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Studying the Storage Duration Effect on Some Properties of Olive Oil Samples in Western Libya

Ahmed B Ibrahim[a], Najat A Bahroun[b*], Marwa N Al-Zuqait[c]
[a,b,c] *Department of Chemistry, Faculty of science, University of Zawia, Zawia, Libya*
**Corresponding author:*
E-mail address: n.bahroun@zu.edu.ly

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A B S T R A C T
Olive oil has high nutritional and therapeutic benefits compared to other vegetable oils due to its content of vitamins, unsaturated fatty acids and the antioxidants. This study examined the chemical properties of eight olive oil samples stored for periods ranging from 3 months to 30 years, collected from western Libya. The study assessed various quality indicators, including moisture content, acidity, peroxide number, saponification number, and reducing power, comparing them to the standards set by the International Olive Council. The results showed that the moisture content in two samples (3-year Yafran and 30-year Zawia) was within the permissible limit of 0.2%, while the rest exceeded it. Acidity levels did not surpass the 17% limit in any of the samples. Interestingly, the 30-year Zawia sample had the highest oleic acid content at 32%. Peroxide values remained below the 20 meq/kg threshold. The most significant finding was that the 30-year Zawia sample exhibited the strongest reducing power of 0.077 ± 0.7, highlighting olive oil's potential in protecting cells from chronic diseases and inhibiting the spread of cancer cells.

Keywords: Olive oil, Fatty acids, Antioxidant ,Peroxide number.

دراسة تأثير مدة التخزين على بعض خواص عينات زيت الزيتون في غرب ليبيا

أحمد بلقاسم أحمد أبراهيم[a]، نجاة الهادي عمر بحرون[b]، مروة نجيب الهادي الزقيط[c]

[a,b,c] قسم الكيمياء، كلية العلوم، جامعة الزاوية، الزاوية، ليبيا

الملخص
لزيت الزيتون فوائد غذائية وعلاجية عالية مقارنة بالزيوت النباتية الاخرى وذلك لاحتوائه على فيتامينات وأحماض دهنية غير مشبعة و مواد تعمل كمضادات للأكسدة. ثماني عينات زيت زيتون مخزنة فترات زمنية متباينة تراوحت من 3 أشهر الى 30 سنة جمعت من مناطق غرب ليبيا لدراسة خواصها الكيميائية. في هذ الدراسة تم تقدير بعض مؤشرات الجودة ومقارنتها بالقيم المرجعية التي حددها المجلس الدولي لزيت الزيتون. ومن هذه المؤشرات نسبة الرطوبة ونسبة الحموضة ورقم البيروكسيد ورقم التصبن. بالإضافة الى قياس خاصية القوة الاختزالية. بينت هذه الدراسة أن نسبة الرطوبة في عينات مدينة يفرن المخزنة لثلاث سنوات والزاوية المخزنة 30 سنة بلغت على التوالي 0.0828 % و 0.0973 % وفي كلاهما لم تتجاوز الحد المسموح به حسب مقاييس المجلس الدولي لزيت الزيتون و التي تبلغ 0.2 %. بينما باقي العينات بلغت الرطوبة نسبة أعلى من الحدود المسموح بها. نسبة الحموضة للعينات المدروسة لم تتجاوز الحد المسموح به والمحددة بقيمة 17 %. وجد في هذا البحث أن اعلى نسبة لحمض الاوليبك كانت لعينة زيت الزاوية المخزنة 30 سنة حيث بلغت نسبته 32 %. ومن النتائج المتحصل عليها لقيمة البيروكسيد يتضح انها لم تتجاوز الحد المسموح به وهو 20 ملي مكافئ لكل كيلوجرام. من اهم نتائج هذه الدراسة ان مستخلص عينة زيت زيتون الزاوية المخزن لفترة 30 سنة يمتلك أكبر قوة اختزالية و بلغت 0.077 ± 0.7 مما يعزز اهمية زيت الزيتون في حماية الخلايا من العديد من الامراض المزمنة و الحد من نشوء وانتشار الخلايا السرطانية.

الكلمات المفتاحية: زيت الزيتون ,الأحماض الدهنية ,مضادات الأكسدة ,عدد البيروكسيد.

