Title: Traditional techniques applied in olive oil production results in lower quality products in Northern Cyprus

Key words: Olive oil, Analysis, Oxidation, Northern Cyprus

Abbreviations: IOOC; International Olive Oil Council, ISO; International Organization for Standardization, FFA; Free fatty acid, TPC; Total phenol content, FAAE; Fatty acid alkyl ester.

Abstract

The manner of olive oil production and its dietary is one of the characteristics of the Cyprus Island, including the Northern Cyprus. Despite its extensive consumption, there has not been known scientific research carried out so far to qualify the olive oil traditionally produced and consumed within the Northern Cyprus. Therefore, in the present study, we aimed to screen the quality of the olive oil produced and consumed regionally. The guidelines and the related methods offered by International olive oil council (IOOC) and International Organization for Standardization (ISO) were employed to screen the quality indices of the olive oil produced employing traditional methods without the presence of industrialized techniques. In contrast to the regional belief and consideration, the results have indicated that the olive oil produced locally is highly exposed to oxidation and therefore, it is of lower quality according to the ISO guidelines.

Özet

Zeytinyağı üretiminin ve beslenmesinin tarzı Kuzey Kıbrısın da dahil olduğu Kıbrıs Adasının karakteristik özelliklerinden biridir. Yoğun tüketimine rağmen, Kuzey Kıbrıs'ta geleneksel olarak üretilen ve tüketilen zeytinyağının niteliklerini belirlemek için yapılan bilinen bir bilimsel çalışma bulunmamaktadır. Bu nedenle, bu çalışmada, bölgesel olarak üretilen ve tüketilen zeytinyağının kalitesini araştırmayı hedefledik. Uluslararası zeytinyağı konseyi (IOOC) ve Uluslararası Standartlar Organizasyonu (ISO) tarafından sunulan prensipler ve ilgili yöntemler, sanayileşmiş teknikler olmadan geleneksel yöntemlerle üretilen zeytinyağı kalite endekslerini taramak için kullanılmıştır. Bölgesel inanç ve değerlendirmenin aksine, sonuçlar, yerel olarak üretilen zeytinyağının oksidasyona aşırı maruz kaldığını ve dolayısıyla ISO talimatlarına göre daha düşük kalitede olduğunu ortaya koymuştur.

1. Introduction

There is no doubt that olive oil consumption has been a significant component of daily dietary in the Mediterranean region.[1] Indeed, the olive tree (*Olea Europea* L.) agriculture, and the following olive oil production is a typical and one of the oldest traditions in various countries in the region.[1-2] Starting from the second half of the last century, the employment of Mediterranean diet in non-Mediterranean areas has led to the production of olive oil in higher amounts to respond to the worldwide demand which, in turn, has forced the establishment of industrialization for the olive oil production to guarantee the quality.[3-4] This has further been regulated and warranted by both the producer countries and the International Organization for Standardization.[5-7]

As a part of Cyprus, Northern Cyprus, also referred to as Turkish Republic of Northern Cyprus, in the middle of the Mediterranean Sea, is one of those countries continuing to harvest thousand tons of the olive fruit and the olive oil annually. Even, the archaeological ruins from the Neolithic period (i.e., 8200 BC) in the region indicate the processing of olive fruits since from that time.[8] Records, currently, estimate the presence of approximately 1 million olive trees (*Olea Europea* L.), many of which are cultivated to obtain the olive fruits (i.e., a typical breakfast dietary), and the olive oil.[9] Furthermore, the current data also indicates that olive oil consumption per person is around 25-50 mL/day in the region.[10] Indeed, almost all of the local restaurants in the region serve olive oil as one of the appetizers, even without charge.

Besides the existence of a few producers that utilize modern industrial subsidiaries, the majority employs traditional methods for the olive oil production in Northern Cyprus. In public, these techniques are classified as either hot or cold procedures. As implied, cold and hot refers to the temperature in the extraction phase (i.e., lower than 28°C temperature of aqueous phase for the cold procedure and above 28°C temperature of aqueous phase for the hot procedure). It is considered that the hot procedure accelerates the extraction phase, therefore aids in the yield and stability with respect to the time spent; on the other hand, it is a disadvantage for the transfer of various beneficial chemicals (e.g., stabilizers, and antioxidants) to the aqueous phase depending on the change of solubility at varying temperatures.[11-13] Moreover, the majority of the public rely on olive oil products produced through the traditional

techniques rather than the industrial products, since they find it more natural. Indeed, the industrial local products are generally produced to export them to other countries concomitant to analysis certificates guaranteeing the quality, whereas, almost all of the local traditionally produced products reach to consumers, even, without an apparent label on. The main difference with the current traditional techniques is that they do not obey the manufacturing rules strictly regulated in the industrialized techniques. The exposure to air and light during the production phase, the non-employment of protective equipment or systems to prevent the oxidation, and the related degeneration of olive oil, as well as the insufficient containers utilized (e.g., non-opaque glass material without a correct label) make the traditional method produced olive oils susceptible. Even, some producers still use stone-mills for malaxation.

So far, there has not been a scientific research conducted to screen the quality indices of the olive oil traditionally produced in the Northern Cyprus. From the curiosity of this point of view, this research aimed to investigate, for the first time, the basic quality parameters of the olive oil produced employing the traditional techniques within the Northern Cyprus. Therefore, the quality indices (i.e., free fatty acid percentage, peroxide value, UV-specific extinctions at 232 and 272 nm, total phenol, chlorophyll, and carotenoid contents, and the fatty acid alkyl ester compositions) of the samples collected were aimed to be determined.

