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Effect of duration of olive storage on the quality of olive oil produced in west Libya

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ABSTRACT

Olive oil is known for its health benefits due to its richness in monounsaturated fats, antioxidants like polyphenols, and vitamin E. Regular consumption lowers risks of cardiovascular diseases, inflammation, and certain cancers, benefiting public health in Libya. Olive oil quality is influenced by factors such as the time from harvest to processing. This research aimed to study the effect of olive storage duration on the oil's chemical composition. Therefore, ten samples of olive fruits were collected from different regions of the Libyan coast and western mountain regions during October 2018 to evaluate the quality of the olive oil by conducting analyses of acidity, peroxide values and absorption coefficients, as well as estimating the content of phenolic compounds. The results showed that the acidity percentage in the Zliten Azdo oil samples which were stored for 2 months and the Nalut sample which were stored for 3 months exceeded the values specified by International Olive Oil Council (3%), while the acid number and peroxide number for all samples were within international standard limits (17) and (20) respectively. The results from this study demonstrated a range of K232 and K270 values across different olive oil samples, highlighting the influence of storage conditions on oil quality. Delaying the pressing of olives after harvesting increases free acidity, and raises the peroxide index.
This study provides guidelines to minimize the storage duration from harvesting to processing, aiming to preserve both the chemical and sensory quality of the oil throughout its shelf life.

Keywords

Olive oil, acidity, peroxide, phenolic compound, olive storage duration.

1. INTRODUCTION

Olive oil trees hold multifaceted importance in Libya, encompassing economic, cultural, culinary, nutritional, and environmental dimensions. Libya has historically been one of North Africa's leading producers of olive oil, contributing significantly to its agricultural exports. The Olive sector in Libya has many positive aspects including good indigenous varieties, hand picking, minimum use of pesticides and chemical fertilizers and modern mills [1]. The olive fruit has been traditionally used in the production of olive oil.

Olive oil is renowned for its health-promoting properties, particularly in the context of the Mediterranean diet. The role of olive oil in human health is related to its high content of monounsaturated fatty acids (MUSFA) such as oliec acid, which reduces the risk of cardiovascular disease, lowers the level of bad cholesterol (LDL) and raises the level of good cholesterol (HDL) [2]. In addition, olive oil plays a significant role in human health due to its high content of antioxidants, such as vitamin E, phenolic and polyphenol compounds, which help reduce oxidative damage in the body. The antioxidants in olive oil may have anti-cancer properties. They can reduce oxidative damage due to free radicals, which is believed to be a leading driver of cancer [3], [4]. Moreover, olive oil contributes to health as its anti-inflammatory effects are similar to those of ibuprofen. Chronic inflammation is thought to be a leading driver of many diseases, including heart disease, cancer, metabolic syndrome, diabetes, and Alzheimer's [4]. Despite being high in calories, olive oil may help with weight management. It promotes a feeling of fullness and can reduce the risk of overeating [5], [6]. Furthermore, olive oil can help improve insulin sensitivity and blood sugar levels, which is beneficial for managing and preventing type 2 diabetes [7],[8]. Some studies suggest that olive oil can help preserve bone density and may be beneficial in the prevention of osteoporosis [9],[10].
Several factors significantly influence the quality and composition of olive oil. These factors can be categorized into agricultural practices, environmental conditions, harvesting methods, time from harvest to processing, processing techniques, and storage conditions. The interval between olive harvesting and processing (milling) is crucial. Delayed processing can lead to enzymatic degradation, affecting oil quality. Shortening the time from harvest to milling helps maintain freshness and minimize oxidative damage [11]. This research aims to evaluate the impact of different storage durations of harvested olive fruits on the quality of the resulting olive oil. Specifically, the study seeks to assess how varying time intervals between harvesting and milling affect the oil's chemical composition, sensory properties, and overall quality indicators such as acidity, peroxide value, polyphenol content, and the presence of defects.

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2. MATERIALS AND METHODS

2.1 SAMPLES COLLECTION

Ten samples of olive fruits were collected from different regions of the Libyan coast and western mountain regions in October 2018. The fruits from different regions were stored for a while before processing in a dry, away from direct exposure to the sun in a special bag. The olive oil samples were collected directly from olive oil presses in dark glass bottles and transferred to the laboratory for analysis. The names of the samples and the storage duration before processing are shown in **Table 1**. All samples were analyzed to measure some chemical properties and determine polyphenol compounds.