1. Introduction

The cultivation of olive trees is widespread in the countries of the Mediterranean countries. Olive oil is one of the vegetable oils used in human food. It is the first oil known and extracted and one of the most palatable vegetable oils because of its taste, flavor and distinctive color. It is the only oil that can be consumed directly without the need to refine it [1].

Despite the development of the production of other vegetable oils in the modern era, scientific studies have proven to the world that olive oil is not comparable in terms of its benefits and high nutritional value to any other oils due to its content of vitamins and monounsaturated fatty acids as well as its high content of antioxidant agents [1]. Olive oil consists of various compounds that contribute to its unique properties and health benefits. These compounds can be divided into two parts: saponifiable compounds and non-saponifiable compounds. The saponifiable part accounts for up to 98% of triglycerides and is the most important part of the saponifiable part. The non-saponifiable portion represents about 2 % of the total weight of the oil. It consists of a complex of compounds belonging to chemical families such as aliphatic and terpene alcohols, sterols, hydrocarbons, phenolic compounds, dyes, vitamin E and some volatile compounds [2]-[6].

The saponified portion of olive oil is rich in unsaturated fatty acids, especially oleic and linoleic acids (monounsaturated), and contains a moderate amount of saturated acids such as palmitic and stearic acids and a small amount of linoleic and linolenic acids (polyunsaturated) [1], [7].

The compounds constituting the non-saponified part of olive oil are very necessary to give the distinctive taste and flavor of the oil, increase the stability of the oil for oxidizing agents, prevent undesirable reactions that occur to the oils, and also give the oil therapeutic and medicinal properties [8], thus eliminating free radicals, providing adequate protection against peroxidation, and reducing the development of atherosclerosis [9].

Olive oil varies in its fatty acid content and composition from sample to sample. Among the main factors affecting the formation of fatty acids are the geographical origin of olive oil, climate, genetic factors (diversity) and the degree of ripeness of harvested olives where the maturity of the fruits is accompanied by a change in the color of the crust from green to purple due to a gradual decrease in the content of chlorophyll and carotenoids, and an increase in the content of anthocyanins [10].

The composition of olive oil can vary depending on the variety, latitude, approved agricultural techniques, level of olive ripeness at harvest, olive oil extraction system and storage conditions. The quality and originality of olive oil can be assessed from the free acidity coefficients and peroxide indices, as well as from the composition of fatty acids, sterols and candles. These coefficients can be influenced by factors such as maturity, storage, enzymatic action, olive quality, oil production system, refining grade and purity [8], [11], [12].

Failure to follow scientific methods during olive harvesting, oil extraction, operations and conditions, transport and storage often lead to a change in the taste of the oil and lose its properties. This encouraged thinking about a scientific search for olive oil and testing samples from different areas and in different storage periods and transparent or opaque storage containers to give an idea of the correct methods to maintain the quality of this product. In this study, samples of olive oil will be analyzed to study their biochemical indicators and to find out the effect of the storage method on the chemical content of the samples by measuring several coefficients, including an antioxidant property. In this study, the biochemical indicators obtained will be compared with the values determined by the International Olive Oil Council.

2. Methods

2.1 Chemicals and reagents

Sodium Hydroxide 0.1 N, Potassium Hydroxide 0.5 N, Ethanol, Phenol Phthalin Manual 1%, Potassium Iodide were obtained from Sigma Aldrich Ltd. (Gillingham, UK), while Ice Acetic Acid, Chloroform, Sodium Thiosulfate 0.01 N, Starch Solution 1%, Hydrochloric Acid 0.5 N, Sulfuric Acid, Ammonium Molybdate, Sodium Phosphate, Ether was obtained from Fluka Ltd., (Gillingham, UK).

2.2 Sample Collection

The samples were collected in polyethylene bottles and stored at room temperature in a dark place until the time of analysis.

Table 1 below shows the important information about olive oil samples collected from local areas for the purpose of the study.

2.3 Preparation of olive oil sample extracts

Olive oil samples were extracted by dissolving them in chloroform acidified with ice acetic acid so that the oil does not separate when mixed with the reagents prepared with water.

