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THE EFFECT OF ULTRASOUND ON THE ANTIOXIDANT PROPERTIES, PHENOLIC COMPOUNDS AND SOLUBLE VITAMINS IN SUNFLOWER OIL

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Abstract
The current study intended to increase sunflower oil extraction by subjecting the oil to an ultrasonic device at a frequency of 20 Hz for varied times (0, 5, 10, 15) minutes and then extracting the oil with hexane. The total phenolic compounds were investigated, and there was a considerable rise in the phenolic compounds of sunflower oil, with the maximum percentage being (30.56 mg/mm at time (10) and the lowest being 22.32 (mg/mm) at time (0.) The antioxidant properties were also studied, with the highest percentage being (41.2, 49.8, 69.7, 84.5, 94.5%) at time (15) (20. 6, 30.5, 44.5, 55.4, 73.5%) for each of the concentrations (30, 60, 120, 250, (ppm500) respectively, and the lowest percentage at time (0) for each of the concentrations (30, 60, 120, 250, 500 ppm) respectively. The soluble vitamins in the oil were studied, and the highest concentration was found (49.57%) at the time (10) and the lowest concentration was found (39.28%) at the time (0).

Keywords: ultrasound, total phenolic compounds, total flavonoids, antioxidants, vitamins

Introduction

Because it is an oil crop, the sunflower is one of the most significant industrial agricultural crops. According to statistics, there is a rising demand each year on the international markets for its seeds and the oil that is derived from them. This crop comes in the second place after the soybean crop, and it contains 30% oil (Belitz et al., 2009).

There are three main kinds of sunflower oil. The first is oleic oil, which has an oleic acid content of 80%. The second is mid oleic oil, which has an oleic acid content of 65% and an appropriate amount of linoleic acid. The third type is linoleic oil, which

has a linoleic acid content of 69% and an oleic acid content of 20%. (Warner et al., 2010).

The use of ultrasonic technology in food processing is a novel and intriguing technique that is frequently an addition to traditional techniques and is effective in extraction, emulsification, pasteurisation, and sterilizing, resulting in shortened extraction times and more effectiveness (Mason, 1998). When compared to other extraction techniques, this technology has a number of benefits, including the use of less organic solvents, lower temperatures, less energy use, and higher extraction rates (Kumar et al., 2021).

Due to the disruptions and molecular absorption that happen as a result of the medium's lack of viscosity, which causes gas bubbles to develop, ultrasound waves generate heat when they travel through the medium as kinetic energy (Zisu et al. 2010). High-intensity ultrasound technology in particular has generated a lot of interest since it has led to the creation of novel procedures for enhancing the quality and safety of processed foods (OSullivan, Murraym, 2016).

In recent years, there has been increased interest in antioxidants due to the detrimental influence of fat oxidation on food quality, which promotes the deterioration of oils and fatty meals, resulting in the loss of nutritional content (Bondi et al., 2017). Many studies have shown that antioxidants can act as antivirals, bacteria, and fungi (Wang et al., 2009).

In general, antioxidants are important because of their capacity to bind metals while eliminating free radicals and blocking numerous lipid peroxides (Manosroi et al., 2012). Sunflower oil is also high in soluble vitamins, such as vitamin E, which can scavenge free radicals and prevent oxidation (Dutta, 2003).

Materials and Methods

Sample Sources

In this study, samples of sunflower seeds collected from commercial markets in Salah El-Din Governorate were used, and dust and impurities were removed from them.

Processing of Sunflower Seed Powder

The sunflower seed powder was made using the method (Hua and Mao 2012). The whole seeds were crushed using an electric grinder to obtain seed powder, which was then sieved using a 300 mm micron sieve before being packed in bags, sealed, and refrigerated until use.

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Ultrasound Treatment

A hexane-sunflower seed powder solution was prepared, in which the sunflower seed powder was treated with 10% hexane with continuous stirring by a magnetic stirrer for three hours, followed by ultrasonic treatment according to the method recommended by Malik et al. In the basin of the ultrasonic device type (power Sonic 405), at a frequency of 20 Hz for times (0, 5, 10, 15) minutes at room temperature with the addition of ice every 3 minutes to maintain the temperature, the samples were left to rest for half an hour after the ultrasonic exposure process was completed (Jamal and Kareem,2022)

