JOURNAL OF PHARMACEUTICAL ANALYSIS

STUDY OF EXTRA VIRGIN OLIVE OILS: EFFECTS OF THE FORMATION OF PYROPHEOPHYTIN A AND ITS RELATIONSHIP WITH THE QUALITY LOSSES OF THESE OILS

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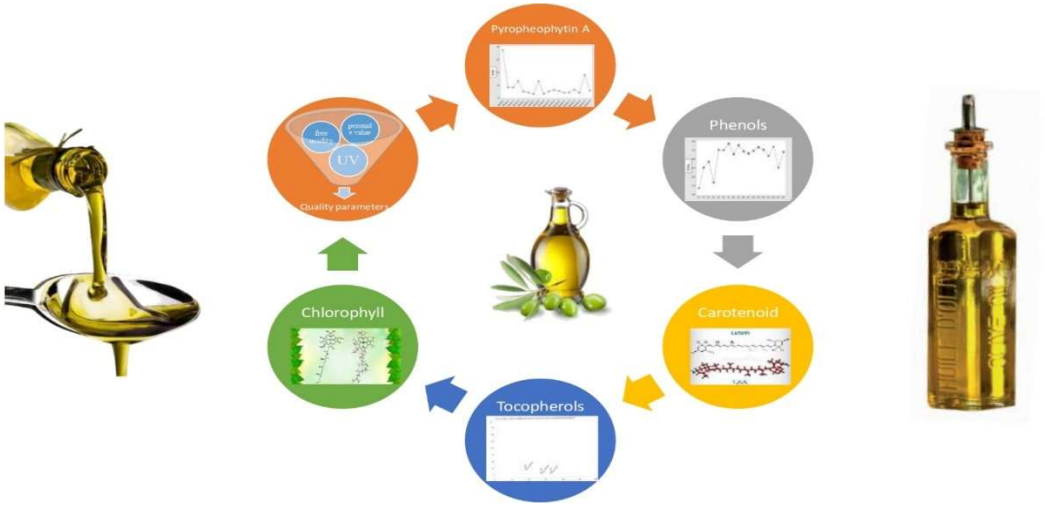
Abstract
Pyropheophytin A (PPP), generated at high levels through heat treatment, can significantly impact the quality of virgin oil. To address this, we devised a novel chromatographic method employing HPLC with UV-Visible detection to quantify PPP levels. We conducted an analysis on eighteen samples of extra virgin olive oils sourced from various regions in Morocco, assessing parameters such as free acidity, peroxide value, specific UV extinction, total phenols content, chlorophyll and carotenoid contents, tocopherols, and PPP percentage. Our results were revealing, indicating that certain samples surpassed the maximum acceptable PPP level of 17%, thereby posing a notable threat to oil quality.
Keywords: Extra virgin olive oils; pyropheophytin A; total phenols; chlorophyll; carotenoid; tocopherols.

Graphic abstract



1. Introduction

Olive oil is a vegetable oil that has been produced in Mediterranean countries for centuries. There are different types of olive oils, such as extra virgin, virgin, and lampante, which are categorized based on their physico-chemical composition and sensory characteristics. Due to the high risk of fraud in the olive oil industry, legal regulations have been put in place to protect consumers [1]. These regulations set maximum limits for various chemical and physico-chemical

parameters, including free acidity, peroxide index, specific extinction coefficients, fatty acid composition, and sterol composition. Sensory evaluation is also considered, which involves assessing for the presence of any organoleptic defects and the presence of positive fruity sensations [2,3].

The literature describes several analysis techniques that can be used to detect and verify potential frauds in the production and marketing of olive oil. This is done to ensure consumer confidence and minimize the risk of unfair competition among producers. The American Oil Chemists' Society has established official methods and recommended practices for evaluating olive oil quality [4]. However, some of these methods are expensive, non-ecological, and require standardized procedures to ensure accuracy. Extra virgin olive oils have a wide range of characteristics that can be influenced by factors such as agronomy/environment, technology, and storage conditions. As a result, maximizing the quality of extra virgin olive oil can be costly. High-quality, niche extra virgin olive oils can command much higher prices than oils produced in large-scale industrial facilities due to factors such as manual labor requirements, limited availability of raw materials, and other requirements. Although the retail price of extra virgin olive oil may vary depending on market demand and other factors, there will always be differences in terms of chemical composition, quality, and price between niche and industrial products, even within the same market and time period.

Light, oxygen, and heat can significantly impact the chemical composition and sensory properties of oil, affecting its shelf life stability [5]. One particular group of compounds that can be affected by these factors are the chlorophyll pigments, specifically pheophytin (PP) and pyropheophytin A (PPP) [6] (Figure 1). The composition of these pigments is largely dependent on the genetic basis and maturity of the fruit, with chlorophyll content being about three times higher than that of chlorophyll b [7,8]. During the extraction process, plant tissues are broken down and most of the chlorophyll pigments are converted into pheophytins, which can then be decarbomethoxylated into pyropheophytins [9]. In freshly made extra virgin olive oil, levels of pyropheophytin are either non-existent or very low [7]. Over time, the level of pyropheophytin increases depending on storage conditions and whether the oil has undergone heat treatments during the refining process, such as deodorization [5,7]. As such, this conversion serves as a sensitive indicator of stress during oil production and storage [6].

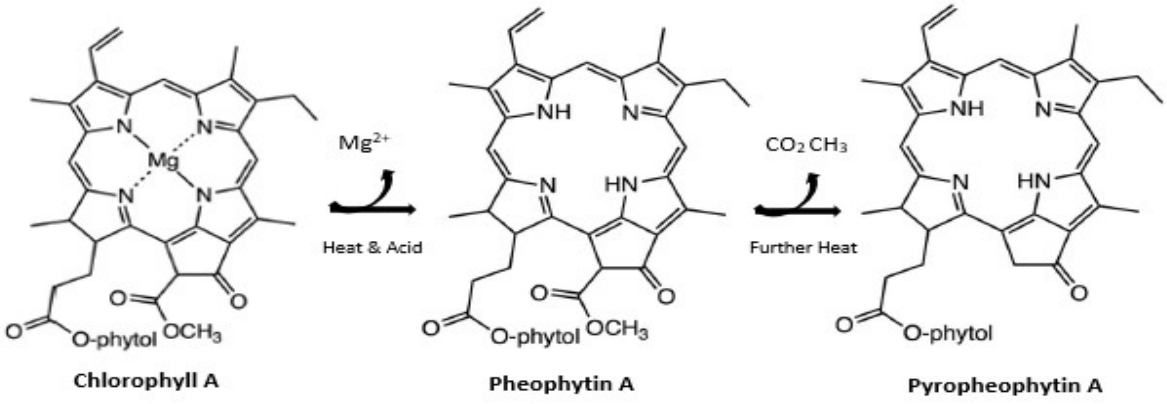


Figure 1. Chemical structures of Chrolophyll A, Pheophytin A and Pyropheophytin A.

The objective of this article is to study the quality and authenticity of eighteen samples of extra virgin olive oils collected during the olive growing campaign (2020/2021) according to

geographical origin (Fez/Meknes, Tangier/Tetouan/Al Hoceima, L'Oriental, Beni-Mellal/Khenifra and Marrakech/Safi). The chemical analyzes carried out on these samples of virgin olive oils, namely: physicochemical, bioactive parameters (free acidity, peroxide index, specific UV extinction, total content of phenols, chlorophyll, content of carotenoids, tocopherols and the percentage of pyropheophytin A) and its impact on the quality of these oils.

2. Methods and materials

2.1. Sampling

The study focused on eighteen bottles of extra virgin olive oil from four varieties grown in the five regions of Morocco produced during the 2020-2021 agricultural season (Figure 2).

2.2. Determination of pyropheophytin A content

The methodology utilized in this study is based on the International Standard Organization's technique [10], which is commonly used to determine pheophytin A and pyropheophytin A. To summarize the procedure, a small beaker is used to weigh approximately 300 mg of the test sample, which is then dissolved in 1 ml of n-heptane or n-hexane and transferred onto a silica cartridge column. The beaker is rinsed twice with 1 ml of petroleum ether and the washes are also poured onto the column. Once the solvent reaches the top of the column, nonpolar substances are eluted twice using a 5 ml petroleum ether/ethyl ether solvent mixture. The pheophytin fraction is then eluted twice with 5 ml of acetone and collected in a conical flask, shielded from light. The solvent is evaporated using a rotary evaporator at a maximum temperature of 20°C and the residue is dissolved in 200 µl of acetone. The solution is immediately introduced into an HPLC setup for analysis of chlorophyll pigments using an HP Agilent 1100 Liquid Chromatograph and HP Chem Station System Software Manager. The HPLC assays utilize a Spherisorb ODS2 C18 RP column (250 mm 4.6 mm internal diameter, 3 mm particle diameter), an injection volume of 20 ml, and isocratic elution conditions: water/methanol /acetone (4/36/60, v/v/v), at a flow rate of 1 ml/min. A photometric detector is used to perform sequential detection at 410 nm. The pyropheophytin A content is quantified using the peak areas to calculate the relative proportions of the analysts in the sample solution, assuming equal response factors for all pigments.