2. Materials and methods

2.1. Chemicals and Reagents

Hexane (99.0%), cyclohexane (99.5%), ethanol (99.9%), methanol (99.9%), diethyl ether (99.0%), sodium carbonate, sodium thiosulfate, potassium hydroxide (85.0%), potassium iodide (99.0%), sodium hydroxide (99.0%), acetic acid (99.0%), hydrochloric acid (37%), gallic acid were obtained from Merck (Germany). Folin-coicalteus phenol was purchased from Sigma-Aldrich (Darmstadt, Germany).

2.2. Samples

Although there are quite a lot number of producers, particularly within the western part of the region, the majority of them produce limited amount for their own use. Therefore, we have collected sample olive oils from the thirteen olive oil producers who also sell their traditionally produced olive oils beside their own use. From this point of view, 26

samples in capped non-opaque glass materials (i.e., a classical way of packing and marketing of producers) from 13 different producers were purchased directly from the producers (i.e., 2 samples from the same production of the same producer). Each sample from 13 producers was subcategorized in the way that the first group to be analyzed in their 3rd month, and the second group to be analyzed in their 6 month of production. The samples to be analyzed in these periods were kept in light-free shelves at room temperature until analysis. In order to make a comparison with a reference product coming from industrial production, a commercial extra virgin olive oil at the 3rd month of its production (i.e., the data on the label was directly utilized) was also purchased from a supermarket and employed in the same analysis. Each sample was analyzed for three times and the results were expressed as the mean ± standard deviation. Table 1 shows the codes, the place obtained, and the production method (i.e., cold or hot) of the samples analyzed.

2.3. Determination of free fatty acid content (FFA)

Free fatty acid content (FFA), expressed as the percentage content of the free fatty acids in olive oil, was determined through titration using potassium hydroxide according to proposed procedure by ISO660.[14] Accordingly, 500 mg of olive oil sample was dissolved in 15 mL of ethanol and diethyl ether (solvent mixture), which was previously neutralized by potassium hydroxide. Then this solution was titrated by 0.1N potassium hydroxide. Acidity, expressed as a percentage of fat type, was calculated according to the given formula:

Acidity= (V×N×F×M)/(10×m)

wherein:

V= The volume of 0.1N KOH consumed, F= Factor of 0.1N KOH, N= Normality of KOH (i.e., 0.1), M= Molar mass of oil in gram per mole (i.e., 256 g/mol) and m= the mass in gram of the test portion.

2.4. Assessment of peroxide value (PV)

Peroxide value, as stated in miliequivalent of $O_2.kg^{-1}$ (meq O_2/kg oil), was determined according to the method described by ISO3960.[15] Briefly, 5 g olive oil sample was dissolved in glacial acetic acid-hexane (6:4) solution. Then 0.5 mL of saturated potassium iodide was added and swirled exactly one minute. Immediately after, 100 mL distilled water was introduced to the flask and shaken vigorously. Finally the

mixture was titrated with 0.01 N sodium thiosulfate. Peroxide value (meqO_2/kg oil) was calculated based on the formula described below:

$PV= ((V-V_0)\times N\times F\times 1000)/m$

wherein;

V: The volume of sodium thiosulfate consumed for the samle, V_0: The volume of sodium thiosulfate consumed for the titration of the blank (without olive oil sample), N: The normality of sodium thiosulfate (i.e., 0.01N), and m: Mass (weight) of sample in gram.

2.5. Determination of oxidation satus of olive oils (K232 and K270)

The experiment to determine the oxidation status of olive oils was carried out measuring their absorption at specific wavelengths (i.e., 232 and 270 nm).[16] Briefly, 0.25 g olive oil sample was dissolved in cyclohexane in a 25 mL graduated flask to prepare 1% w/v. Then, the specific extinctions at 232 and 270 nm were examined.

2.6. Carotenoids and chlorophyll content assays

Carotenoid and chlorophyll (mg/kg of oil) contents were determined employing a UV based procedure.[17] As described above, 0.25 g olive oil sample was dissolved in cyclohexane (i.e., 1% w/v) and the specific extinctions were determined at 470 and 670 nm, respectively for the carotenoid, and chlorophyll contents .

2.7. Detection of total phenol content (TPC)

The Folin-Ciocalteaus method, an assay in which the results are expressed in terms of gallic acid as mg of gallic acid/kg olive oil depending on the spectrophotometric measurements conducted at 765 nm, was employed for the determination of the total phenol content.[18] Accordingly, 10 g of olive oil was dissolved in 50 mL of hexane and extracted three times with 80% aqueous methanol. Then, the extract was added distilled water to a final volume of 100 mL aqueous methanol and kept overnight. 5 mL folin-ciocalteus phenol reagent was added to 1 mL of aliquot extract, then shaken well and let to stand for 5 minutes. 1 mL of saturated sodium carbonate was added and swirled. After 1 hour at room temperature, absorption was read at 765 nm. 1 mL aliquot of 0.05, 0.2, 0.4, 0.5 and 0.6 mmol/l aqueous gallic acid solution were mixed with 5 mL folin-ciocalteaus reagent and 1 mL saturated sodium carbonate solution. Then, the

absorption was measured at 725 nm to obtain the calibration curve. Finally, the total concentration of polyphenol in olives oil samples was determined as ppm of Gallic acid.

2.8. Determination of fatty acid alkyl esters (FAAEs)

For the determination of fatty acid alkyl esters, the European Official Methods of Analysis (EEC), suggesting a GC assay, was used.[19] Accordingly, 100mg olive oil sample was dissolved in 10 mL n-hexane in 20 mL test tube and 100 µL of 2 N potassium hydroxide in methanol was added. The prepared sample solution was vortexed for 30 seconds and centrifuged for 15 min. Afterward, supernatant phase was transferred into 2 mL autosampler vial for chromatographic analysis.