Preparation of the Oil Extract

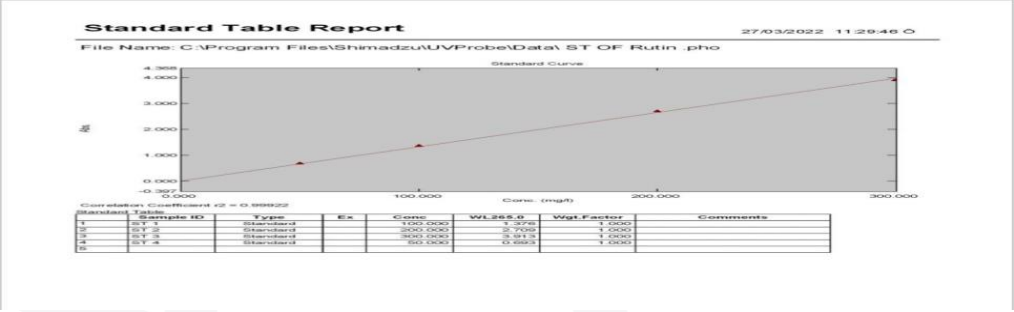
To separate the filtrate from the sediment, the samples were filtered using filter paper No. (1), and the product was collected from the extraction process and placed in a rotary evaporator to get the pure oil extract. Fuad and colleagues, 2016.

3. Determination of Total Phenols (TPC) of Sunflower Oil

The Ayoola et al. method was used to determine the total phenolic content (TPC) (2008). This procedure involved adding 2.5 ml of Folin-Ciocalteu reagent, 3 ml of distilled water, and 2 ml of oil to a 10 ml volumetric flask. Following a 4-minute shaking, 2 ml of sodium carbonate solution was added to the flask. The volume was then filled to the mark, the combination was mixed, and the mixture was allowed to sit for two hours before the absorbance was measured using a spectrophotometer at a 750 nm wavelength.

1-11-3 Standard Curve of Phenols

As a standard solution, gallic acid was used. The standard curve was created by dissolving 0.5 g of gallic acid in 10 ml of the experiment's alcohol and filling the volume to 100 ml with distilled water, then taking 1,2,5,10 and filling the volume to 100 with distilled water to produce Concentrations 50,100,250,500 (Shpelev et al., 2016).



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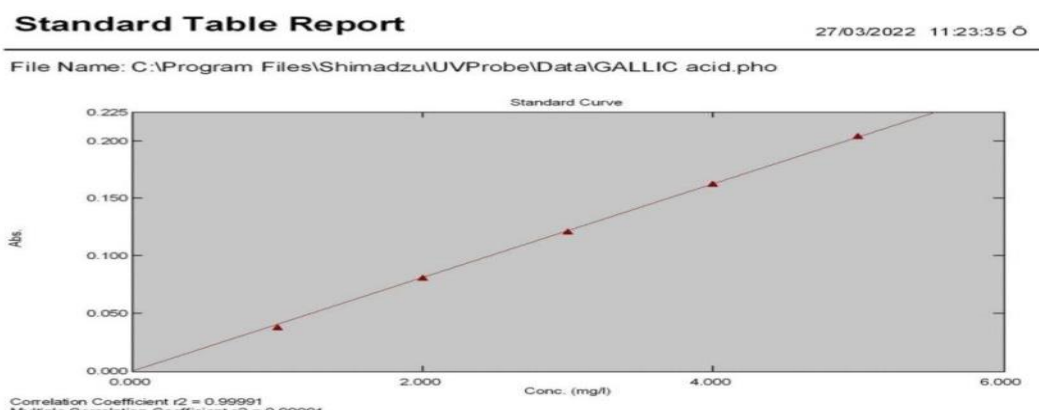
3. Determination of Total Flavonoids

According to Ayoola et al., (2008), the amount of flavonoids in the studied sunflower oil was estimated by taking 2 ml of 2% aluminium chloride and adding it to 2 ml of extracts, shaking the mixture well, and leaving it for two hours at room temperature (25 C), and then measuring the absorbance at 420 nm.

1-12-3 Standard Curve for Flavonoids

The amount of flavonoids of the studied sunflower oil was estimated based on the graphic relationship between the concentration of the compound and the absorbance, as several different concentrations were prepared from the standard routine.