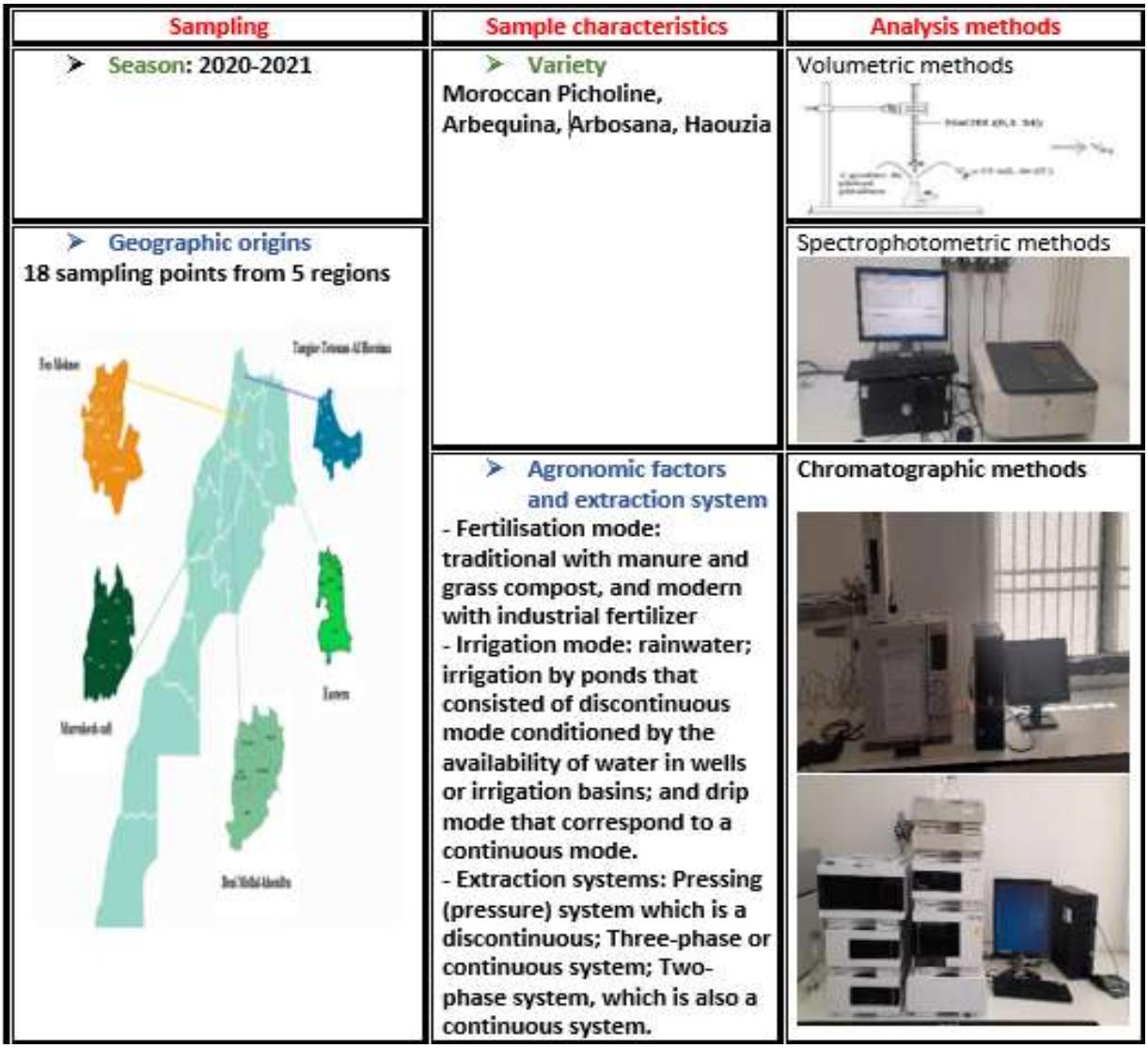


Figure 2. Geographic origins, sample characteristics and analysis methods.

The pyropheophytin A (Figure 3) content is expressed as a percentage of mass fraction (*wpppa*) based on the peak area ratio.

$$wpppa(\%) = \frac{A_{pppa}}{\left(A_{pppa} + \sum A_{pp}\right)} \times 100 \qquad (1)$$

Where A_{pppa} is the peak area of pyropheophytin A; $\sum A_{pp}$ is the sum of the areas of pheophytin peaks A and A'; Report the result to one decimal place.

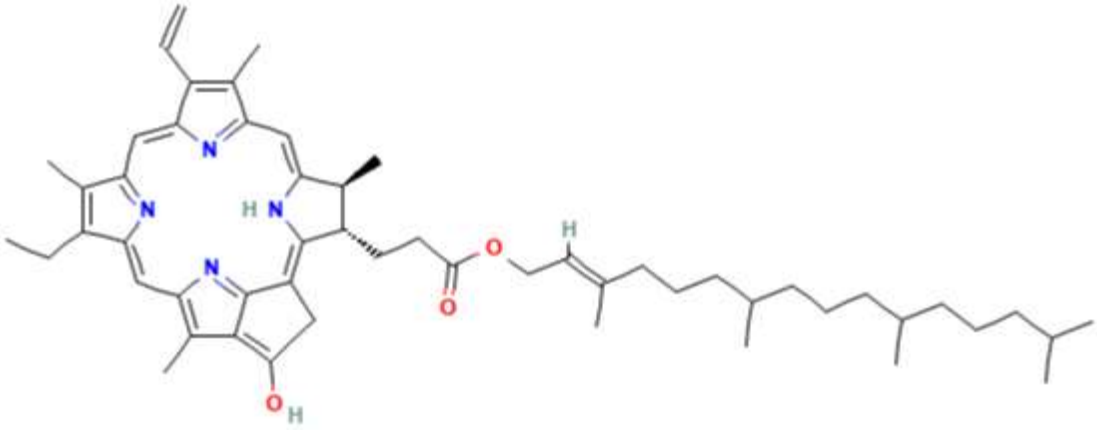


Figure 3. Chemical structure of Pyropheophytin A.

2.3. Determination of free acidity

To determine the free acidity level in a 20 g test sample of olive oil, the sample was dissolved in a mixture of 100 ml ethanol and ethyl ether (50/50). The mixture was then titrated with a 0.1 N potassium hydroxide solution in the presence of phenolphthalein, using the method outlined by the International Organization for Standardization [11].

2.4. Determination of peroxide value

A 5 g sample of olive oil was dissolved in 50 ml of a mixture consisting of acetic acid/chloroform (3:2, v/v). To the resulting mixture, 0.5 ml of a saturated potassium iodide solution was added, and the mixture was left in the dark for 5 minutes. Subsequently, 100 ml of distilled water and 0.5 ml of a starch solution were added, resulting in a purple color. The mixture was then titrated with a 0.01 N sodium thiosulfate solution, using the method described by the International Standard Organization [12].

2.5. Spectrophotometric analysis in the ultraviolet

0.25 g of the sample is dissolved in 25 ml of cyclohexane. After homogenization, the extinctions are measured at wavelengths 232 nm and 270 nm.

