



Quality Assessment and Biological Activity Evaluation of Different Brands of Olive Oil

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IEMAE and AEAMA designed the study, performed the literature search and statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IEMAE, NMES and MI carried out the experimental work. All authors read and approved the final manuscript.

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ABSTRACT

Background: The quality of medicinal/food products is directly related to the consumer's safety. Virgin Olive Oil (VOO) is a widely used oil in Saudi Arabia for cooking, frying, and salad dressing. It is also an ingredient of many pharmaceutical products. Therefore, its regular quality assessment is essential for the consumer's safety.

Objective: To assess standard quality parameters of the marketed VOO brands in the Rafha City of Saudi Arabia and to perform their antioxidant activity evaluation.

Methodology: The different brands of VOO sold in the Rafha City of Saudi Arabia were collected from two supermarkets and one local shop. The quality of the different brands of the VOO was assessed for their physical appearance, solubility, relative density, refractive index, absorbance, acid value, and peroxide value. The standard procedures provided in the British Pharmacopoeia

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(BP) were used to assess these parameters. The antioxidant activity of the oils was performed by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method.

Results: The VOO sample test results revealed no significant change in the studied parameter's standard values. The VOO samples presented compliance with the specification of VOO provided in the BP. The VOOs were also free of Sesame oil, which is one of the possible adulterants of the VOO. The antioxidant activity data of the VOO samples (IC_{50} = 107 to 110 mg oil) also matched with antioxidant activity data of the reference VOO (IC_{50} = 105 mg oil). The antimicrobial activity of the VOO samples and the reference VOO was also comparable.

Conclusion: The different brands of the marketed VOO comply with the specification of VOO provided in the BP. They were suitable for their intended use/application.

Keywords: Virgin olive oil; quality; safety; antioxidant activity; DPPH; antimicrobial activity.

1. INTRODUCTION

Virgin Olive Oil (VOO), a fixed fatty oil, is obtained from the ripe drupes of *Olea europaea* L. (Family: Oleaceae), which is cultivated in almost all parts of the world. VOO is popular for its organoleptic properties and health benefits [1]. It is a widely used oil in Saudi Arabia for cooking, frying, and salad dressing. VOO is also an ingredient of many pharmaceutical products. It further possesses numerous beneficial properties such as anti-infective activity, antioxidant activity, anticancer activity, antidiabetic activity, antihypertensive activity, antiplatelet activity, and anti-inflammatory activity [1-10]. All these beneficial effects of the VOO are due to the chemical composition of this oil, for example, the phenolic compounds present in the VOO [1-3].

VOO is a non-prescription oil and can be purchased from ordinary shops, online markets, along with the pharmacy. However, an ordinary person working in a shop may not maintain the quality of VOO as per the specified standards, for example, British Pharmacopeia (BP) and the United States Pharmacopeia (USP). The inappropriate storage condition of this oil may spoil it and make it unsuitable for consumption. Exposure to sunlight, oxygen, and higher temperatures (> 25-30°C) cause the degradation of chemical compounds (pigments, Vitamin-E, and phenolic compounds) of VOO. The packaging materials made up of polypropylene and polyethylene are not recommended because they have increased oxygen permeability and can lead to oxidative degradation of the oil. The shelf life (9-18 months) is also a critical VOO quality parameter, which also depends on the storage of the VOO [11-14]. The low-quality VOO may become less bioactive (less anti-oxidative) [12], may pose safety issues with the consumer (due to the generation of unwanted products in the oil itself), and may also lead to undesirable

consequences (adverse effects) [13,14]. Therefore, continuous monitoring of the quality of VOO is essential for the consumer's safety. The quality of the VOO oil may be assessed by monitoring its physical properties (appearance, solubility, relative density, and refractive index, etc.) and chemical properties (acid value, peroxide value, etc.) in comparison to the standard values mentioned in the USP and BP [15,16]. Based on the above facts, it was decided to perform the quality assessment and a comparative antioxidant activity evaluation of the different VOO brands marketed in the Rafha City of Saudi Arabia, believing that the outcomes of this study will be beneficial to ensure the safety of the consumers.