The Chromatographic analyses were performed on an Agilent 6890 GC (Agilent Technologies, Santa Clara, USA) fitted with a FID detector. The column used was a capillary HP-88 J&W 112-88A7 (length 100 m, id 0.25 mm and film thickness 0.2 µm). The operating conditions were as follows: the inlet temperature was 250 ºC; injection volume was 2 µL; the carrier gas was helium with a flow rate of 2 mL/min and 1:50 split ratio; oven temperature was set to 120 ºC for 1min initially and then it was first increased up to 175 ºC (i.e., 10 ºC/min rate), then increased to 220 ºC (i.e., 3 ºC/min rate) where it was maintained for 5 min; detector temperature was set to 280 ºC.

2.9. Statistical analysis

Statistical analysis was performed using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Mean scores and standard deviations were calculated with respect to the assay results practiced in triplicate. Paired samples t-test was employed in order to show the statistical significance between the mean scores of 3 and 6 months samples.

3. Results and Discussion

In order to determine the basic quality indices of the samples collected, the percent free acid, peroxide values, and the specific absorption coefficients were measured first.

The results obtained for the percent free acid of the samples and the reference are shown in Figure 1. Mainly, IOOC defines and designates the classification of olive oil mainly according to their free fatty acid content.[20] Accordingly, none of the three month samples can be classified as extra virgin olive oil. Since the results for the free acid content for each 3 month sample was found less than 2 grams per 100 grams oil, each were categorized as virgin olive oil. With respect to the free fatty acid content at 6 month samples, the categorization as virgin olive oil was saved for the majority of the

samples, although 2 of them (i.e., 5O, and 6O, both of which were cold procedure products) appeared to be ordinary virgin olive oil according to the IOOC guidelines. Furthermore, the reference commercial sample was shown to be keeping its extra virgin olive oil property in the first 6 months following its production. It is noteworthy to state that the increase in percent FFA content in each sample and the reference was found statistically significant (i.e., $p<0.05$).

One of the major parameters that shows the quality of olive oil is the peroxide value, defined as the measure of total peroxides in olive oil expressed as miliequivalent of O_2/kg oil. The upper standard for the peroxide is 20 meq/kg oil.[21-22] Beside the sample 11F, each sample was shown to possess peroxide level less than the upper standard in the 3rd month analysis (i.e., Figure 2). However, there is a serious increase in terms of peroxide value, an indication of oxidation in all of the samples tested. Indeed, more than half of the samples tested (i.e., 1O, 2O, 4O, 5O, 6O, 7O, 10O, and 11O) were shown to have peroxide levels less than 20 meq/kg oil, in their 6 month analysis. Beside the sample 3O, all of the cold procedure products were found to possess higher tendency for oxidation. The increase in the peroxide value for the reference sample was also established, however, it did not reach to the upper standard peroxide level (i.e., 20 meq/kg oil), even at the 6th month. Moreover, the increase observed for peroxide value of each sample, including the reference, was found statistically significant (i.e., $p<0.05$). The oxidation status of each sample was also analyzed with other experiments.

The measurement of absorptions at 232 nm (i.e., K_{232}) and 270 nm (i.e., K_{270}) are important parameters for the estimation of oxidation stage of olive oil. The increase in the number of conjugated diene and trienes contribute to K_{232}, while the secondary oxidation resulting in the formation of aldehydes and ketones is effective for K_{270}.[23] The European Regulation standard limit value for olive oil expresses $K_{232} \leq 2.5$ for extra virgin olive oil and $K_{232} \leq 2.6$ for both virgin olive oil, and ordinary olive oil. On the other hand, K_{270} values are restricted to ≤ 0.2 for extra virgin olive oil and ≤ 0.25 for both virgin olive oil, and ordinary olive oil.[24] As seen in Figure 3, all of the samples, regardless of their analysis time and extraction procedure, have K_{232} levels less than 2.5. However, it is obvious that there is an increase in the K_{232} levels from 3rd to 6th month samples making the K_{232} levels getting closer to upper limit of 2.5, and 2.6 for the extra virgin olive oil, virgin olive oil, and ordinary olive oil, respectively. On the other

hand, the K_{270} measurements at 3 month samples classified almost all of the samples (i.e., beside the sample 9F) under the extra virgin olive oil quality (i.e., Figure 4). However, similar to the observation obtained for the K_{232} values, all of the samples tested in their 6th month obviously indicated an increase all above 0.25. This is an absolute indication of oxidation determined by UV studies (i.e., K_{232} and K_{270} measurements) concomitant to the results obtained for the peroxide measurements. Besides the K_{232} value change for the samples 2 (i.e., 2F and 2O), and 4 (i.e., 4F and 2O) and K_{270} value changes for the samples 5 (i.e., 5F and 5O), and 9 (i.e., 9F and 9O), and the reference, all changes for the rest of the samples were found to be statistically significant (i.e., $p<0.05$).

Phenolic compounds are also present in olive oil. They are not only important for the biological systems with respect to their antioxidant capacity but also significant parameters that show the level of oxidation in olive oil.[25-26] According to the results we have obtained for the total phenol contents of the samples (i.e., Figure 5), it was obvious that the total phenol content of the samples were quite low in comparison to industrially produced reference olive oil product in both 3rd and 6th month analysis. Furthermore, hot and cold extraction techniques does not make difference in terms of the presence of phenolic compounds, since both technique employed products have a total phenol content ranging around 70 ppm to very low 10 ppm. It is implying that the missing control systems in the production of traditionally produced olive oils (e.g., high exposure to light and air) and the insufficiency of the packaging result in oxidation of phenolic compounds. This is totally consistent with the previous results obtained in previous experiments (i.e., PV values, K232, and K270 measurements) displaying the high exposure of samples to oxidation. It is also clear that the decrease in TPC was also found statistically significant for each sample, including the reference (i.e., $p<0.05$). In contrast to the regional belief of public, this status also questions the nutritional level of the olive oil produced under primitive conditions without the presence of industrialized systems. The percent free fatty acid, peroxide, and K_{232} and K_{270} measurements of the samples and the reference concomitant to statistical analyses are provided in detail on Table 2.