2.6. Determination of chlorophyll and carotenoid content

To evaluate the chlorophyll and carotenoid fractions of a 7.5 g sample, it was dissolved in 25 ml of cyclohexane and the absorbance was measured at λ = 670 nm and λ = 470 nm, respectively, in accordance with [13]. The specific extinction coefficients used for the calculations were E0 = 613 for the chlorophyll fraction, where pheophytin was considered the major component, and E0 = 2000 for the carotenoid fraction, where lutein was considered the major component. The pigment contents were then calculated using formulas 2 and 3.

$$\text{Chlorophylls } (\text{mg.kg}^{-1}) = \frac{A_{670} \times 10^6}{613 \times 100 \times d} \quad (2)$$

$$\text{Carotenoids } (\text{mg.kg}^{-1}) = \frac{A_{470} \times 10^6}{2000 \times 100 \times d} \quad (3)$$

2.7. Total phenol content

The Folin-Ciocalteu method, as described in [14], was used to determine the total polyphenol content in olive oils. In brief, 2.5 g of oil was dissolved in 2.5 ml of hexane and

extracted three times for 20 minutes with 2.5 ml of methanol-water (80:20 v/v) under magnetic stirring. The resulting supernatants were collected, washed twice with 5 ml of hexane, and stored in a 50 ml volumetric flask. To the flask, a 2.5 ml aliquot of Folin-Ciocalteu reagent and 2.5 ml of saturated sodium carbonate solution were added and the solution was brought to a volume of 50 ml with distilled water. The mixture was allowed to react for 120 minutes at room temperature in the dark, after which the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimazu). The total content of phenolic compounds was then calculated and expressed in mg of gallic acid equivalent per kg of oil.

2.8. Determination of tocopherols

The Tocopherol content is the mass fraction of the different tocopherols, determined according to the method described by [15]. The tocopherols (α, β, γ and δ) were analyzed by HPLC (Figure 4). The oil samples were weighed (at 0.25 g) and dissolved in 25 ml of n-heptane then mixed using the vortex and directly injected into the HPLC column (250 mm x 4 mm column, packed with silica microparticles grafted with diol groups with an average diameter of approximately 5 µm).

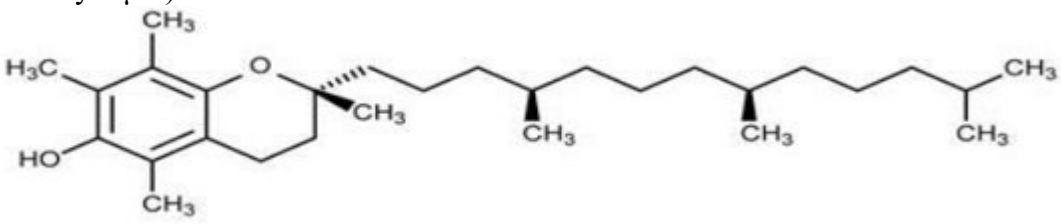


Figure 4. Chemical structure of α Tocopherol.

2.9. Statistical analysis

The chemical analyses were conducted in triplicate and the results were expressed as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was performed on the mean values to determine significant differences ($p < 0.05$), followed by the Tukey test. The statistical analysis was conducted using Minitab v19 software.

3. Results and discussion

3.1. Oil quality parameters

It is evident from

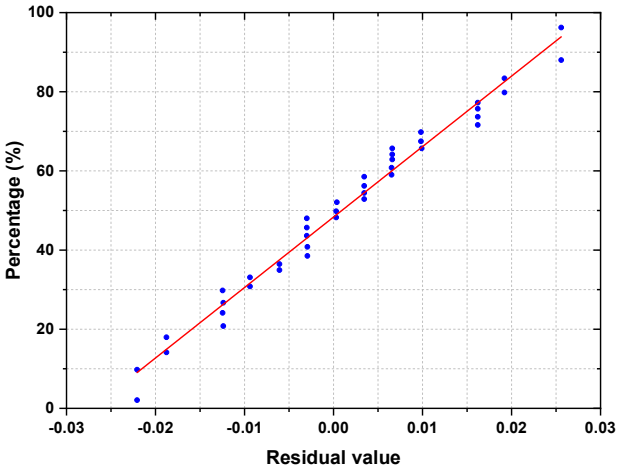
Table 1 that all the samples analyzed met the requirements for being categorized as extra virgin olive oil, as their values for free acidity (Figure 5), peroxide value (Figure 6), K232 (Figure 7), K270 (Figure 8), and ΔK (Figure 9) were within the limits established by regulatory standards. The low values of free acidity (ranging from 0.12 to 0.62 %) indicate that the olives used for oil production were of good quality and were processed promptly after harvesting [16]. The peroxide value and specific UV extinction values were also within legal limits (peroxide value < 20 meq O_2 per kg of oil, K232 < 2.50, K270 < 0.22, and ΔK < 0.01), indicating that the oil had not undergone rancidity. The values for peroxide value ranged from 6.2 to 13.6 meq O_2 kg^{-1}, K232 values ranged from 1.64 to 2.38, K270 values ranged from 0.14 to 0.20, and those of ΔK varied between 0.00 and 0.01. The statistical analysis of the data revealed no significant differences ($P < 0.05$) between the samples. These results are consistent with those reported by Giuseppe Di Lecce et al. (2020) [17], who found that all the samples analyzed were classified as extra virgin olive oil according to the International Olive Council (IOC) standards [16].

Table 1. Free acidity, peroxide value and UV absorbance of the studied extra virgin olive oils.

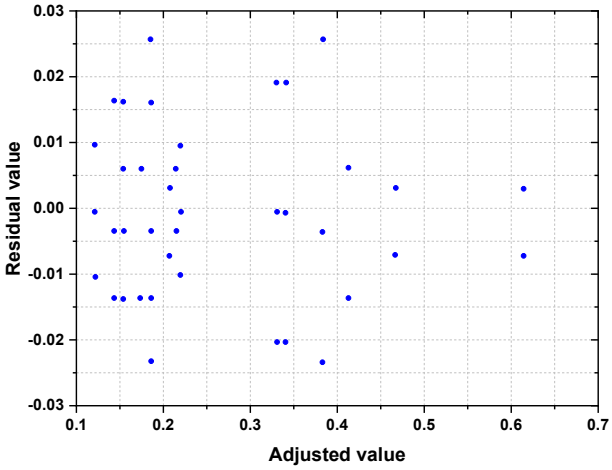
	Free acidity (% C18 :1)	Peroxide value (meq O_2 kg^{-1})	K232	K270	ΔK
Sample 1	0.62 ± 0.01^{a}	13.6 ± 0.1^{a}	2.38 ± 0.01^{a}	0.20 ± 0.01^{a}	0.01 ± 0.01^{a}
Sample 2	0.14 ± 0.02^{hi}	9.4 ± 0.2^{cd}	2.11 ± 0.03^{c}	0.18 ± 0.01^{ab}	0.00 ± 0.00^{b}
Sample 3	0.33 ± 0.02^{e}	9.2 ± 0.2^{cde}	2.08 ± 0.03^{c}	0.18 ± 0.01^{ab}	0.00 ± 0.00^{b}
Sample 4	0.38 ± 0.03^{cd}	10.1 ± 0.2^{bc}	2.21 ± 0.02^{b}	0.19 ± 0.01^{ab}	0.01 ± 0.01^{ab}
Sample 5	0.34 ± 0.02^{de}	7.8 ± 0.3^{fghi}	1.84 ± 0.02^{gh}	0.16 ± 0.03^{ab}	0.00 ± 0.01^{ab}
Sample 6	0.21 ± 0.01^{fg}	7.1 ± 0.6^{hij}	1.74 ± 0.03^{ij}	0.15 ± 0.03^{ab}	0.00 ± 0.01^{ab}
Sample 7	0.18 ± 0.03^{fgh}	6.2 ± 0.5^{j}	1.64 ± 0.02^{k}	0.14 ± 0.02^{b}	0.00 ± 0.00^{b}
Sample 8	0.41 ± 0.01^{c}	10.1 ± 0.1^{bc}	2.22 ± 0.01^{b}	0.19 ± 0.01^{a}	0.01 ± 0.01^{ab}
Sample 9	0.15 ± 0.02^{hi}	6.3 ± 0.4^{j}	1.64 ± 0.02^{k}	0.14 ± 0.02^{b}	0.00 ± 0.00^{b}
Sample 10	0.17 ± 0.01^{gh}	7.9 ± 0.5^{fgh}	1.86 ± 0.03^{fgh}	0.16 ± 0.02^{ab}	0.00 ± 0.00^{b}
Sample 11	0.22 ± 0.01^{f}	8.7 ± 0.3^{def}	1.97 ± 0.04^{de}	0.18 ± 0.01^{ab}	0.00 ± 0.00^{b}
Sample 12	0.21 ± 0.01^{fg}	8.2 ± 0.4^{efg}	1.91 ± 0.02^{efg}	0.17 ± 0.02^{ab}	0.00 ± 0.01^{ab}
Sample 13	0.18 ± 0.02^{fgh}	6.8 ± 0.5^{ij}	1.69 ± 0.02^{jk}	0.15 ± 0.02^{ab}	0.00 ± 0.00^{b}
Sample 14	0.12 ± 0.01^{i}	7.5 ± 0.5^{ghi}	1.79 ± 0.05^{hi}	0.16 ± 0.02^{ab}	0.00 ± 0.01^{ab}
Sample 15	0.12 ± 0.01^{i}	8.8 ± 0.3^{def}	1.99 ± 0.04^{d}	0.18 ± 0.02^{ab}	0.00 ± 0.00^{b}
Sample 16	0.14 ± 0.02^{hi}	6.9 ± 0.4^{hij}	1.71 ± 0.03^{ijk}	0.15 ± 0.02^{ab}	0.00 ± 0.00^{b}