2. MATERIALS AND METHODS

2.1 General

The different VOO brands in the Rafha City of Saudi Arabia were collected from two supermarkets and one local shop. These were designated as Brand A, Brand B, Brand C, Brand D, and Brand E. The BP grade reference VOO and DPPH were procured from Aldrich, USA. During the study, all VOO samples and the reference VOO were kept at the same place and temperature (about 25°C). The analytical grade solvents/reagents were used for the analysis of the oils.

2.2 Physical Examination

It was performed by the naked eye, and the observations were compared with the BP's information.

2.3 Solubility Determination at Room Temperature

VOO (0.5 mL) was mixed with 96% ethanol (5 mL) and light petroleum ether (5 mL), in separate

test tubes. The test tubes were shaken, and the observation was made after five minutes. The observations were compared with the information provided in the BP.

2.4 Determination of the Relative Density

The density bottle (5 mL) was cleaned with water, followed by absolute ethanol, and dried. The bottle's weight was noted down, and it was filled up to the mark with distilled water. The water-filled bottle was weighed, and the weight of the water was calculated. The density of the water was calculated. In the same manner, the density of the VOO samples was also determined. The oil's relative density was calculated by dividing the sample oil density by the density of distilled water.

2.5 Determination of the Absorbance

VOO sample absorbance was determined by a UV-Visible spectrophotometer (APEL, PD-303UV, Japan) at 270 nm and 232 nm. A 1% solution of VOO samples was prepared in cyclohexane. The sample absorbance was measured at 270 nm (A_{270}) and 232 nm (A_{232}). The ratio of the A_{270}/A_{232} was also calculated.

2.6 Determination of the Acid Value

VOO (10 g) was mixed with 50 mL mixture (1:1) of the ethanol (95%) and ether. The mixture was shaken to make a solution. Phenolphthalein (1 ml) was added to the solution. The solution was titrated with 0.1M KOH solution to get the endpoint (pink color). The acid value was calculated as follows.

$$\text{Acid value} = (56.1 \times \text{Volume of KOH} \times \text{Molarity of KOH solution}) / (\text{Weight of VOO in g})$$

2.7 Determination of the Peroxide Value

VOO (5 g) was dissolved in a 30 mL mixture (3:2) of glacial acetic acid and chloroform. Saturated potassium iodide solution (0.5 mL) was added to the solution, and it was standing for 1 minute with occasional shaking. Water (30 mL) was added to the mixture, and the resultant mixture was titrated with sodium thiosulphate (0.1M) till the yellow color almost disappears. Starch solution (0.5) mL was added, and the titration was continued to get a blue color. A blank titration without a sample was also performed. The peroxide value was calculated as follows.

$$\text{Peroxide value} = ((0.1 \times (\text{mL of the test-mL of the blank})) / \text{Weight of oil in g}) \times 100$$

2.8 Determination of the Sesame Oil (Adulterant)

VOO (10 mL) was taken in a ground-glass-stoppered cylinder. A mixture of 0.5 mL of a 0.35% (v/v) solution of furfural in acetic anhydride and 4.5 mL of acetic anhydride was added to the flask. The contents were shaken vigorously. The solution was filtered through filter paper impregnated with acetic anhydride. Sulfuric acid (0.2 mL) was added to the filtrate. No bluish-green color developed. This showed the absence of Sesame oil in the VOO.

2.9 Determination of the Refractive Index (RI)

It was determined by the KRUSS refractometer (DR6000-T, Germany). In short, the instrument was switched on and left for 5-10 minutes. It was calibrated with water. Two to three drops of distilled water were poured on the specified surface. The RI of water (RI = 1.3325 at 25°) was read from the display unit after 2 minutes. In the same manner, the RI of the VOO samples was also determined.

The quality assessment data (appearance, solubility, relative density, absorbance, acid value, peroxide value, and refractive index) of the VOO are provided in Table 1.