Beside their function for the coloration, pigment contents (i.e., chlorophylls and carotenoids), present in olive oil, are not only critical for the stability of the olive oil but also for their antioxidant activity.[27] Therefore, the change in the level of these

compounds is another indication to measure the level of oxidation in olive oil samples. As shown in Figure 6, each of the samples tested in both 3rd and 6th months was found to possess a quite lower chlorophyll amount in comparison to the reference olive oil. On the other hand, the carotenoid levels were also found quite lower in comparison to the reference product (i.e., Figure 7). In general, chlorophyll, and carotenoid levels are expected to be around 1-3ppm range.[28] Therefore, the levels obtained for the sample olive oils definitely show their lower content in terms of these pigment contents. Besides the samples 7, 8, 13, and the reference, the changes for the chlorophyll, and the carotenoid levels were all found statistically insignificant (i.e., $p>0.05$). Similar to the results obtained for total phenol content of the samples analyzed, this status implies the insufficiency in both production conditions and packaging systems making the olive oil products highly susceptible to oxidation. The measurements on total phenol and pigment content of the samples concomitant to statistical analyses are provided in detail on Table 3.

The composition of the fatty acids in the samples tested was measured via a GC method. Table 4 represents the results obtained for the six major fatty acids considered in this study (i.e., palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), and linoleic acid (C18:2), and linolenic acid (C18:3)). At first, it is noteworthy to state that there is a decrease in the fatty acid composition of the samples analyzed from three to six month analysis, regardless of the fatty acid type. The percentage of oleic acid content of olive oil samples varied from 30 to 46% both at their 3rd and 6th month analysis (i.e., Figure 8). This indicates that none of the samples can be classified as extra virgin olive oil, since IOOC confirms 55-83% olive oil presence in extra virgin olive oils.[20] Furthermore, the analysis of other fatty acids also pointed that their ratio is at the lower limits of appreciable amounts, expressed by IOCC. The presence of oxidation proven via several methods followed might clearly explain the loss of fatty acids in the samples to be oxidized to other ingredients such as polyenes in the first state, and aldehydes and ketone in the second stage. In contrast to samples, the reference product was shown to possess extra virgin olive oil quality at both 3rd and 6th month analysis. Even, beside the change for palmitic acid (C16:0), the rest of the changes for each fatty acid analyzed was found insignificant (i.e., $p>0.05$).

Conclusion

Although the olive oil production and consumption has been very popular in Northern Cyprus, the quality indices of the oil prepared via traditional methods were found to be lower with respect to the results of this study. Indeed, in almost all tests employed, the exposure to oxidation was quite unique to these products regardless of the extraction procedure employed (i.e., hot and cold extractions). This absolutely implies the insufficiency in production techniques, which may be mainly attributed to the control insufficiency from light and air, during the production process. Furthermore, the deficiencies regarding the packaging of the oil produced are another drawback to limit the shelf-life of these products.

This study, known to be conducted for the first time in Northern Cyprus, has pointed out to the high oxidation exposure of olive oil produced within the country employing the traditional techniques. Therefore, the results will contribute to the awareness of both producers and consumers. Particularly, it will possess a significant effect on producers to change their production characteristics in terms of paying attention to control systems (e.g., harvesting and production periods, packaging, and appropriate labeling) to prevent the continuous oxidation in olive oil produced.

Acknowledgements

This work was supported by Faculty of Pharmacy, Eastern Mediterranean University, research funds.

Conflict of Interest Statement

The authors have declared no conflict of interest.

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Table 1: The origin, extraction type, and the coding of the olive oil samples employed.

Code	Place of origin	Extraction type
1	Bostanci (Zodia)	Cold
2	Camlikoy (Camlikoy)	Cold
3	Yesilirmak (Limnidi)	Cold
4	Yesilirmak (Limnidi)	Cold
5	Guzelyurt (Morphou)	Cold
6	Guzelyurt (Morphou)	Cold
7	Yedidalga (Potamos du Gambo)	Hot
8	Yedidalga (Potamos du Gambo)	Hot
9	Yedidalga (Potamos du Gambo)	Hot
10	Yesilirmak (Limnidi)	Hot
11	Yesilyurt (Pentagia)	Hot
12	Yesilyurt (Pentagia)	Hot
13	Yesilyurt (Pentagia)	Hot