Sample 17	0.47 ± 0.01[b]	10.8 ± 0.1[b]	2.31 ± 0.01[a]	0.20 ± 0.01[a]	0.01 ± 0.01[a]
Sample 18	0.15 ± 0.01[hi]	8.4 ± 0.3[defg]	1.93 ± 0.03[def]	0.17 ± 0.02[ab]	0.00 ± 0.01[ab]
EVOO *	≤ 0.8	≤ 20	≤ 2.50	≤ 0.22	≤ 0.01

The values are the means of the eighteen different VOO samples ± the standard deviations. Significant differences in the same column are indicated by different varieties of letters (a–k) (P < 0,05). **Extra virgin olive oil quality criteria, Values limits set by International Olive Oil Council*



(a)



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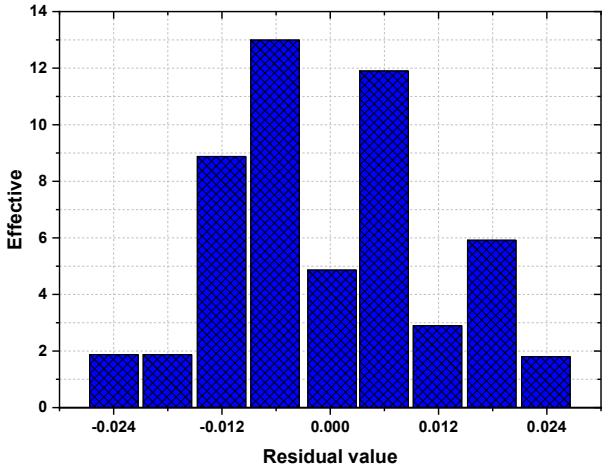


(c)

Figure 5. Residual value of acidity, (a) Line of Henry, (b) Adjusted values, (c) Histogram.

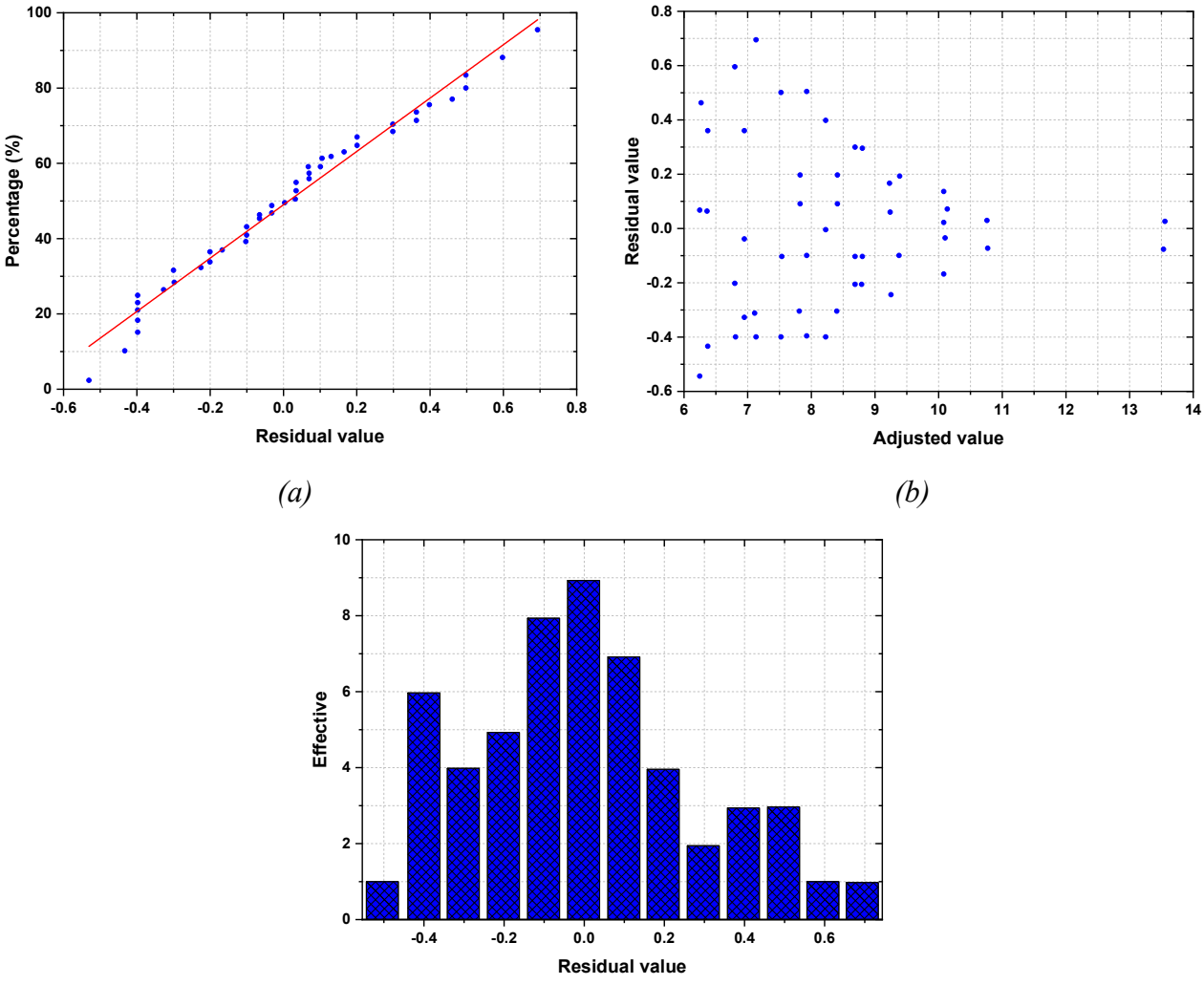
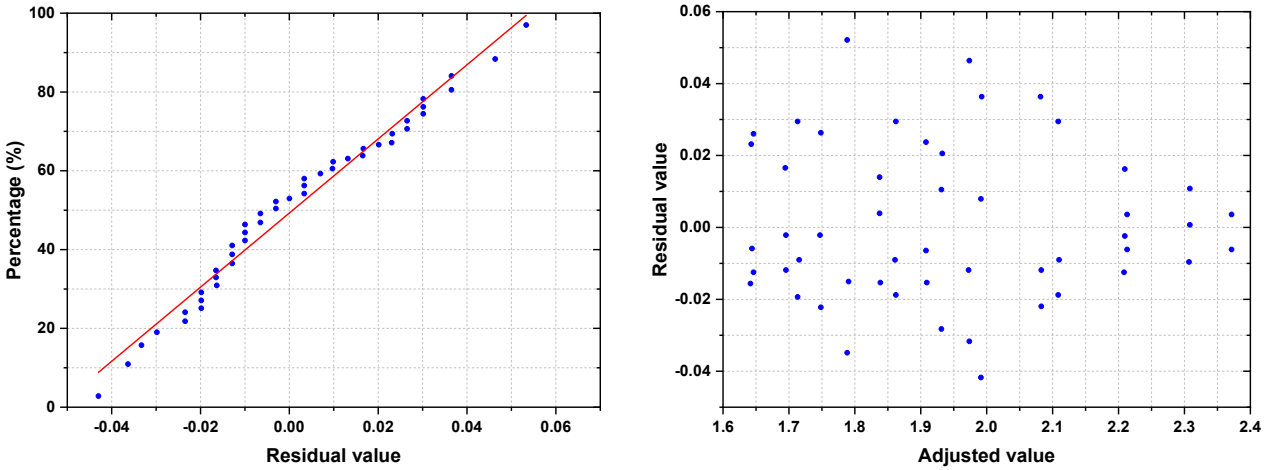
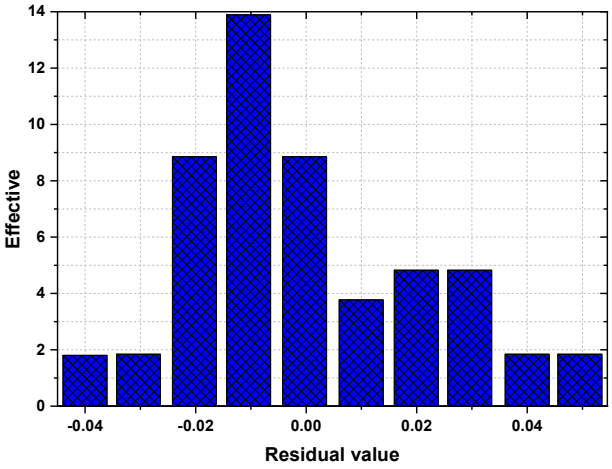


Figure 6. Residual value of peroxide, (a) Line of Henry, (b) Adjusted values, (c) Histogram.