2.10 Determination of the Antioxidant Activity

The DPPH (2, 2-Diphenyl-1-picrylhydrazyl) method was employed to assess the VOO sample's antioxidant activity [17,18]. The solutions of the DPPH (0.1 mM) and VOO (different concentrations in mg of oil/mL) in ethanol and diethyl ether mixture (4:1) were prepared. The final solution's absorbance was read at 517 nm (UV-Visible spectrophotometer, APEL, PD-303UV, Japan). The results are expressed as the half-maximal inhibitory concentration (IC_{50}) [18,19]. The results are provided in Table 2 and graphically presented in Fig. 3.

2.11 Determination of the Total Phenolic Contents

VOO (2.5 g) was mixed with n-hexane (10 mL). The mixture was extracted with a mixture of

methanol and water (8:2, 5 mL). The extracted mass was centrifuged for 5 minutes (5000 rpm). The Folin-Ciocalteu reagent (1 mL), Na₂CO₃ solution (1 mL, 7.5%), and deionized water (7 mL) were added. The mixture was homogenized, kept overnight, and analyzed spectrophotometrically at 765 nm. A calibration curve (absorbance vs concentration) for caffeic acid (0.04 to 0.18 mg/mL) was prepared. The concentration of the caffeic acid (mg/kg of the oil) was calculated from the curve for each sample of VOO and the reference VOO [20].

2.12 Antimicrobial Activity Evaluation

It was performed by following the method mentioned in our previous publication [19]. Several dilutions of the VOO (5%, 10%, 15%, 20%, 25%, 30%) were made in the sterile dimethylsulfoxide (DMSO). Likewise, several dilutions of fluconazole (5 to 25 µg/ml) and ofloxacin (5 to 25 µg/ml) were also made in DMSO. The data of the antimicrobial activity are mentioned in Table 3.

2.13 Statistical Analysis

The SPSS software (version 20) was used for the statistical analysis. The *p*-value < 0.5 represents the statistically significant result.

3. RESULTS AND DISCUSSION

Many Olive Oil varieties are present in the market, for example, VOO, Extra VOO, and Refined Olive Oil. These oils differ with respect to their manufacturing processes. A direct mechanical procedure is used to obtain VOO from the ripe fruits of *O. europaea*. This process does not use any solvent for the extraction and keeps all the critical properties (phenolic components and vitamin E) of the VOO intact. Therefore, this VOO is suitable for human consumption. The Extra VOO is more expensive than VOO because it does not have taste defects like VOO and have a lower acid value than VOO [21]. VOO is one of the widely used oils in Saudi Arabia. Continuous monitoring of its quality is essential for the consumer's safety. Accordingly, the authors carried out the titled research work. The results of the studied quality parameters are provided in Table 1, whereas Fig. 1 and Fig. 2 provide the graphical representation of the results of Table 1.

3.1 Appearance and Solubility

Table 1 showed that the physical examination and the solubility aspects of the VOO complied with the BP.

3.2 Peroxide Value

The peroxide value is related to the chemical constituents produced after oxygen reacts with the oil, especially during the storage condition. It measures the presence of the active oxygen species in the oil, which leads to the rancidity of the oil. As the peroxide value increases (generally > 10), the oil's stability and its shelf-life decrease. Storing the oil at a higher temperature, exposure to sunlight and oxygen increases the oil's peroxide value [22]. The results have shown that the sample's peroxide value was in the range of 7.57 to 7.98 meq O₂/Kg compared to the reference (6.6 meq O₂/Kg). The samples and reference's peroxide values were less than the standard value (maximum 20 meq O₂/Kg) mentioned in the BP [16].

3.3 Acid Value

The decomposition/degradation of an oil causes the generation of fatty acids in the oil. The acid value is one of the measures to assess the oil's rancidity due to higher levels of the fatty acids [22,23]. The samples' acid values were observed in the range of 0.79% to 0.89% compared to the reference (0.76%). The samples' acid values and reference were less than the standard value (maximum 2%) mentioned in the BP [16]. These observations suggest that the peroxide/acid values of the samples comply with the standard values.