Table 1 The period in months (m), years(y) and place of storage for oil samples

N.0	Collected area	Storage period	Stored container
1	Emsalata	2 m	Transparent Plastic Bottle (PET)
2	Gharyan	4 m	Transparent Plastic Bottle (PET)
3	Zawia	3 m	Transparent Plastic Bottle (PET)
4	Gharyan	20 y	Transparent Plastic Bottle (PET)
5	Al Jumayl	3 m	Transparent Plastic Bottle (PET)
6	Sabratha	2 m	Transparent Plastic Bottle (PET)
7	Yafran	3 y	Transparent Plastic Bottle (PET)
8	Zawia	30 y	Porcelain opaque jar

2.4 Estimation of moisture content in olive oil samples

A 5 grams of each of the olive oil samples under study were taken and put them in an aluminum crucible. These samples were dried in an oven at 105°C for 2 hours. Then placed inside the dissector to cool it apart from the moisture. Dried oil samples were weighed to find the percentage of moisture and volatiles in the oil samples under study.

The percentage of moisture and volatiles is calculated by the Equation

$$M\% = W2 - W3/W1 * 100. \quad (1)$$

Where:

M% is Moisture percentage of oil samples

W_1 is sample weight

W_2 is weight of crucible and sample before drying

W_3 is weight of crucible and sample after drying

2.5 Estimation of acidity in olive oil samples

Acidity is defined as the number of milligrams of potassium hydroxide needed to neutralize free fatty acids in one gram of oil. This test is used to determine the suitability of olive oil for human consumption [13].

A 1 gram of the oil sample was taken and a 30 ml of ethanol and ether mixture was added in a ratio of 1:1. After adding drops of phenolphthalein, the sample was titrated with a 0.1 standard potassium hydroxide solution. The acidity and mean standard deviation were calculated for each sample using the Equation

$$Acidity\% = 65.1\ (A - B)\ N\ /W, \quad (2)$$

where

W= weight of sample, A is the volume of potassium hydroxide during oil sample calibration, B is the volume of potassium hydroxide during plank calibration, and N is the concentration of potassium hydroxide.

2.6 Determination of saponification number in olive oil samples

The saponification value of fats and oils is one of the most common quality indicators. The saponification number is a measure of the average molecular weight (or chain length) of all fatty acids present as triglycerides in the sample. The smaller the average length of fatty acids, the lower the average molecular weight of triglycerides and the greater the saponification value.

The value of saponification was determined using the Okparanta [14] method by taking 0.5 grams of the oil sample and adding 15 ml of 0.5 standard alcoholic potassium hydroxide (prepared using 50% ethanol). Heat the mixture under a condenser for half an hour until the process of dissolving and saponification is complete. Allow the mixture to cool at room temperature and titrate with 0.5 N HCl in the presence of phenolphthalein until the calibration end point is reached.

The average volume of hydrochloric acid consumed and the standard deviation were calculated for each titration step using Equation:

$$SV = (v° - v)\ x\ N\ 56.1/\ m \quad (3)$$

Whereas:

$v0$ is the volume consumed during blank calibration

v is the volume consumed during calibration of oil samples

N is the hydrochloric acid concentration (Normality)

m is the sample weight in grams

2.7 Estimation of peroxide number in olive oil samples

Peroxide number is defined as the number of milliequivalents of peroxide in one kilogram of oil. This test determines the degree to which rancidity and oil taste change occur as a result of oxidation during processing or storage. The main factors that cause rancidity in addition to moisture, bacteria and enzymes are light, heat, air and some types of minerals that can be made from the storage container [14]. In this method, a known weight of olive oil sample was taken and dissolved in 2 ml of a mixture of chloroform and acetic acid and 0.5 ml of potassium iodide. This sample then left in the dark for 2 minutes, and then the solution was diluted with distilled water. The amount of liberated iodine by oxidative action of the peroxides present in the oil was determined by titration with sodium thiosulfate 0.01N in the presence of starch solution as indicator.

The average volume of 0.01N sodium thiosulfate solution consumed and the standard deviation were calculated. The peroxide value was calculated using the Equation

$$PV = 1000\,S\,N/W \quad (4)$$

Whereas:

S is volume consumed of sodium thiosulfate solution.

N is standard sodium thiosulfate solution $0.01N$

W is weight of the oil sample under study.