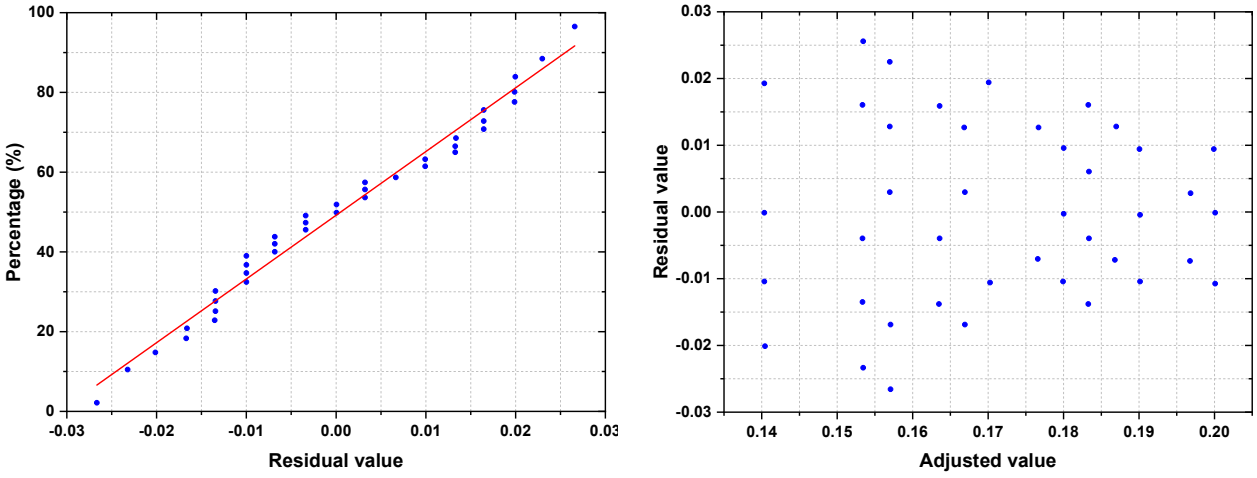


(a) *(b)*



(c)

Figure 7. Residual value of K232, (a) Line of Henry, (b) Adjusted values, (c) Histogram.



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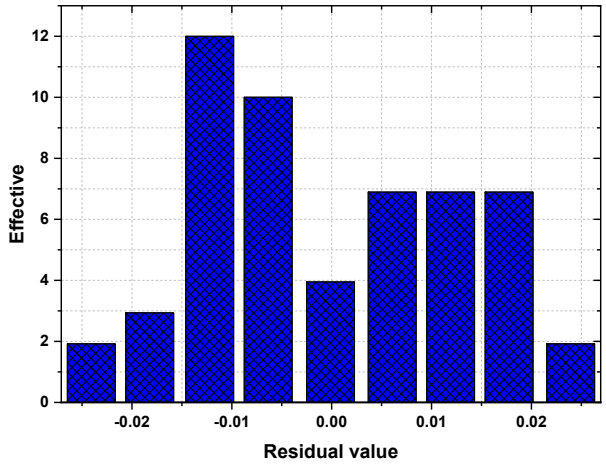

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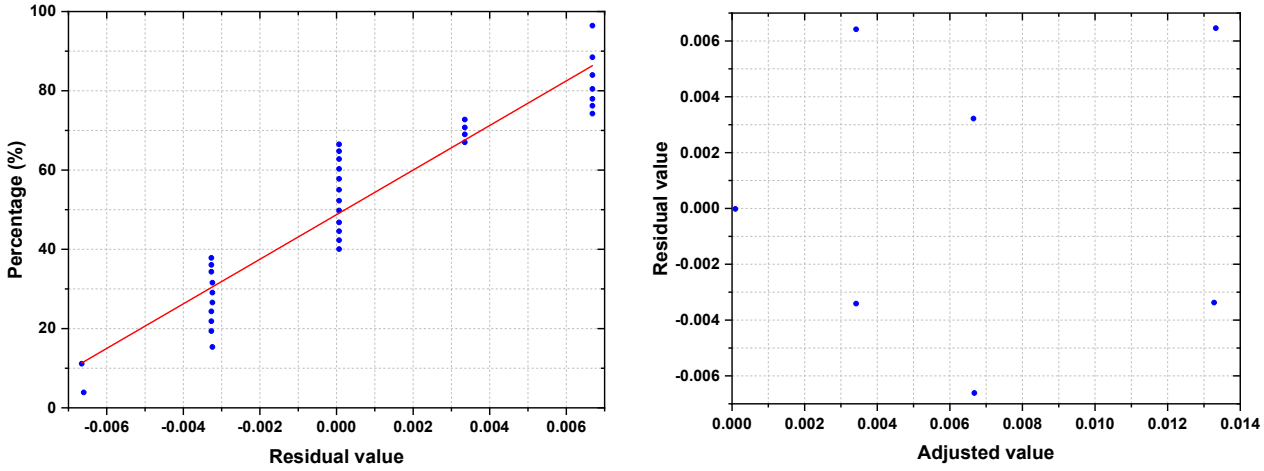
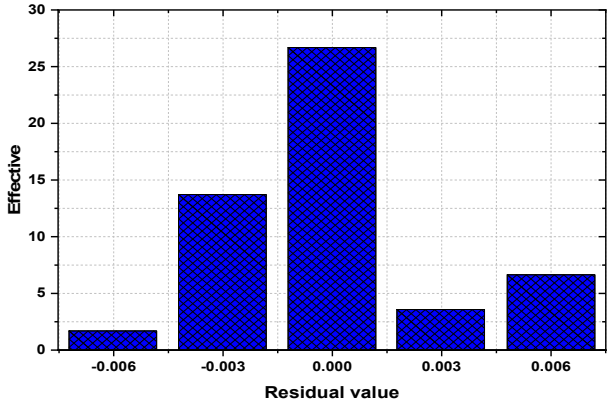
Figure 8. Residual value of K270, (a) Line of Henry, (b) Adjusted values, (c) Histogram.



(a) *(b)*



(c)

Figure 9. Residual value of delta K, (a) Line of Henry, (b) Adjusted values, (c) Histogram.

3.2. Pyropheophytin A

Pyropheophytin A is a degradation product of chlorophyll pigments that can be used as an indicator of the state of freshness of olive oils. As the oil ages or is exposed to heat, the amount of pyropheophytin A increases. Therefore, measuring the percentage of pyropheophytin A can be useful to determine the quality and freshness of the oil, and it could also be used in commercial exchanges between operators. However, it's worth noting that an increase in pyropheophytin A content is sometimes observed after six months of storage, so it's important to take storage conditions into account when interpreting these results.

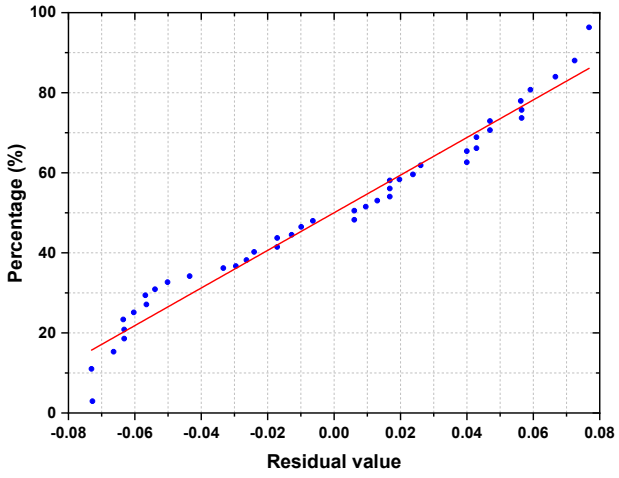
The Pyropheophytin A content results shown in Table 2 vary from 2.77 to 37.26 %. These results show significant differences ($P < 0.05$) between the virgin olive oils studied. Our results (Figure 10) agree with those obtained for Italian olive oils which reported values ranging from 208 to 396 % [18].