Table 2: Percent free fatty acid, peroxide, and K232, and K270 measurements

Samples	FFA %	p (FFA)	PV ($meqO_2/Kg$)	p (PV)	K_{232}	p (K_{232})	K_{270}	p (K_{270})
1F	1.06 ± 0.07	**0.009** *	17.66 ± 0.36	**0.001** *	0.48 ± 0.02	**0.005** *	0.08 ± 0.01	**0.002** *
1O	1.65 ± 0.08		28.63 ± 0.34		1.29 ± 0.08		0.49 ± 0.02	
2F	1.69 ± 0.04	**0.008** *	15.21 ± 0.08	**0.000** *	1.04 ± 0.03	**0.002** *	0.16 ± 0.02	**0.053** *
2O	2.38 ± 0.12		32.60 ± 0.26		1.71 ± 0.02		0.27 ± 0.03	
3F	1.19 ± 0.09	**0.002** *	14.76 ± 0.27	**0.004** *	0.90 ± 0.03	**0.001** *	0.13 ± 0.02	**0.002** *
3O	2.15 ± 0.10		17.37 ± 0.31		1.34 ± 0.05		0.47 ± 0.03	
4F	1.19 ± 0.11	**0.025** *	13.48 ± 0.36	**0.003** *	0.95 ± 0.06	**0.003** *	0.14 ± 0.02	**0.003** *
4O	1.70 ± 0.05		22.76 ± 0.54		1.48 ± 0.01		0.25 ± 0.02	
5F	0.97 ± 0.06	**0.011** *	13.90 ± 0.18	**0.001** *	0.67 ± 0.01	**0.000** *	0.17 ± 0.02	0.057
5O	1.22 ± 0.06		21.81 ± 0.51		1.57 ± 0.02		0.26 ± 0.03	
6F	1.06 ± 0.08	**0.009** *	17.66 ± 0.36	**0.001** *	0.49 ± 0.02	**0.005** *	0.08 ± 0.01	**0.002** *
6O	1.66 ± 0.09		28.63 ± 0.34		1.30 ± 0.08		0.49 ± 0.02	
7F	1.38 ± 0.05	**0.009** *	12.47 ± 0.38	**0.000** *	0.81 ± 0.08	0.054	0.16 ± 0.04	**0.004** *
7O	1.66 ± 0.06		28.76 ± 0.28		1.08 ± 0.04		0.31 ± 0.02	
8F	1.17 ± 0.02	**0.001** *	13.43 ± 0.61	**0.028** *	0.85 ± 0.03	**0.008** *	0.15 ± 0.02	**0.016** *
8O	1.84 ± 0.03		17.16 ± 0.50		1.20 ± 0.08		0.25 ± 0.02	
9F	1.53 ± 0.03	**0.010** *	12.27 ± 0.21	**0.013** *	0.86 ± 0.05	0.054	0.23 ± 0.02	0.146
9O	1.91 ± 0.10		16.92 ± 0.79		1.19 ± 0.09		0.29 ± 0.05	
10F	1.09 ± 0.07	**0.003** *	17.56 ± 0.28	**0.003** *	0.76 ± 0.03	**0.003** *	0.09 ± 0.03	**0.004** *
10O	1.43 ± 0.07		23.50 ± 0.33		1.37 ± 0.06		0.25 ± 0.03	
11F	1.12 ± 0.05	**0.001** *	21.60 ± 0.20	**0.000** *	1.07 ± 0.03	**0.002** *	0.15 ± 0.03	**0.028** *
11O	1.59 ± 0.06		28.20 ± 0.17		1.64 ± 0.06		0.24 ± 0.02	
12F	0.92 ± 0.04	**0.001** *	8.96 ± 0.78	**0.005** *	0.81 ± 0.04	0.063	0.12 ± 0.03	**0.000** *
12O	1.59 ± 0.05		17.56 ± 0.30		1.56 ± 0.04		0.22 ± 0.03	
13F	1.05 ± 0.03	**0.001** *	10.26 ± 0.13	**0.002** *	0.86 ± 0.03	**0.003** *	0.12 ± 0.02	**0.002** *
13O	1.89 ± 0.04		17.42 ± 0.45		1.82 ± 0.08		0.51 ± 0.04	
Reference F	0.39 ± 0.05	**0.027** *	10.02 ± 0.20	**0.000** *	0.58 ± 0.03	**0.014** *	0.10 ± 0.02	0.138
Reference O	0.66 ± 0.04		15.94 ± 0.15		1.11 ± 0.09		0.15 ± 0.02	

F, O, and R represent 3 month, 6 month and reference samples, respectively.

*: significant if $p<0.05$

Table 3: Total phenol and pigment content

Samples	TPC (ppm)	p (TPC)	Chlorophylls (ppm)	p (Chlp)	Carotenoids (ppm)	p (Car)
1F	30.27 ± 0.81	**0,000***	0.14 ± 0.05	0,074	1.27 ± 0.06	0,530
1O	6.16 ± 0.25		0.14 ± 0.03		1.20 ± 0.20	
2F	21.40 ± 0.10	**0,000***	0.11 ± 0.01	0,423	0.99 ± 0.08	0,131
2O	14.13 ± 0.06		0.10 ± 0.01		0.91 ± 0.03	
3F	75.03 ± 1.14	**0,002***	0.14 ± 0.01	0,122	1.08 ± 0.13	0,236
3O	33.03 ± 1.97		0.10 ± 0.02		0.97 ± 0.17	
4F	67.77 ± 0.65	**0,000***	0.13 ± 0.01	0,225	0.90 ± 0.00	0,339
4O	31.57 ± 0.61		0.12 ± 0.01		0.86 ± 0.06	
5F	22.53 ± 0.67	**0,003***	0.15 ± 0.02	0,213	0.86 ± 0.06	0,093
5O	18.90 ± 0.82		0.11 ± 0.03		0.82 ± 0.04	
6F	30.27 ± 0.81	**0,000***	0.14 ± 0.05	0,742	1.27 ± 0.06	0,529
6O	6.16 ± 0.25		0.14 ± 0.03		1.20 ± 0.20	
7F	51.70 ± 0.36	**0,002***	0.13 ± 0.04	0,173	1.17 ± 0.06	**0,020***
7O	41.33 ± 0.45		0.09 ± 0.03		0.93 ± 0.06	
8F	37.30 ± 1.71	**0,013***	0.15 ± 0.01	**0,010***	1.10 ± 0.17	0,529
8O	26.13 ± 1.16		0.11 ± 0.01		1.03 ± 0.06	
9F	17.87 ± 0.49	0,051	0.13 ± 0.03	0,576	1.17 ± 0.06	0,199
9O	15.77 ± 0.49		0.11 ± 0.04		1.00 ± 0.10	
10F	20.60 ± 0.53	**0,001***	0.15 ± 0.02	0,474	1.02 ± 0.04	0,303
10O	12.40 ± 0.20		0.12 ± 0.05		0.98 ± 0.02	
11F	18.97 ± 0.25	**0,001***	0.10 ± 0.02	0,860	1.13 ± 0.06	0,055
11O	8.43 ± 0.31		0.11 ± 0.01		0.96 ± 0.04	
12F	76.83 ± 0.64	**0,000***	0.12 ± 0.03	0,383	1.20 ± 0.10	0,067
12O	15.00 ± 0.10		0.09 ± 0.02		0.94 ± 0.04	
13F	27.20 ± 0.36	**0,000***	0.11 ± 0.01	0,095	1.17 ± 0.02	**0,016***
13O	9.90 ± 0.20		0.08 ± 0.01		0.83 ± 0.06	
Reference F	263.73 ± 1.35	**0,001***	1.08 ± 0.02	**0,009***	2.53 ± 0.04	**0,014***
Reference O	212.83 ± 2.32		1.02 ± 0.03		2.48 ± 0.03	