Table 1 Sample information

No	*Sample name*	*Storage duration*
1	Alajailat	1day
2	Zliten Mager	2 days
3	Wadi Alhay	3 days
4	Daphnia	4 days
5	Zaltan	5 days
6	Qasr Alahyar	30 days
7	Qamata	40 days
8	Zliten Zdo	60 days
9	Bani Walid	75 days
10	Nalut	90 days

2.2 Determination of free fatty acids and acid number

The free fatty acids and acid number determination by titration with an alkaline solution of known standard according to method AOCS Ca 5a – 40/93 [12].

A sample of one gram of olive oil was weighed accurately and 10 ml of warm ethyl alcohol was added. This step was to dissolve the oil sample and make a homogenous mixture of oil and the aqueous reagents. Some drops of phenolphthalein were added. This solution of the sample was then titrated with 0.01 N potassium hydroxide solution. The light pink colour appears in the solution as the equivalent point and then the volume of potassium hydroxide consumed is recorded in ml. Free fatty acids in the oil samples then were calculated using the **Equation**.خطأ! لم يتم العثور على مصدر المرجع **.** The free fatty acid is expressed as g/100 g of oleic acid. The acid number is calculated from **Equation 2** as a milligram of KOH for each gram of oil sample.

$$Free\ fatty\ acid\ \% = \frac{282.47\ VN}{w} \qquad (1)$$

Where:

V: Volume in ml of potassium hydroxide

N: Concentration of potassium hydroxide (0.01N)

W: Weight of oil sample in grams

282.47: Molecular mass of oleic acid

$$Acid\ number\ [mg(KOH)/g] = \frac{56.11\ VN}{w} \qquad (2)$$

A. 2.3 Determination of oxidative stability - peroxide value (PV).

This test determines the degree to which rancidity and oil taste change occur as a result of oxidation during processing or storage. The peroxide value expresses the oil content of peroxide compounds, it is determined as in the method of AOCSCD 8-53 [13], by reacting potassium iodide in an acidic solution with the oxygen bound in the form of peroxide in the oil. The amount of liberated iodine by oxidative action of the peroxides present in the oil samples was determined by titration using a standard solution of potassium thiosulphate. Chloroform was used to dissolve the oil samples. The estimated peroxide numbers were expressed as milliequivalents of peroxide in one kilogram of oil sample.

Five grams of oil samples were dissolved in 15 ml of 60% glacial acetic acid with 40% chloroform. Then 2.5 ml of saturated potassium iodide solution was added. After stirring the solution in a circular motion for 15 minutes, 100 ml of distilled water was added carefully. The sample solution then was titrated with sodium thiosulfate 0.01 N using starch as an indicator.

The peroxide numbers were calculated using **Equation 3**. The peroxide number is expressed as mEq O_2kg^{-1} oil.

$$PV = 1000\ S\ N/W \qquad (3)$$

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.

2.4. Specific absorption coefficients (K232 and K270)

Among the various methods used to assess olive oil quality, spectrophotometric indices such as K232 and K270 play a pivotal role. These indices are part of the standards established by Regulation EC 2568/91 of the European Union, which sets the criteria for the classification and quality control of olive oil.

The presence of primary and secondary oxidation products of the oil were evaluated using UV-spectrophotometric indices (K232, K270) with official methods described in Regulation EC 2568/91

and [14], [15]. Spectrophotometric analysis involves measuring the absorption of ultraviolet (UV) light by the oil at specific wavelengths. The indices K232 and K270 refer to the absorbance values at 232 nm and 270 nm, respectively.

These wavelengths are significant because they correspond to specific compounds in olive oil that can indicate its quality and level of degradation. According to Regulation EC 2568/91, olive oils are classified into different categories, such as extra virgin, virgin, and lampante olive oil. Each category has specific limits for K232 and K270 values. For instance, extra virgin olive oil typically must have K232 and K270 values below 2.50 and 0.22, respectively. An appropriate amount of olive oil (0.25 g) was weighted and placed in a volumetric flask. The organic solvent cyclohexane is added to the final volume. This results in a specific concentration of the oil in the solvent. Quartz cuvette was filled with the solvent (used as a blank) and absorbance was measured at 232 nm and 270 nm. The absorbance of the blank should be set to zero to account for any background absorption by the solvent. The diluted olive oil sample was then transferred into a clean quartz cuvette. Then the absorbance was measured at 232 nm. Similarly, the absorbance at 270 nm was measured.

