</sup>	13.84 <sup>a</sup>	29.76 <sup>a</sup>
0.625 %	5.35 <sup>a</sup>	10.65 <sup>b</sup>	21.39 <sup>b</sup>
1.25%	5.35 <sup>a</sup>	9.46 <sup>b</sup>	17.85 <sup>c</sup>
2.5%	5.35 <sup>a</sup>	7.87 <sup>c</sup>	12.27 <sup>d</sup>
BHT 0.02%	5.34 <sup>b</sup>	7.13 <sup>c</sup>	10.43 <sup>d</sup>
L.S.D.	0.021	1.88	3.16

-Means in same column with different lowercase letters indicate significant difference ( $P < 0.05$ ) between the studied groups.



## CONCLUSION

This study was dedicated to assess the potential application of Sweet pepper seeds in food preservation instead of discarding it as a solid waste. Firstly, the sweet pepper seed oil was extracted and its physicochemical properties were determined with special attention to its chemical profile using GC-MS technique. Further more, the antioxidant activity of sweet pepper seed oil was evaluated in beef burger patties as a food model. The results revealed presence of many potent bioactive compounds including different fatty acids like cis-Palmitoleic acid, Myristic acid and Pentadecylic acid, in addition to presence of esters of some fatty acids and other bioactive chemical compounds. Therefore, through this study, sweet pepper seed oils is highly recommended as a potent source of antioxidants for food application as a natural preservative in different food models.

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**Authors' contributions:** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Mohammed Ziyarah Eskander], [Khalid Hassak Abdel Hassan], [Ali Sh. Alshara], [Eman M. Taher] and [Zeinab Ibrahim Ali Ali]. The first draft of the manuscript was written by [Mohammed Ziyarah Eskander] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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