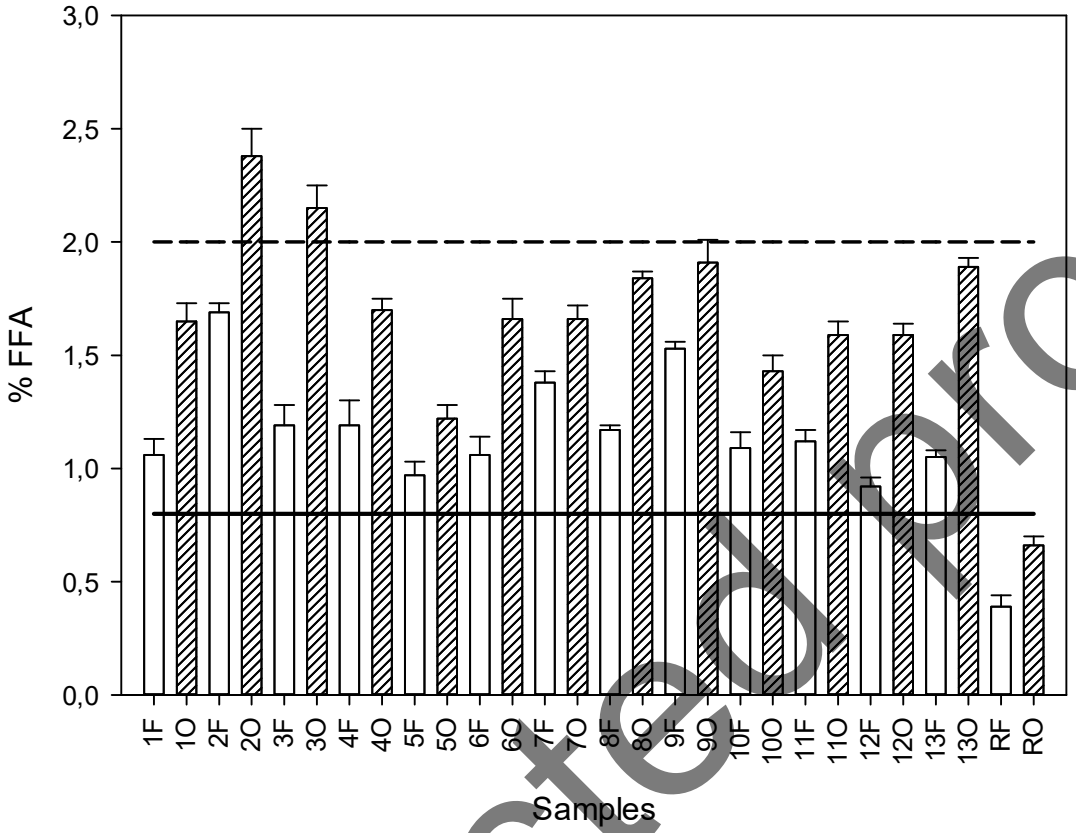
F, O, and R represent 3 month, 6 month and reference samples, respectively.

*: significant if $p<0.05$

Table 4: Fatty acids composition of the samples and the reference.