2.5. Determination of total phenol content

The determination of total phenol content in olive oil is a crucial analysis for assessing its quality and health benefits. Phenolic compounds are significant due to their antioxidant properties, which contribute to the oil's stability and health-promoting effects. The most common method for determining total phenol content in olive oil is the Folin-Ciocalteu assay, a colourimetric method that measures the antioxidant capacity of the phenolic compounds [16]. The Folin-Ciocalteu assay is based on the reduction of the Folin-Ciocalteu reagent by phenolic compounds under alkaline conditions, resulting in a blue complex that can be quantified by measuring its absorbance [17].

A 10 g of oil sample was dissolved in hexane solution (10g/50g) and the phenolic fraction was extracted in triplicate using a mixture of water and methanol (60:40). The combined extract was brought to dryness through a rotary evaporator and then suspended in 2 mL 50% methanolic solution. Then 0.5 ml of Folin-Ciocaltean reagent was added. Allow the reaction to proceed for 5 minutes. After five minutes, 2 ml of saturated sodium carbonate solution (35%) was added to neutralize the reaction. Dilute with distilled water to a final volume (10 mL). Incubate the test tubes at room temperature in the dark for 30-60 minutes to allow the color to develop fully. The absorbance of each solution was measured at 750 nm using a UV-Vis spectrophotometer spectroscopy cray 60, Penang, Malaysia [18], [19].

3 RESULTS AND DISCUSSION

The samples of olives were collected in October of 2018. It was assessed that the storage method of olives and the correspondent olive oil samples collected were the same for all the analyzed samples. Olive storage duration before technological transformation ranged from 1 day to 3 months: chemical data were thus processed dividing them into four classes of storage times: 1day, 2 – 5 days, ≥ 1 month, 2-3 months. Only 10% of olive samples were processed within 24 h, while 40% of olive samples were stored for 2-5 days. And 20% of olive samples were processed within 30-40 days. Finally, 30% of olive samples were stored between 2 - 3 months of storage.

3.1 Fatty acid composition

Free fatty acids (FFA) and acid number (AN) are both measures used to assess the quality of oils, including olive oil. FFA measures the amount of fatty acids that are not bound to glycerol in the oil. It is often expressed as a percentage of oleic acid. The acid number measures the total acidity of the oil, which includes both free fatty acids and other acidic components. Both FFA and AN are indicators of oil quality and degradation. Higher values typically indicate poorer quality and greater oxidation or hydrolysis.

Free fatty acids standard values) sets by the International Olive Council (IOC) [12] are as follows:

Extra Virgin Olive Oil: $\geqslant$ 0.8%

Virgin Olive Oil: $\geqslant$ 2.0%

Ordinary Virgin Olive Oil: $\geqslant$ 3.3%

Lampante Virgin Olive Oil: > 3.3%

While the IOC primarily uses FFA rather than the AN to set standards for olive oil quality, one can estimate the AN when having the FFA value, as both are related to the amount of free fatty acids in the oil using **Equation 4.**

$$Acid\ Number = FFA \times 1.99 \quad (4)$$

However, the International Olive Oil Council, shows that the acidity of oil suitable for human consumption does not exceed 17% [12], [20].

Low acidity refers to the exposure of olive fruits to less damage and gives high-quality oil. The fatty acid percentage of studied oiamples ranges from 0.97 to 4.48 %, as shown in **Figure 1**. The number 11 in Fig 1 and all the figures are related to the limit allowed by the International Olive Oil Council.l. The results show that the highest percent of fatty acids in the study samples was found in Zliten Zdo sample (4.48 ± 0.04) which the olives were stored for 60 days, this result is expected due to the autolysis of the triglycerides forming the oil as a result of the length of the storage period [21]. In addition, the results presented in **Figure 2** show that the percentage of free fatty acids in all olive oil samples studied was at the natural level set by the International Olive Oil Council $\leq 17\%$ [12]. 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. The oils with high levels of FFA are more susceptible to oxidative aging, they become rancid more quickly. The FFA should be removed during a refining process for chemical and sensory oil quality [22]. The unexpected result of acidity was found in samples of (3, 7, and 9) Wadi Alhay, Qamata, and BaniWalid. These samples, although stored longer, gave a lower result than the samples that were stored for a short period of time. This variation could be due to other factors effecting the oil quality such as fruit infestation level [23], [24], [25].