Table 2. Pyropheophytin A, chlorophyll, carotenoid, total phenol and α-tocopherol contents of the studied extra virgin olive oils.

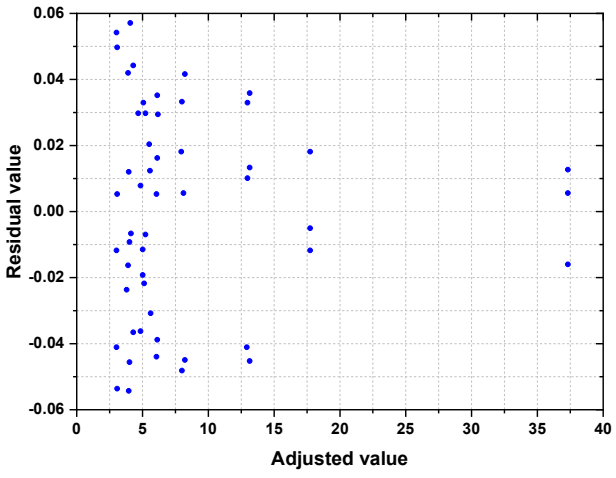
	Pyropheophytin A (%)	**Chlorophylls (mg kg^{-1})**	**Carotenoids (mg kg^{-1})**	**Total phenols (mg gallic acid kg–1 oil)**	**α-Tocopherol (µg/kg)**
Sample 1	37.26 ± 0.02^{a}	3.28 ± 0.03^{l}	2.08 ± 0.04^{j}	267 ± 1.73^{l}	155 ± 2.65^{l}
Sample 2	8.11 ± 0.06^{d}	4.25± 0.02^{j}	3.01± 0.03^{g}	325 ± 2.65^{j}	167 ± 1.73^{k}
Sample 3	7.91 ± 0.06^{e}	4.32 ± 0.04^{j}	3.08 ± 0.03^{g}	342 ± 2.52^{i}	178 ± 1.15^{j}
Sample 4	12.97 ± 0.06^{c}	4.07 ± 0.04^{k}	2.26 ± 0.04^{i}	284 ± 2.65^{k}	162 ± 2.08^{k}
Sample 5	4.77 ± 0.05^{i}	5.46 ± 0.03^{d}	3.49 ± 0.02^{cd}	375 ± 2.08^{de}	211 ± 2.31^{de}
Sample 6	3.93 ± 0.07^{j}	6.08 ± 0.05^{bc}	3.54 ± 0.03^{bc}	374 ± 2.08^{e}	217 ± 2.65^{cd}
Sample 7	2.77 ± 0.07^{l}	6.82 ± 0.04^{a}	4.03 ± 0.05^{a}	392 ± 3.06^{a}	229 ± 2.08^{a}
Sample 8	12.93 ± 0.05^{c}	4.09 ± 0.03^{k}	2.46 ± 0.04^{h}	362 ± 3.21^{gh}	174 ± 2.00^{j}
Sample 9	2.91 ± 0.07^{l}	6.79 ± 0.02^{a}	4.01 ± 0.05^{a}	388 ± 3.06^{ab}	228 ± 2.08^{ab}

Sample 10	4.95 ± 0.04^{h}	5.31 ± 0.04^{e}	3.47 ± 0.03^{cd}	371 ± 3.00^{ef}	205 ± 2.65^{efg}
Sample 11	5.93 ± 0.05^{f}	4.83 ± 0.03^{h}	3.31 ± 0.04^{f}	364 ± 3.51^{fgh}	193 ± 1.15^{hi}
Sample 12	5.04 ± 0.04^{h}	5.17 ± 0.02^{f}	3.42 ± 0.03^{de}	372 ± 2.65^{ef}	203 ± 2.31^{fg}
Sample 13	3.69 ± 0.05^{k}	6.16 ± 0.03^{b}	3.62 ± 0.02^{b}	383 ± 3.61^{bcd}	221 ± 2.31^{bc}
Sample 14	4.05 ± 0.06^{j}	6.04 ± 0.03^{c}	3.52 ± 0.02^{bcd}	376 ± 3.21^{cde}	209 ± 2.31^{ef}
Sample 15	6.06 ± 0.05^{f}	4.52 ± 0.04^{i}	3.29 ± 0.04^{f}	358 ± 2.08^{h}	189 ± 2.65^{i}
Sample 16	3.76 ± 0.07^{k}	6.12 ± 0.05^{bc}	3.61 ± 0.03^{b}	384 ± 1.15^{bc}	221 ± 2.08^{bc}
Sample 17	17.70 ± 0.02^{b}	3.34 ± 0.03^{l}	2.12 ± 0.05^{j}	325 ± 2.00^{j}	164 ± 2.65^{k}
Sample 18	5.50 ± 0.04^{g}	4.96 ± 0.02^{g}	3.34 ± 0.02^{ef}	369 ± 2.65^{efg}	198 ± 2.52^{gh}
NAOOA**	≤ 17.00	-	-	-	-

The values are the means of the eighteen different VOO samples ± the standard deviations. Significant differences in the same column are indicated by different varieties of letters (a–l) ($P < 0.05$). ** *American Olive Oil Producers Association*



(a)



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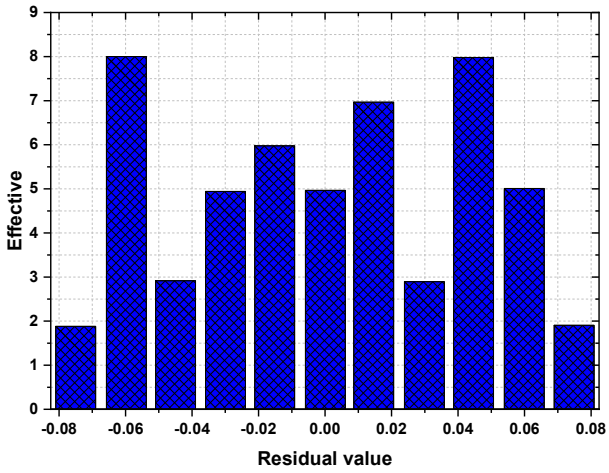
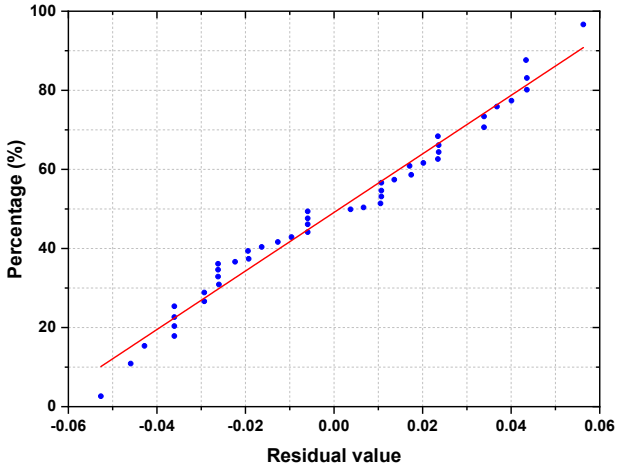


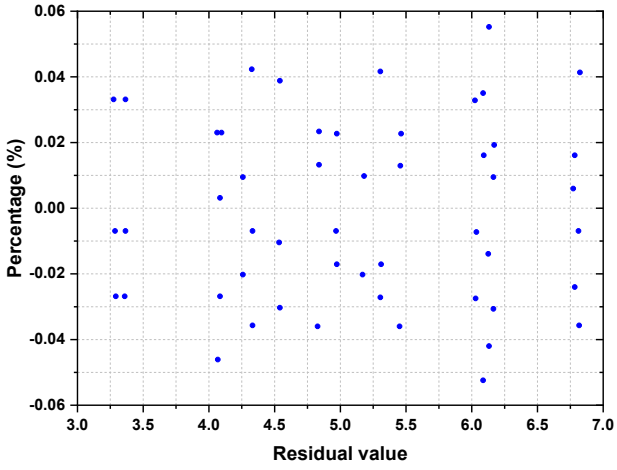
(c)

Figure 10. Residual value of pyropheophytin A, (a) Line of Henry, (b) Adjusted values, (c) Histogram.