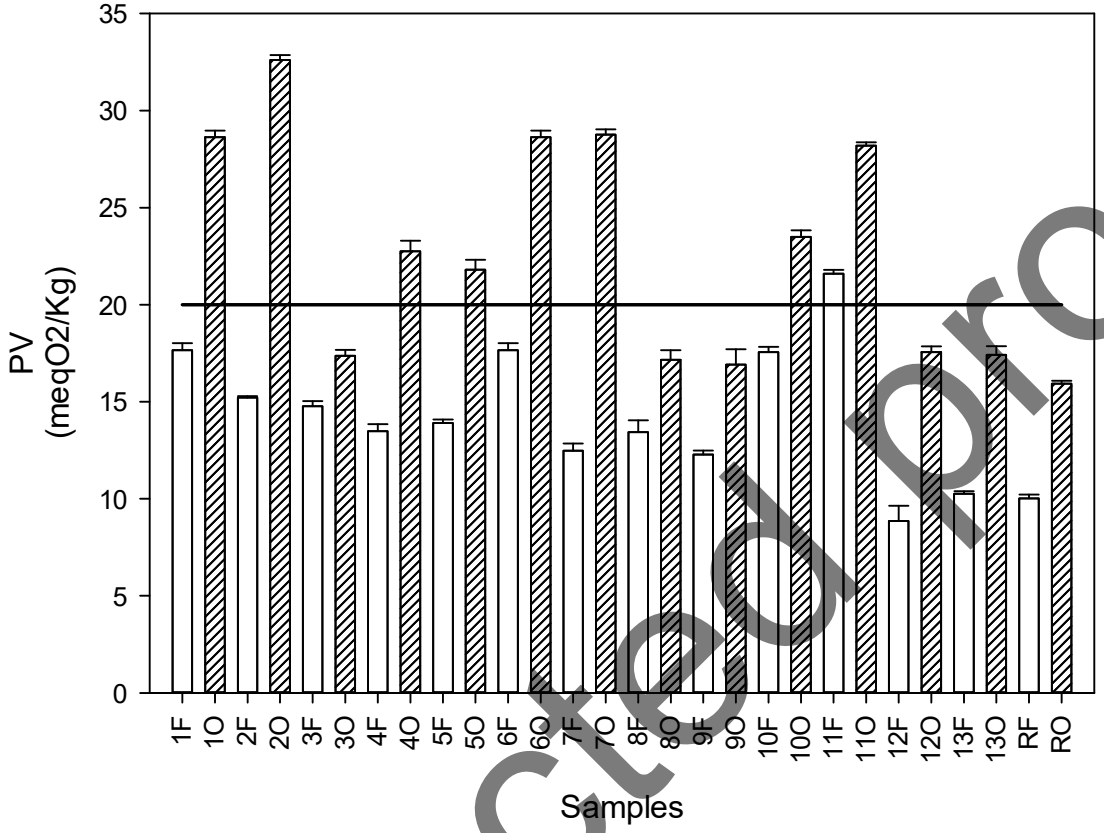
Samples	C16:0 (%)	p (C16:0)	C16:1 (%)	p (C16:1)	C18:0 (%)	p (C18:0)	C18:1 (%)	p (C18:1)	C18:2 (%)	p (C18:2)	C18:3 (%)	p (C18:3)
1F	6.79 ± 0.17	0.071	0.39 ± 0.02	**0.011***	1.57 ± 0.02	**0.000***	43.94 ± 0.13	**0.000***	5.56 ± 0.06	**0.000***	0.18 ± 0.01	0.130
1O	6.02 ± 0.21		0.27 ± 0.04		1.33 ± 0.02		34.83 ± 0.04		3.92 ± 0.06		0.16 ± 0.02	
2F	7.87 ± 0.06	**0.000***	0.33 ± 0.01	**0.014***	2.29 ± 0.04	**0.023***	34.63 ± 0.10	**0.007***	7. 52 ± 0.18	0.255	0.22 ± 0.02	**0.017***
2O	5.96 ± 0.06		0.26 ± 0.02		1.75 ± 0.10		31.21 ± 0.46		7.14 ± 0.26		0.15 ± 0.03	
3F	7.22 ± 0.03	**0.038***	0.35 ± 0.02	**0.037***	2.49 ± 0.08	**0.022***	41.58 ± 1.55	0.156	6.26 ± 0.10	**0.004***	0.26 ± 0.02	0.078
3O	7.02 ± 0.10		0.21 ± 0.04		1.99 ± 0.06		38.07 ± 1.20		5.25 ± 0.04		0.19 ± 0.02	
4F	10.24 ± 0.10	**0.010***	0.59 ± 0.03	**0.032***	2.38 ± 0.04	**0.015***	43.67 ± 0.49	**0.002***	7.61 ± 0.31	**0.002***	0.32 ± 0.02	**0.001***
4O	7.69 ± 0.45		0.38 ± 0.04		1.90 ± 0.10		32.10 ± 1.04		5.02 ± 0.19		0.15 ± 0.01	
5F	8.50 ± 0.11	**0.003***	0.44 ± 0.04	0.059	2.05 ± 0.08	0.176	40.27 ± 0.45	**0.005***	7.82 ± 0.21	**0.026***	0.25 ± 0.02	0.324
5O	7.41 ± 0.19		0.32 ± 0.03		1.89 ± 0.19		35.79 ± 0.31		6.97 ± 0.22		0.22 ± 0.03	
6F	6.79 ± 0.17	0.071	0.39 ± 0.02	**0.011***	1.57 ± 0.02	**0.000***	43.94 ± 0.13	**0.000***	5.56 ± 0.06	**0.000***	0.18 ± 0.01	0.130
6O	6.02 ± 0.21		0.27 ± 0.04		1.33 ± 0.02		34.83 ± 0.04		3.92 ± 0.06		0.16 ± 0.02	
7F	10.06 ± 0.14	**0.045***	0.76 ± 0.05	**0.015***	2.51 ± 0.09	**0.001***	43.73 ± 0.35	0.178	8.34 ± 0.29	**0.001***	0.31 ± 0.02	0.058
7O	9.13 ± 0.47		0.51 ± 0.03		1.59 ± 0.08		41.86 ± 1.50		4.93 ± 0.16		0.21 ± 0.05	
8F	9.50 ± 0.10	0.065	0.54 ± 0.07	**0.019***	2.47 ± 0.04	**0.006***	41.63 ± 0.38	**0.015***	7.57 ± 0.42	0.280	0.29 ± 0.03	0.069
8O	8.30 ± 0.46		0.40 ± 0.04		2.06 ± 0.08		38.60 ± 0.26		6.84 ± 0.57		0.25 ± 0.03	
9F	8.97 ± 0.15	0.069	0.67 ± 0.04	**0.046***	2.22 ± 0.11	0.083	41.73 ± 0.76	**0.015***	7.55 ± 0.05	**0.003***	0.28 ± 0.03	**0.034***
9O	8.23 ± 0.32		0.45 ± 0.06		1.81 ± 0.13		36.97 ± 0.93		6.15 ± 0.17		0.18 ± 0.01	
10F	7.84 ± 0.07	**0.001***	0.45 ± 0.04	0.148	1.95 ± 0.06	0.054	36.83 ± 0.31	**0.003***	5.38 ± 0.08	**0.001***	0.18 ± 0.01	0.157
10O	5.68 ± 0.20		0.35 ± 0.05		1.59 ± 0.11		31.50 ± 0.26		4.77 ± 0.05		0.13 ± 0.03	
11F	7.44 ± 0.48	0.434	0.52 ± 0.04	0.119	1.41 ± 0.09	**0.026**	36.43 ± 0.27	**0.000***	5.61 ± 0.07	**0.001***	0.18 ± 0.02	0.188
11O	7.13 ± 0.08		0.37 ± 0.08		1.30 ± 0.11		33.53 ± 0.21		4.76 ± 0.04		0.15 ± 0.01	
12F	6.90 ± 0.19	**0.006***	0.39 ± 0.04	**0.012***	1.65 ± 0.08	0.148	39.77 ± 0.31	0.067	4.46 ± 0.09	**0.003***	0.18 ± 0.02	0.192
12O	6.01 ± 0.11		0.30 ± 0.03		1.52 ± 0.05		37.63 ± 0.99		3.33 ± 0.12		0.15 ± 0.01	
13F	9.92 ± 0.30	**0.005***	0.77 ± 0.05	**0.007***	2.16 ± 0.06	**0.023***	46.60 ± 0.26	**0.001***	6.48 ± 0.07	**0.001***	0.28 ± 0.01	**0.006***
13O	8.33 ± 0.46		0.49 ± 0.06		1.80 ± 0.04		30.60 ± 0.70		4.40 ± 0.11		0.16 ± 0.01	
Reference F	9.28 ± 0.03	**0.015***	1.18 ± 0.01	0.115	3.44 ± 0.05	0.313	62,57 ± 0.72	0.457	8.89 ± 0.02	0.097	0.50 ± 0.04	0.742
Reference O	9.20 ± 0.03		1.11 ± 0.06		3.37 ± 0.06		61,50 ± 1.48		8.75 ± 0.06		0.52 ± 0.06	