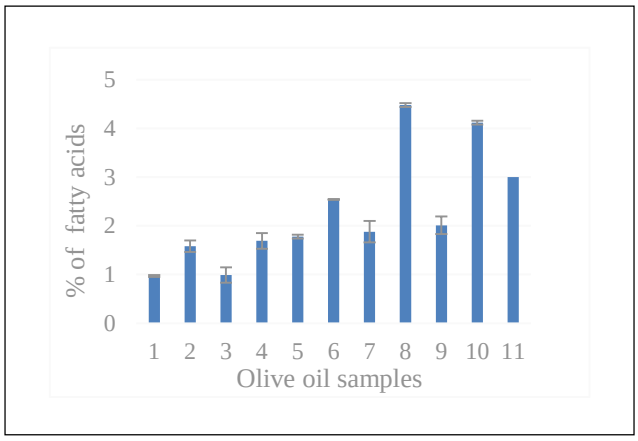

Fig 1. The percentage of fatty acids in the studied oil samples

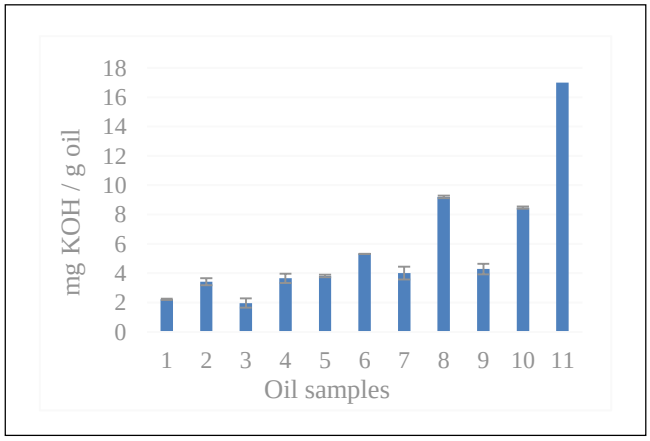

Fig 2. The acid number (acidity) of the oil sample

3.2 Peroxide value (PV)

The peroxide value (PV) is a key indicator used to assess the extent of lipid oxidation in olive oil, reflecting its freshness, quality, and suitability for consumption. 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. The presence of peroxides in olive oil is a consequence of the initial steps of lipid oxidation, during which unsaturated fatty acids react with oxygen. **Figure 3** shows the results obtained for the value of peroxide in the extract of the samples under study. The maximum value of peroxide was (17.20 ± 0.28 $mEqO_2/Kg$) found in a sample of Nalut that was stored for 3 months, followed by a sample of Daphnia stored for 4 days. which is (9.60 ± 0.00 $mEqO_2/Kg$). These values did not exceed the limit allowed by the International Olive Oil Council [12], [26] of 20 $mEqO_2/kg$.

Several factors can influence the peroxide value of olive oil, including the type of olive, and growing conditions. Different olive cultivars have varying resistance to oxidation, which affects their initial peroxide values and overall stability. Environmental factors such as temperature, humidity, and sunlight during the cultivation of olive trees can impact the oil's oxidative stability. These factors may contribute to the observed variation in the peroxide values of the samples under investigation. The findings presented in this study exhibit broader range values in comparison to the results published by Gagour et al., (2024) [27], who documented a considerably lower value (0.85–4.01 mEq O_2/kg oil).

This parameter depends on several external factors, such as light and temperature, high oxygen availability, the presence of prooxidants such as chlorophylls, metal ions (Fe^{3+} and Cu^{2+}), and heavy metals, as well as other factors related to cultivation and harvesting conditions [28], [29].