2.8 Determination of Antioxidant Property of Olive Oil Samples

The antioxidant capacity of olive oil is closely related to the quantity and content of the oil's phenolic compounds. This content of compounds was measured using the phosphomoribdenum method [15], [16]. The quaternary molybdenum is reduced to the pentagon, thereby oxidizing one of the components of the oil. Which is evidenced by the appearance of green color. The more intense the color, the higher the antioxidant content in the oil sample.

In this method, the samples were prepared in the form of olive oil extract with chloroform in a ratio of 2:1 and left for 48 hours. Then 0.5 ml of this extract was taken and the reagent consisting of 0.6 N sulphuric acid, 28 mM of sodium phosphate and 4 mM ammonium molybdate was added. The mixture was placed in a water bath at a temperature of $95\,°\,C$ for 90 minutes. The absorbance was then measured at a wavelength of $695\,nm$ using a spectrophotometer, and the higher the optical density, the greater the reduction power.

3. Results and Discussion

3.1 Moisture content in olive oil samples

The obtained results, as shown in Table 2 shown that most of the olive oil samples under study contain a high percentage of moisture, with the exception of the olive oil samples from the Yafran area stored for a period of 3 years and the olive oil sample from the Zawia area stored for 30 years, where the percentage of humidity was (0.082% and 0.0973%) respectively. These percentages did not exceed the value of 0.2% specified for the validity of olive oil according to the Libyan standard specifications. The high percent of humidity may spoil the oils and cause the formation of an unpleasant smell and flavor, which is called rancidity. Three different mechanisms of oil rancidity may occur: oxidation, hydrolysis, and ketolysis. Oxidative rancidity from the degradation of peroxides resulting from the oxidation of unsaturated fats. Compounds resulting from the degradation of peroxides include aldehydes, ketones, and hydrocarbons. These compounds help produce flavours and aromas associated with stinkiness and oxidative rancidity. The abnormal properties of rancid oil are a pungent smell similar to paint and an abnormal taste (rancidity) and sometimes the color of the oil does not change from normal. Humidity is one of the main factors that cause the rancidity of olive oil. There are other factors that cause the rancidity of oil, including bacteria, enzymes, light, heat, air and some types of minerals. Rancid oil forms harmful free radicals in the body, which are known to cause cellular damage causing diabetes, Alzheimer's disease, and other conditions. Spoiled (rancid) oils can also cause gastrointestinal distress and deplete the body of vitamins, especially vitamin E and B [14].

Table 2 Percentage of moisture in the studied olive oil samples

N.0	Collected area	Storage period	Moisture %
1	Emsalata	2 m	0.9191
2	Gharyan	4 m	0,6970
3	Zawia	3 m	0.7450
4	Gharyan	20 y	0.3880
5	Al Jumayl	3 m	0.360
6	Sabratha	2 m	0.8115
7	Yafran	3 y	0.0828
8	Zawia	30 y	0.0973

3.2 Acidity of olive oil samples

In this study, as shown in Figure 1 it was found that the percentage of acidity (percentage of free fatty acids) in the extract of the olive oil samples of Gharyan 4 months was almost equal to the acidity of olive oil Zawia 3 months and equal to 0.558 ± 0.10% and 0.585 ± 0.392%, respectively. The acidity of Emsalata sample of olive oil stored for a period of two months was 1.048 ± 0.261%. While the lowest acidity value was 0.493 ± 0.261% of the 20-year Gharyan oil sample. The acidity of Yafran sample extract stored for 3 years was 3.199 ± 0.211%. It was found that the highest acidity in this study founded in a sample of olive oil stored for 30 years (6.745 ± 0.732), which is

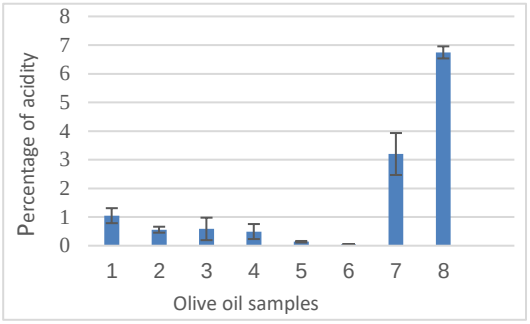

Figure 1 The percentage of acidity in the oil samples under study

expected due to the autolysis of the triglycerides forming the oil as a result of the length of the storage period. However, this result is still at the natural level set by the International Olive Oil Council [17], which shows that the acidity of oil suitable for human consumption does not exceed 17%. It is known that the acidity increases with the increase in the degree of rancidity, which increases the percentage of free fatty acids in the oil sample.