	Sample ID	Type	Ex	Conc	WL264.0	Wgt.Factor	Comments
1	G.A 1	Standard		1.000	0.038	1.000	
2	G.A 2	Standard		2.000	0.081	1.000	
3	G.A 3	Standard		3.000	0.121	1.000	
4	G.A 4	Standard		4.000	0.163	1.000	
5	G.A 5	Standard		5.000	0.204	1.000	
6							

3. Determination of the antioxidant DPPH of sunflower oil

According to what was mentioned by Okunade (2002), 0.04 g of DPPH was dissolved in 100 ml of methanol, noting that the concentration of DPPH was 400 µg/ml. The standard solution (vitamin C) and sample were prepared by mixing 0.5 g of vitamin C with 100 ml of methanol and distilled water. The standard solution's concentration was (5000 ppm), and other concentrations were made using the dilution law at 30, 60, 120, 250, and 500 ppm of vitamin C (Vaidyaratnam, 2002). After 30 minutes of stagnation at room temperature, the mixture was strongly shaken, and the absorbance was calculated at 517 nm using a spectrophotometer. The concentration of the sample necessary to block 50% of free radicals DPPH was estimated as the sample's C50 value. The logarithmic dosage inhibition curve shows that more free radical activity is indicated by a lower absorption of the reaction mixture (Koleva, 2011). The following equation was used to calculate the DPPH radical scavenging activity:

DPPH scavenging activity (%)=(1-(A1-A2)A3)100
where :
A1 is the absorbance of the oil sample and DPPH solution
A2 is the absorbance of the oil and methanol sample (without DPPH)
A3 is the absorbance for DPPH solution and absolute methanol (without oil)

3 Determination of the soluble vitamins in the oil

Using a high-efficiency liquid column chromatography (HPLC) type Shimadzu (Japanese) and a C18 type separating column with a size of 5 microns, i.e., the method described in (Ixtaina, et al., 2011), the soluble vitamins in sunflower seed oil were determined (250 mm x 4.6 mm). 0.25 g of oil was collected and combined with 5 ml of hexane in the mobile phase, which was 100% Aceton Nitril with a flow rate of 1.5 and a temperature of 25 °C. Next, 20 microliters of the reagent were injected, and the reagent was set at 290 nm. Retention time of the typical compound was compared with the appearance time of the standard compound.

Results and Discussion

Phenolic Compounds and Total Flavonoids

The impact of ultrasound on the total phenols and total flavonoids in sunflower oil is shown in Tables (1-4). At different points (0, 5, 10, 15), the amount of total phenols was (22.32, 24.72, 30.56, and 26.38 mg/mm). Results revealed that time (10) performed better than the other times with a value of (30.56 mg/mm), while time (0) had the lowest value at (22.32 mg/mm), with a significant difference at the probability level (P 0.05) between them. Several factors, including extraction time, temperature, UV radiation, and extraction process, can explain these results (Lopes et al (2021).
According to Kenari et al. (2020), the type of solvent and its polarity have a greater impact on the phenol content. The temperature also increased the extracted phenolic content, and heating can soften plant tissues and lower the cell wall's resistance, which results in the hydrolysis of the bonds between phenols. Finally, the effect of time had a positive impact on the phenol content because it increased the surface area between the solvent and the solid, and the prolonged processes caused solvent evaporation, which in turn decreased the extraction rate and caused the phenolic content to drop as the time was extended. This increase was in line with the findings of Lopes et al. (2021), who found that the percentage of phenolic compounds increased in the ranges of (8.68 and 26.41) and at various durations between (5 and 20 minutes).
In a study employing ultrasound on the thyme and comparing it to the conventional extraction method, Mnayer et al. (2017) discovered that the extraction of phenolic

compounds using sound waves is more than that of conventional extraction methods. They added that phenol concentration was enhanced compared to samples not subjected to the ultrasound equipment, indicating that phenols are not degraded by ultrasound-assisted extraction.

The same figure also showed the value of flavonoids for the different times (0, 5, 10, 15) were (8.62, 10.21, 16.22, 13.29) mg/ml, respectively, and the results showed that the time (10) was superior to the other times as it reached (16.22 mg/mm).), and that the lowest value was at the time (0) where it was (8.62 mg / mm), and a significant difference was noted at the probability level ($P < 0.05$) between the different times.

This rise is in line with findings from Nadeem et al. (2018), who investigated how ultrasound affected the characteristics of phenols, flavonoids, and total antioxidants in a blend of grape and carrot juice. By collapsing through the cavity in the periphery of the particles, ultrasound can enhance the release of these compounds from the cell wall. They discovered that the value of phenolic compounds and flavonoids increases with increasing time, which is contrary to what was found in our study, where the value reached a maximum at time (10) and decreased at time (15th)

Additionally, these results are consistent with those of Wang, et al. (2018), who used ultrasound to improve the extraction of flavonoids from olives at various times (30,40,50,60,70 min). At the time range of 30–50 min, the amount of flavonoids increased from 71.52 to 79.03 mg/ml, but the percentage decreased at 50–70 min. This outcome could be the result of the biologically active mass diffusing from the sample into the solvent.