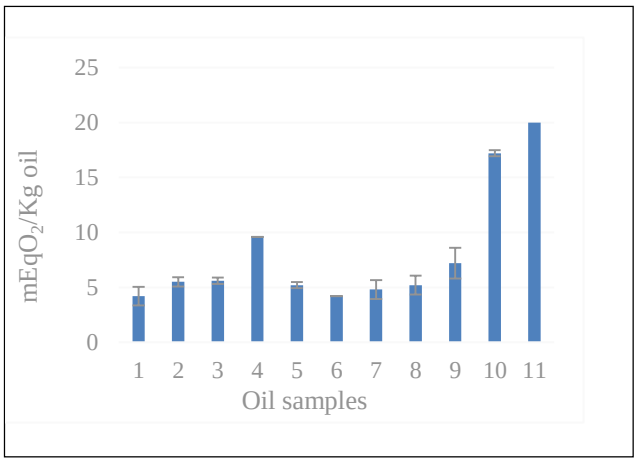

Fig 3. The peroxide number of the oil sample

3.3 Antioxidant properties of olive oil samples

The antioxidant property of olive oil sample extracts was estimated using the reaction with the Folin-Ciocalteu reagent. This method is a reliable and widely used method for this purpose, offering a straightforward approach to quantifying phenolic compounds. Phenolic compounds are used as

quality markers for olive oil and are an important property when estimating oil quality.

The primary antioxidants in olive oil are polyphenols and tocopherols, with polyphenols being the most significant contributors to its antioxidant capacity. Polyphenols are the most significant contributors to its antioxidant capacity [30]. **Figure 4** shows the polyphenolic content of oil samples under investigation. The results showed that the extract of the Daphnia (4) oil sample which was stored for 4 days before proceeding had the greatest reduction power, reaching 322.1 ppm. The lowest value of phenolic compounds (26.1ppm) was detected in the sample of Qasr Alahyar, which was stored for a month. From Figure 4, it is clear that olive fruits pressed a week after harvesting showed the highest content of phenolic compounds compared to fruits stored for more than a month. The phenolic content of olive oil processed a week after harvesting is expected to be significantly higher than that of olive oil stored for a month or more. Therefore, this study found that freshly harvested olives contain higher levels of phenolic compounds, which contribute to the oil's antioxidant properties and overall quality. As the storage period increases, the phenolic content tends to decrease due to oxidation and other degradation processes [31]. Therefore, olive oil processed shortly after harvesting, typically within a week, will have a richer phenolic profile compared to oil produced from olives that have been stored for an extended period. This higher phenolic content enhances the health benefits, flavour, and stability of the oil [32].

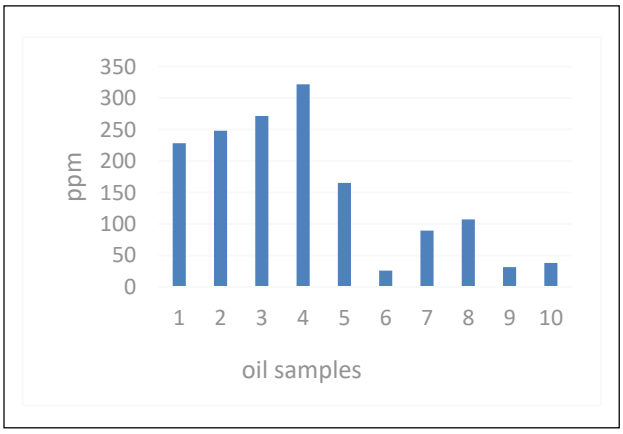

Fig 4. The polyphenolic content of oil samples

3.4 UV–Light Absorption (K232 and K270)

Ultraviolet (UV) light absorption measurements, specifically at wavelengths of 232 nm (K232) and 270 nm (K270), are important analytical techniques used to assess the quality and purity of olive oil. These measurements provide information about the presence of certain compounds that can indicate the level of oxidation and the presence of adulterants in the oil. The wavelength 232 nm is sensitive to conjugated dienes, which are formed during the initial stages of oxidation and are indicative of primary oxidation products in the oil. A high K232 value suggests that the olive oil has undergone oxidation or has been exposed to conditions that promote oxidation, such as exposure to air, light, or heat. This can occur during improper storage or processing [33]. While. The 270 nm wavelength is sensitive to conjugated trienes and secondary oxidation products such as aldehydes and ketones. A high K270 value indicates more advanced stages of oxidation and the presence of secondary oxidation products, which can affect the flavour, aroma, and nutritional quality of the olive oil [34]. According to the International Olive Council (IOC) standards, the K232 value for extra virgin olive oil should not exceed 2.50, and the K270 value should not exceed 0.22. While, for virgin olive oil, the K232 value should not exceed 2.60, and the K270 value should not exceed 0.25 [35]. Lampante oil, is generally higher than 2.60 and 0.25 for K232 and K270 respectively, which is a sign of poor quality [35], [36], [37].