Oleic acid is one of the unsaturated fatty acids that contains one double bond. The percentage of oleic acid in the extracts of the olive oil samples under study was determined, and was varied in these samples. The percentage of oleic acid was calculated by the neutralization method Figure 2.shows the results of oleic acid obtained from the samples under study.

The highest percentage of oleic acid was 32% for the olive oil sample of the Zawia city stored for 30 years. This percentage is followed by 17%, which is for Yafran sample that stored for 3 years. These results are consistent with what researchers Al-Bachir and Sahloul [18] obtained, which found that olive oil is distinguished from other oils by the presence of a high percentage of monounsaturated fatty acids, especially oleic acid. Another previous study [19], [20], in Pakistan found that the percentage of oleic acid reached 65.2% in olive oil samples, which is almost double the percentage obtained in the current study.

3.3 Saponification number in olive oil samples

The saponification number is indicative of the length of the hydrocarbon chain of fatty acids involved in the composition of oils and fats and is inversely proportional to the partial weight of fatty acids. Therefore, the saponification number of oils containing short-chain fatty acids is higher than

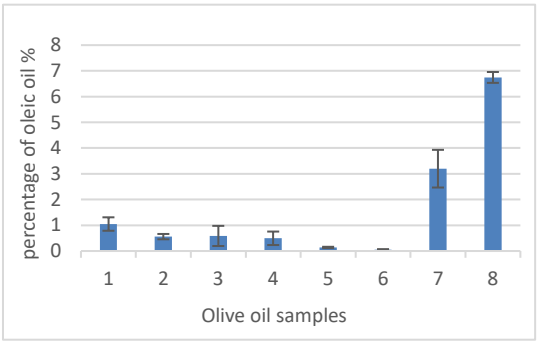

Figure 2 Percentage of oleic acid in the oil samples

the saponification number of oils containing long-chain fatty acids. Thus, it is considered to be to a certain extent indicative of the quality of fatty acids included in the composition of oils [20].

As shown in Figure 3, the highest value of the saponification number is found in the sample of olive oil of Zawia city stored for 30 years, followed by Al Jumayl sample of olive oil stored for 3 months, while the sample of Zawia city stored for 3 months recorded the lowest value among all studied samples. It was noted that by comparing the saponification numbers for Yafran samples 3 years and Gharyan sample 4 months and sample Emsalata 2 months that the composition of the oil in the three samples is similar in terms of the length of fatty acids chain despite the large difference in the age of the oil sample. These results are consistent with previous studies [8], [12], in terms of the variation in the composition of olive oil according to geographical location, the technology used for irrigation and fertilization, the level of ripeness of fruits during olive harvest, as well as the method of extraction of oil and its storage conditions. Therefore, similarity was found in the saponification number for different samples in age and region in this study.

3.4 Peroxide number in olive oil samples

The more olive oil is exposed to light and atmospheric air, the greater the oxidative content in the fatty acids that make up the oil, which increases the value of peroxide in it. Figure 4 shows the results obtained for the value of peroxide in the extract of the samples under study. The maximum value of peroxide was found in a sample of Yafran olive oil stored for 3 years, followed by a sample of olive oil stored for 30 years. These values did not exceed the limit allowed by the International Olive Oil Council [17] of 20 meq/kg. This indicates that all oil samples under study are suitable for human consumption.

3.5 Antioxidant property of olive oil samples

The antioxidant property of olive oil sample extracts was estimated using the phosphomolybdenum method. The results obtained are shown in Figure 5.

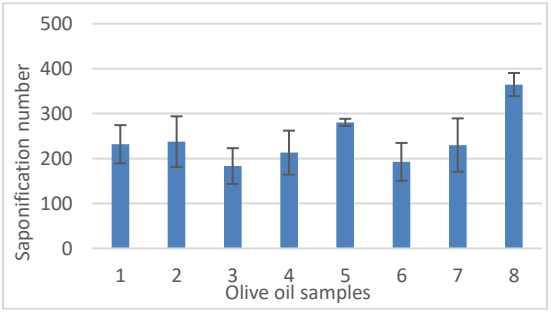

Figure 3 The saponification number in the oil samples

In this study, it was found that the extract of the 30-year-old Zawia city oil sample had the greatest

reduction power, reaching 0.7 ± 0.077, while the extract of the 3-month of Zawia was 0.294± 0.00346. The reduction strength value of the 20 years Gharyan olive oil extract was found to be 0.0426±0.02, which is the lowest reduction strength among all the samples studied.