3.4 Relative Density, Refractive Index, and Absorbance

The relative density, refractive index, and A₂₇₀ are the critical parameters to measure the purity and identity of liquids/oils. A higher acid value and peroxide value of an oil is an indicator of the generation of impurities in the oil. The presence of impurities in the oil increases its relative density [24], refractive index [25] and also affects its absorbance at 270 nm (A₂₇₀) [26] because these impurities increase the atomic numbers of the constituent atoms in the oil. The results provided in the Table 1 revealed that the values of the relative density, refractive index, and A₂₇₀ of the sample oils and the reference oil complied with their standard values.

Table 1. The quality assessment data of VOO samples

Parameter	Reference	Brand A	Brand B	Brand C	Brand D	Brand E	Comment (BP values)
Appearance	Clear, transparent, yellow liquid	Clear, transparent, greenish-yellow liquid	Clear, transparent, greenish-yellow liquid	Clear, transparent, greenish-yellow liquid	Clear, transparent, greenish-yellow liquid	Clear, transparent, greenish-yellow liquid	Compliance
Solubility (25°C)	Insoluble in ethanol; miscible with ether	Insoluble in ethanol; miscible with ether	Insoluble in ethanol; miscible with ether	Insoluble in ethanol; miscible with ether	Insoluble in ethanol; miscible with ether	Insoluble in ethanol; miscible with ether	Compliance
Relative Density (25°C)	0.917±0.11	0.916±0.34	0.916±0.61	0.916±0.26	0.916±0.20	0.916±0.17	About 0.913
Absorbance (A ₂₇₀)	0.15±0.23	0.14±0.12	0.17±0.16	0.17±0.33	0.17±0.41	0.16±0.18	Maximum 0.20
Absorbance (A ₂₃₂)	2.1±0.22	1.89±0.26	2.33±0.31	2.28±0.18	2.32±0.15	2.11±0.40	---
ΔA (A ₂₃₂ /A ₂₇₀)	14±0.22	13.50±0.45	13.70±0.16	13.41±0.25	13.64±0.21	13.18±0.36	> 8
Acid value (%)	0.76±0.34	0.79±0.19	0.83±0.10	0.89±0.23	0.81±0.14	0.83±0.16	Maximum 2.0
Peroxide value (meq O ₂ /Kg)	6.6±0.11	7.89±0.15	7.98±0.18	7.84±0.18	7.62±0.13	7.57±0.12	Maximum 20.0
Refractive Index (25°C)	1.4700±0.42	1.4669±0.32	1.4671±0.20	1.4667±0.39	1.4672±0.31	1.4676±0.15	1.4667-1.4705