Table (1-4) Ratio of total phenols and flavonoids of sunflower oil exposed to ultrasound for different times.

Time / coefficients	0 min	5 min	10 min	15 min
Total phenols in sunflower oil mg/mm	**22.32**	**24.72**	**30.56**	**26.38**
Total flavonoids in sun oil mg/mm	**8.62**	**10.21**	**16.22**	**13.29**

Measurement of the ability to inhibit free radicals DPPH Diphenylpicrylhydrazyl

The ability of the sunflower oil extract subjected to the ultrasonic device to give a hydrogen atom or kanron to suppress the free radicals formed by using DPPH, which is commonly used to estimate the activity of antioxidants, was determined.

This is shown in Table (2-4) A number of vitamin C concentrations were used for the comparison, including 500, 60, 120, 250, 30.5, 44.5, 55.4, and 73.5% ppm (500 if the rate of free radical inhibition over time was 20.6), 30.5, 44.5, 55.4, 73.5%). The percentage of free radical inhibition for time 5 was (29.8,36.2,56.9,70.5, (80.5%). Also, the percentage of free radical inhibition for the time was 10 (33.6,42.6,60.5,77.5, (88.9%).

The percentage of free radical inhibition for the time was 15 (41.2,49.8,69.7,84.5% (94.5) for each of the concentrations (30,60,120,250, (500 ppm) respectively), where the time exceeded 15 in terms of its ability to inhibit the activity of free radicals, and it is noted that with increasing time, the ability to inhibit the effectiveness of free radicals increased and the lowest percentage was at time zero. The results are consistent with what was reported by Mnayer et al. (2017) who extracted green absolute from thyme using ultrasound and sunflower oil.

It also agrees with Goldsmith et al. (2017), who used a wave device to boost by 11% the aqueous extraction of phenolic compounds with antioxidant activity from olives. They showed also that ultrasounds have impact on the plant materials' microstructure. The increase also agreed with Sousa and et al (2022), who indicated that the increase in antioxidant activity increased from 6 to 12 times, but the factors did not agree, as they concluded that time and concentration do not raise the number of antioxidants, indicating that other mechanisms are at work.

Nadeem et al (2018) discovered that increasing the sonication time increases the ability to scavenge free radicals, which is an important characteristic because it is related to consumer health and its presence can lead to tissue and cell damage and accelerate the oxidation of fats that are harmful to human health.

Table (2-4) Antioxidants (DPPH) of sunflower oil exposed to ultrasound for different times.

Time / concentration	Vitamin C	0 min	5 min	10 min	15 min
30 ppm	12.5	20.6	29.8	33.6	41.2
60 ppm	22.4	30.5	36.2	42.6	49.8
120 ppm	36.9	44.5	56.9	60.5	69.7
250 ppm	48.9	55.4	70.5	77.5	84.5
500 ppm	68.9	73.5	80.5	88.9	94.5

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Vitamins Dissolved in Sunflower oil

Tables (3-4) illustrate how ultrasound affected the vitamins dissolved in sunflower oil. The percentage of vitamin E reached (39.28, 44.13, 49.75, 46.57 mg) for times (0 ,5, 10, 15) respectively and achieved a greater concentration at time 10 (49.57 mg), , while the lowest concentration at time (0) was (39.28 mg). The percentage of vitamin A reached (2.57, 3.12, 3.80, 3.59) IU for times (0, 5, 10, 15) respectively, and the highest concentration was at time (10) which reached (3.80 IU), The lowest concentration was (2.57 IU) at time 0. This difference is significant at the level of probability (P 0.05).) between different times.

The reason for the lack of vitamins (K, D) in the extracted sunflower oil is that they might be present, but in very small concentrations in the seeds and were not fully extracted in accordance with the extraction method used in the study. Another possible explanation is the use of a column of type C18 instead of the special column LiChrosorb Si 60, which was used to detect vitamins. (Lxtaina et al., 2011) It could also be attributable to the equipment utilized, the precision of the analysis method, or the circumstances surrounding the sample preparation for the analysis process (Noshi, 2018).

Table (3-4) vitamins dissolved in sunflower oil exposed to ultrasound at different times.

Vitamins dissolved in sunflower oil	0 min	5 min	10 min	15 min
Vitamin E (mg per 100g)	39.28	44.13	49.75	46.57
Vitamin A (IU)	2.57	3.12	3.80	3.59

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