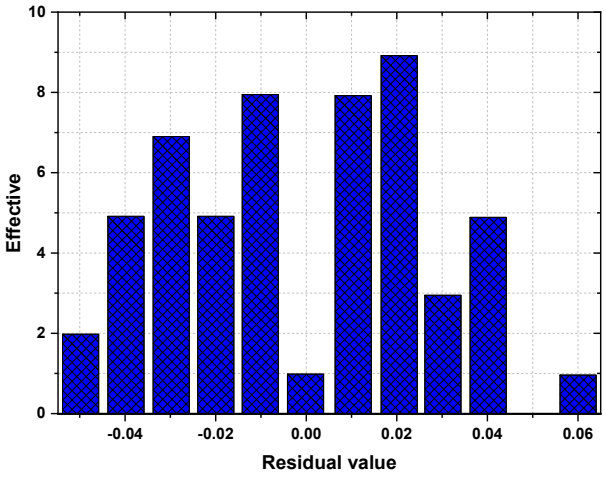
3.3. Chlorophyll and carotenoid contents

The assessment of pigment profiles in olive oil is crucial due to their antioxidant properties. Olive oil contains two types of pigments: chlorophylls (Figure 11) and carotenoids (Figure 12). The chlorophyll content reflects the presence of color substances in the oil and is influenced by olive contamination with leaves, whereas carotenoids are natural chemicals that participate in the oil oxidation mechanisms. Sufficient amounts of carotenoids in oil can delay photo-oxidation and preserve oil quality during storage. The chlorophyll and carotenoid content of the virgin olive oils studied in Table 2 ranged from 3.28 to 6.82 $mg.kg^{-1}$ and 2.08 to 4.03 $mg.kg^{-1}$, respectively, exhibiting significant differences ($P < 0.05$). These results align with previous findings on Brazilian olive oils that reported chlorophyll and carotenoid values ranging from 1.3 to 1.7 $mg.kg^{-1}$ and 2.7 to 3.9 mg kg^{-1}, respectively [19]. The pigment content in olive oil is determined by various factors, such as olive variety, maturity level, oil extraction method, and storage conditions. Carotenoid pigments in olive oil protect against photo-oxidation by deactivating the oxygen produced by chlorophylls, thus inhibiting photo-oxidation.



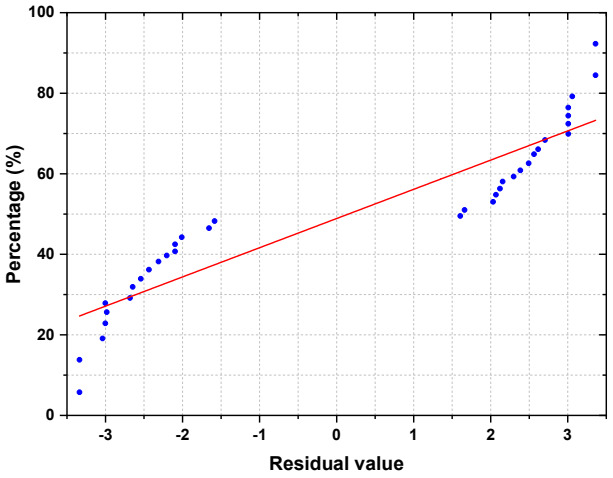


(a) *(b)*

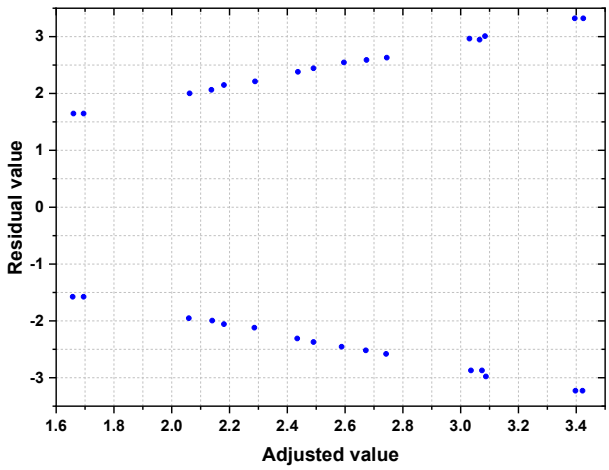


(c)

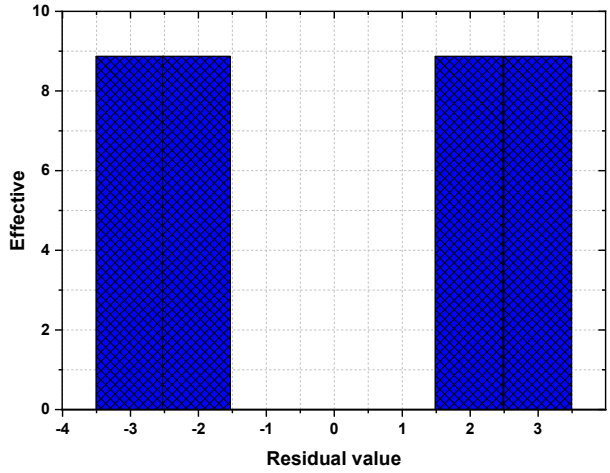
Figure 11. Residual value of chrolophyll, (a) Line of Henry, (b) Adjusted values, (c) Histogram.



(a)



(b)

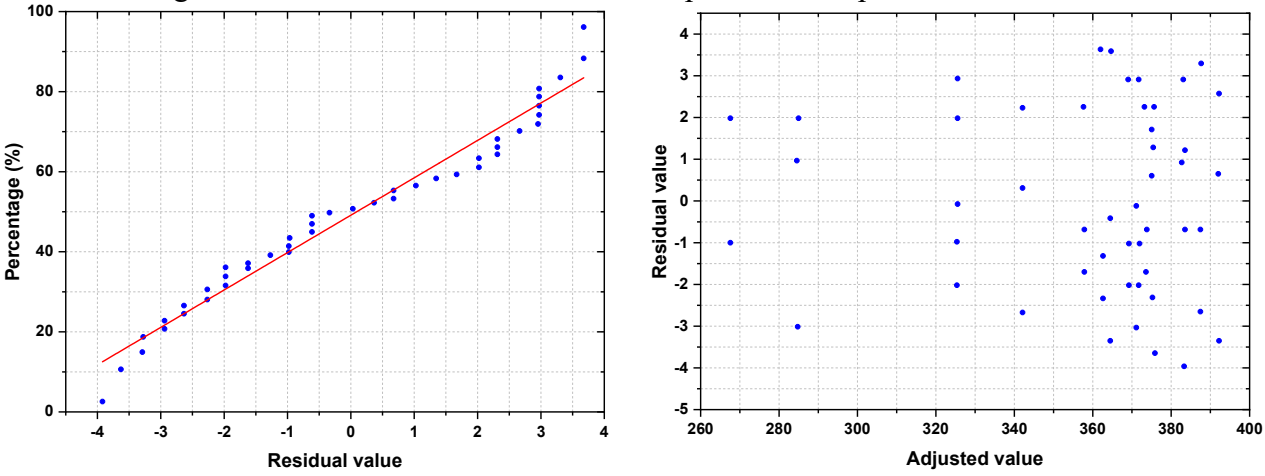


(c)

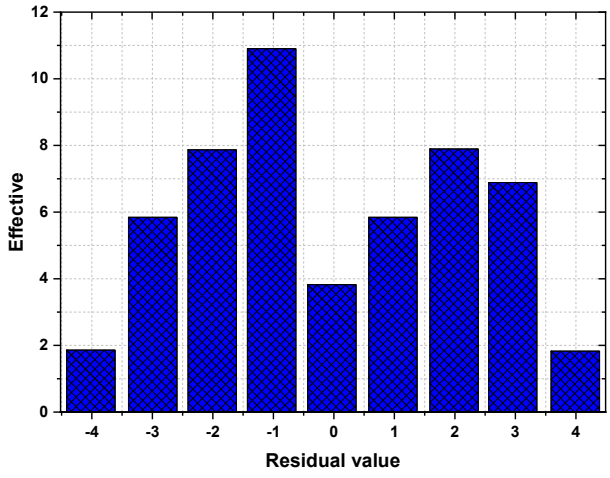
Figure 12. Residual value of carotenoid, (a) Line of Henry, (b) Adjusted values, (c) Histogram.