p < 0.05; SD = Standard deviation; N = 5

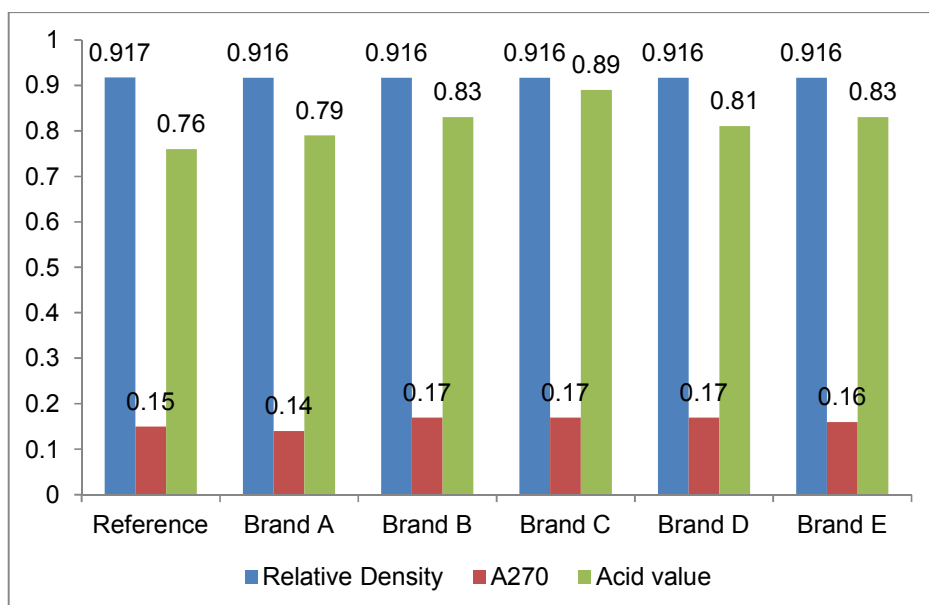


Fig. 1. Relative density, A₂₇₀, and acid value of the VOO samples

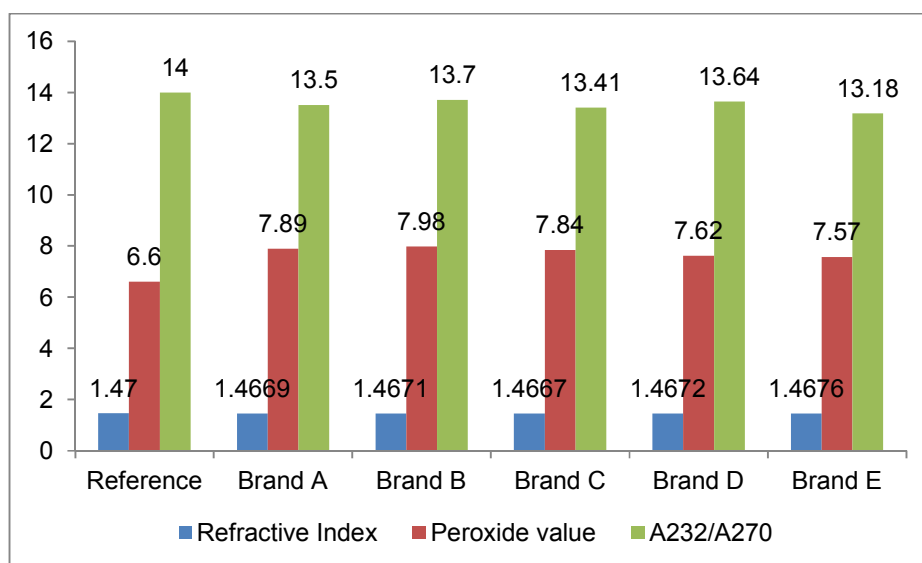


Fig. 2. Refractive index, peroxide value, and A₂₃₂/A₂₇₀ of the VOO samples

Table 2. Antioxidant activity and total phenolic contents of VOO samples

VOO	Antioxidant activity (IC ₅₀ (mg oil) ±SD)	Total phenolic content (mg/kg of the oil)
Reference	105±0.43	710±3.0
Brand A	108±0.22	713±4.0
Brand B	110±0.40	699±5.0
Brand C	107±0.50	703±7.0
Brand D	109±0.14	708±2.0
Brand E	107±0.33	712±5.0