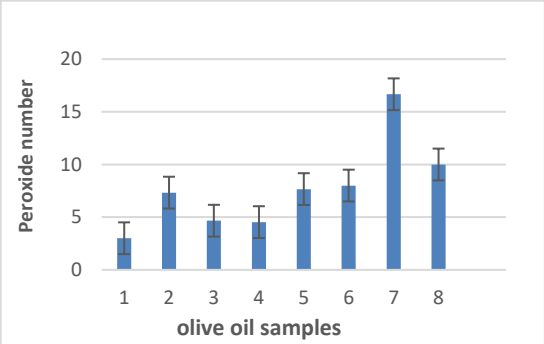

Figure 4 Peroxide number in olive oil samples

Several studies, including El Riachy [21], and Casburi [22], have proven that olive oil is rich in chemical compounds that act as antioxidants such as vitamin E and phenolic compounds such as Hydroxytyrosol and Tyrosol. These compounds are present in simple concentrations in fresh olive oil. The percentage of these important compounds increases as the period of storage of olive oil increases as a result of the decomposition of Secoiridoids [23]. This may explain the results obtained for the 30-years-old olive oil sample, which has the greatest reduction power.

4. Conclusions and recommendations

Olive oil is a very valuable oil because of the ingredients it contains. Often preferred in healthy nutrition menus. This study provides information on some quality indicators, and a comparison of them with what was determined by the International Olive Oil Council. These indicators include percentage of moisture, acidity, saponification number, peroxide number and antioxidant capacity of olive oils collected from six cities of the largest olive oil producers in Libya.

Qualitative and quantitative differences were noted between the oil samples studied, but they did not exceed the permissible limit according to the recommendations of the International Olive Oil Council. The percentages of moisture in the olive oil sample from the Yafran area which stored for a period of 3 years and the olive oil sample from the Zawia area which stored for a period of 30 years were (0.082% and 0.0973%), which is less than the internationally permissible value of 0.2%. The acidity rate did not exceed the permissible limit of 17%.

Olive oil is known to be distinguished from other oils by the presence of a high percentage of

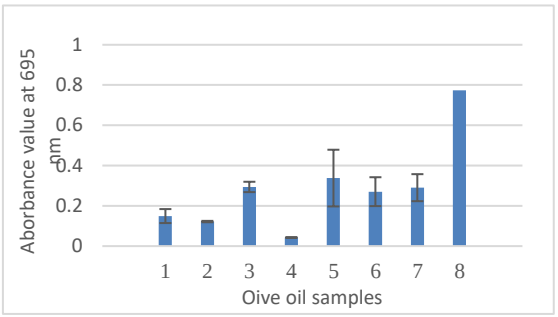

Figure 5 Reductive power of olive oil samples

monounsaturated fatty acids, including oleic acid. The percentage of oleic acid in the olive oil sample collected from the city of Zawia at the age of 30 years was 32%.

The antioxidant capacity of olive oil is closely related to the quantity and content of the oil's phenolic compounds. Phenolic compounds are used as quality markers for olive oil, and are an important property when estimating oil quality. The great importance of phenolic compounds is due to their anti-cancer, antiviral and anti-inflammatory properties [24], [25]. One of the most important results of this study is that the olive oil sample collected from the Zawia city, which reached 30 years old, has the greatest antioxidant strength, reaching 0.077 ± 0.7. The importance of antioxidants in protecting cells from many chronic diseases such as curry disease, aging, and Alzheimer's disease, as well as reducing the development and spread of cancer cells, has been scientifically proven [22].

Based on the results obtained in this research, preserving olive fruits during harvesting and not leaving them exposed to heat or pressure is very important because they are subject to undesirable chemical reactions that affect the quality of the oil. It is also recommended to keep olive oil away from heat and light so as not to activate peroxide formation reactions that affect the quality of the oil. This research also recommends the separation of biochemical compounds found in oils stored for very long periods of time that have a therapeutic effect.

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