3.4. Total phenols content

Phenolic compounds pass into the oil during its extraction. They are considered natural antioxidants that protect the oil against oxidation and give it better storage stability and a bitter flavor. The beneficial effects of olive oil on health depend largely on its content of phenolic compounds by helping the body to strengthen its defense system against abnormalities related to oxidative stress such as cardiovascular diseases, cancer and the inflammatory process [20]. These compounds, like the other minor constituents of olive oil, contribute to the sensory organoleptic properties and to the prevention of auto-oxidation of oil [21]. The results of the total content of phenolic compounds presented in Table 2 vary from 267 to 392 mg of gallic acid/kg of oil. These results show significant differences between the virgin olive oils studied ($P < 0.05$). Our results (Figure 13) agree with those obtained for Italian olive oils which reported values ranging from 208 to 396 mg of gallic acid/kg of oil [17]. The variations in the levels observed may be due to the difference in the degree of maturity of the olives before crushing (early harvest of the olives) but also depend on the variety cultivated and the geographical area [22]. The presence of leaves during olive crushing can also increase the concentration of phenolic compounds in olive oils.



(a) *(b)*



(c)

Figure 13. Residual value of phenols, (a) Line of Henry, (b) Adjusted values, (c) Histogram.

3.5. Tocopherols

Tocopherols are crucial fat-soluble antioxidants present in olive oil that effectively prevent lipid oxidation in food and biological systems [23]. Olive oil contains four tocopherol isomers, namely α, β, γ, and δ, with α-tocopherol (also known as vitamin E) being the dominant fraction of tocopherols (more than 95 % of total tocopherols) [24]. The α-tocopherol content (Figure 14) of virgin olive oils was found to vary from 155 to 229 $\mu g.kg^{-1}$ according to the results presented in Table 2, with significant differences observed between the studied oils ($P < 0.05$). These findings are in agreement with those obtained for Italian olive oils, which reported values ranging from 148 to 229 $\mu g.kg^{-1}$ [25]. Previous research reported lower α-tocopherol contents of Italian extra virgin olive oils and found mean values of 100 to 175 $\mu g.kg^{-1}$, which may be due to the sensitivity of these samples to cold and temperature differences [26]. Tocopherols are the primary dietary sources of olive oil and contribute to the oil's overall quality by providing protection against oxidation and nutritional value [27].

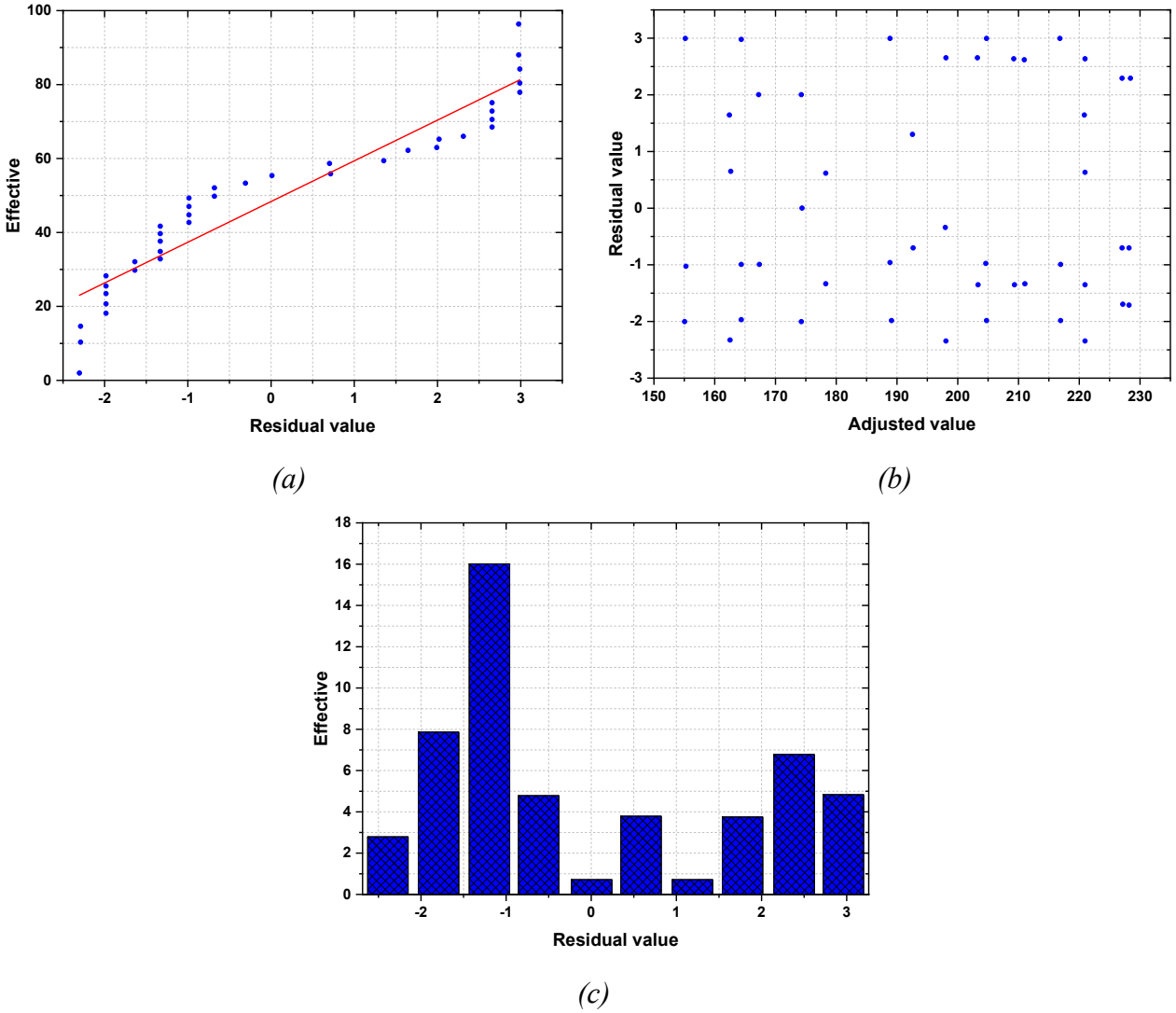


Figure 14. Residual value of tocopherols, (a) Line of Henry, (b) Adjusted values, (c) Histogram.

4. Conclusions

This article reports on the physico-chemical analysis of eighteen extra virgin olive oil samples from five regions of Morocco. The results show that the quality parameters of these oils meet the International Olive Council's standards for high-quality virgin olive oil. However, the study found variations in the levels of antioxidant parameters, such as phenolic compounds, pigments, α-tocopherol, and pyropheophytin A, which are affected by climatic conditions. Pyropheophytin A is a marker of quality in olive oil and is present in trace amounts, increasing with temperature and storage time. The American Olive Oil Producers Association has set a limit of ≤ 17 % for pyropheophytin A content, which was exceeded by two samples in this study (sample N° 1 with PPP = 37.26 % and sample N° 17 with PPP = 17.70 %). This parameter could indicate the performance of the product in the market when subjected to heat treatment.

In the context of promoting olive oil from Morocco, we undertook a comparative analysis of the different physicochemical parameters of olive oil samples gathered from the region. Assessing the quality of olives is a multifaceted task that requires evaluating a broad array of characteristics.

Quality interpretations are often shaped by the viewpoints and objectives of producers, nutritionists, merchants, and consumers. The makeup of olive oil is the result of intricate interactions among various environmental, agronomic, and technological factors that affect the growth and ripening of the fruit, as well as the processes of extraction and storage. In light of the promising findings from this study, it is essential to enhance efforts aimed at increasing the value of this oil. The scientific ramifications of these initiatives could significantly influence the advancement of the olive oil industry. The potential of using %PPP as an indicator of freshness has proven to be particularly intriguing. Notably, alterations in pyropheophytin a exhibited significantly greater sensitivity to temperature fluctuations compared to pheophytin a, which serves as an indicator of secondary oxidation. This metric could be regarded as an early signal of product performance in the market, especially when the anticipated storage temperature of EVOO exceeds 25 °C. Additionally, the analysis of tocopherols produced during the oxidative process, which can be readily assessed using HPLC, has emerged as a valuable new parameter. Building on this promising foundation, further investigations are necessary to validate and improve the robustness of the proposed methodology by examining oils with varying chemical properties. Gaining insights into the variability of Ea values and their connection to the chemical composition of EVOO may aid in the development of a general SL model for EVOO.

Acknowledgments
We would like to extend our gratitude to the University Hassan II Ain Chock Casablanca, Morocco for their valuable contribution to this study.

Declaration of Interest statement
The authors have no conflicts of interest to declare.

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