p < 0.05; SD = Standard deviation; N = 5

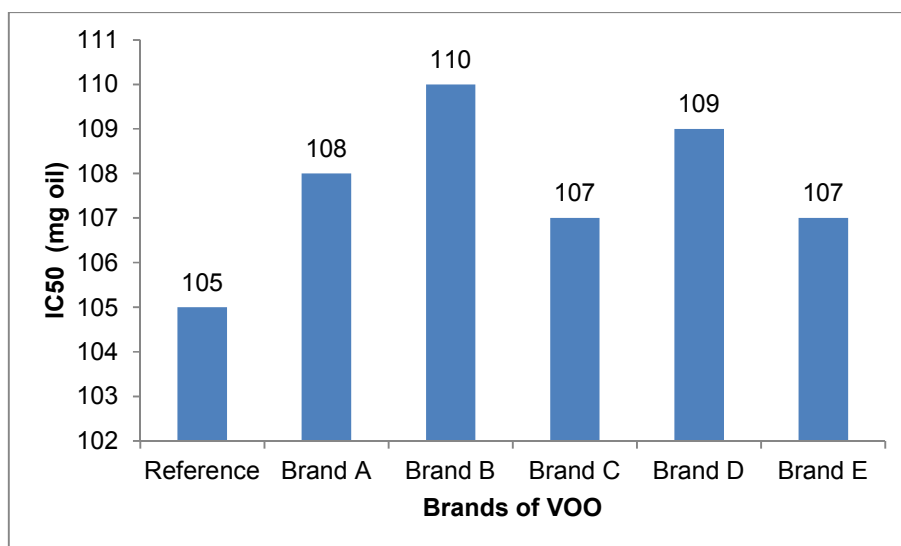


Fig. 3. Antioxidant activity (IC₅₀, mg oil) of the VOO samples

3.5 Adulteration

The VOO can be adulterated with Sesame oil because both oils share similar physical characteristics [16]. Accordingly, the BP also provides a test to ensure the absence of Sesame oil in VOO samples. The sample of VOO and the reference did not show the presence of Sesame oil. This means the tested VOO and reference were not adulterated with Sesame oil.

3.6 Antioxidant activity and the Total Phenolic Contents

The VOO is a good antioxidant due to its phenolic components and vitamin E [21]. However, inappropriate storage may cause oxidation of the phenolic components of VOO and decrease its antioxidant properties. Therefore, the antioxidant activity of the VOO

samples was evaluated. The antioxidant activity data are provided in Table 2, whereas Fig. 3 provides the graphical representation of the antioxidant activity data of Table 2. The data suggest that the samples of the VOO and reference had similar antioxidant properties. This indicates that the phenolic components and vitamin E of the VOO were not degraded. This observation is also supported by the total phenolic contents data (Table 2), wherein the total phenolic contents for VOO samples ranged from 699-713 mg of caffeic acid/kg of the VOO oil compared to the reference VOO (710 mg of caffeic acid/kg of the reference VOO).

3.7 Antimicrobial Activity

The data of Table 3 reveal that the different brands of VOO and the reference VOO had similar MIC values. However, a slight variation in the zone of inhibition was observed.

Table 3. Antimicrobial activity data of different bands of VOO

Samples	MIC (Zone of inhibition)		
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
Brand A	5% (8±0.32)	5% (7±0.30)	5% (7±0.33)
Brand B	5% (9±0.41)	5% (6±0.31)	5% (7±0.20)
Brand C	5% (9±0.50)	5% (8±0.10)	5% (7±0.20)
Brand D	5% (10±0.11)	5% (6±0.47)	5% (7±0.20)
Brand E	5% (7±0.18)	5% (7±0.42)	5% (7±0.20)
Reference	5% (8±0.28)	5% (7±0.16)	5% (7±0.20)
Ofloxacin	20 (24±0.16)	20 (28±0.25)	-
Fluconazole	-	-	15 (22±0.33)

$p < 0.05$; $N = 5$

This observation also indicates that the chemical constituents of different brands of the VOOs and the reference VOO are quantitatively identical. This means that the chemical constituents of the sample VOOs have not changed during their storage. This observation also supports our data related to the physicochemical analysis of the VOOs.

4. CONCLUSION

This study encompasses assessing the quality parameters (physical appearance, solubility, density, refractive index, absorbance, acid value, and peroxide value) of VOO samples and their antioxidant activity evaluation. No significant change in the standard values of the studied VOO samples was observed. These tested samples of VOO complied with the specification of VOO provided in the British Pharmacopeia (BP). The samples of VOO passed the quality tests provided in the BP. The antioxidant activity data (IC₅₀ = 107 to 110 mg oil) also matched the antioxidant activity data of the reference VOO (IC₅₀ = 105 mg oil). The antimicrobial activity of the VOO samples and the reference VOO was also comparable. This suggests that the medicinal value of the VOO samples was intact during their storage. Accordingly, the tested samples qualify for their intended use/purpose. A regular quality assessment of the marketed VOO is also recommended on a regular basis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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