As exposed in **Figure 5**, the absorption values at 232 nm ranged from 1.71nm to 2.70 nm. The oil sample of Zaltan which stored for 5 days represented the lowest absorption value for K232, but the highest value (0.58) for K270 as indicated in **Figure 6**. The olive oil may be relatively fresh (lower K232) but could have been exposed to conditions that led to the formation of more oxidation products, reflected in a higher K270. The oil sample of Nalut which is stored for 3 months shows the highest value (2.71) of K232 which is in the range of the standard value (> 2.60) for Lampante olive oil specified by the International Council of Olive Oil.

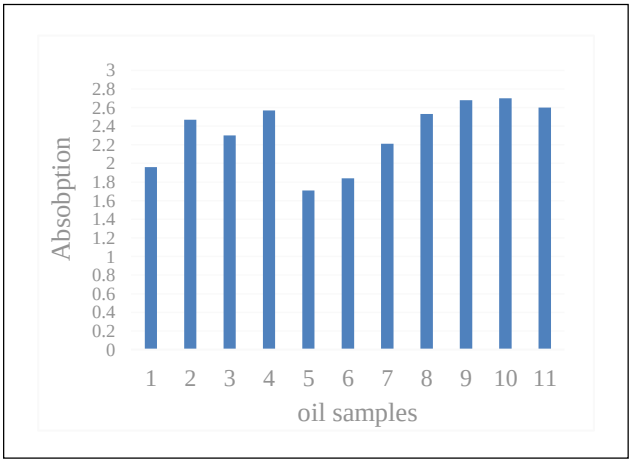

Fig 5. The absorption values at 232 nm for oil samples

The results of the K232 and K270 in this study could potentially be illustrated as different components within the oil can interact differently with oxidation, leading to variations in absorbance values that do not follow a direct correlation [36], [37], [38].

4 CONCLUSION

Olive oil is widely used throughout the world and is considered a very healthy type of oil. The quality of olive oil is largely dependent on the quality of the olives that are used for pressing. The basic quality characteristics of olives and olive oil are influenced by oil sample factors such as the time from harvesting to processing. Optimizing this factor is essential for producing high-quality olive oils that meet consumer expectations.

This research aimed to study the effect of olive storage duration on the oil's chemical composition using 10 olive samples collected from the Libyan coast and western mountain regions in October 2018. Free acidity, peroxide value and spectroscopic indices (K232 and K268) were carried out. The results showed the effect of storing olive fruits on the quality of the oil, as the percentage of free acidity, peroxide, and absorption increased with the increase in the storage of the fruits, while the content of phenolic compounds decreased significantly with increasing the duration of storage of the fruits, and this will affect the quality and stability of the oil. To produce high-quality olive oil, this study recommends pressing the olives within a week of harvesting.

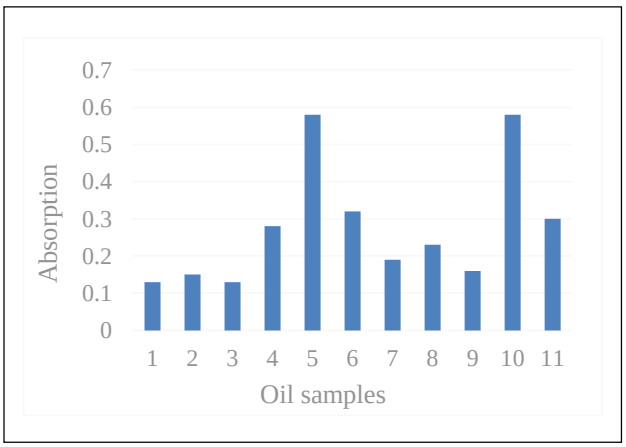

Fig 6. The absorption at 270 nm for oil samples

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