Figure 1: Percent free fatty acid of the samples and the reference



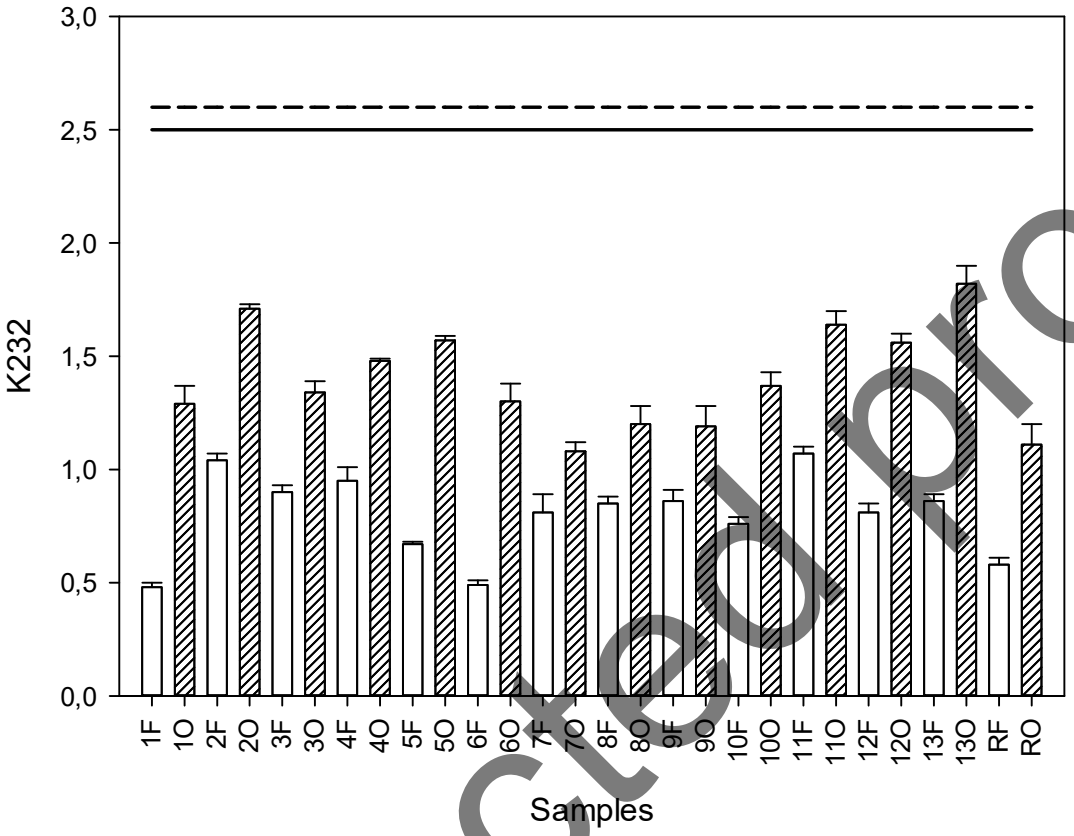
F, O, and R represent 3 month, 6 month and reference samples, respectively. The solid and the dashed lines indicate the highest levels for the free acid content of the extra virgin olive oil, and the virgin olive oil, respectively.

Figure 2: Peroxide value of the samples and the reference



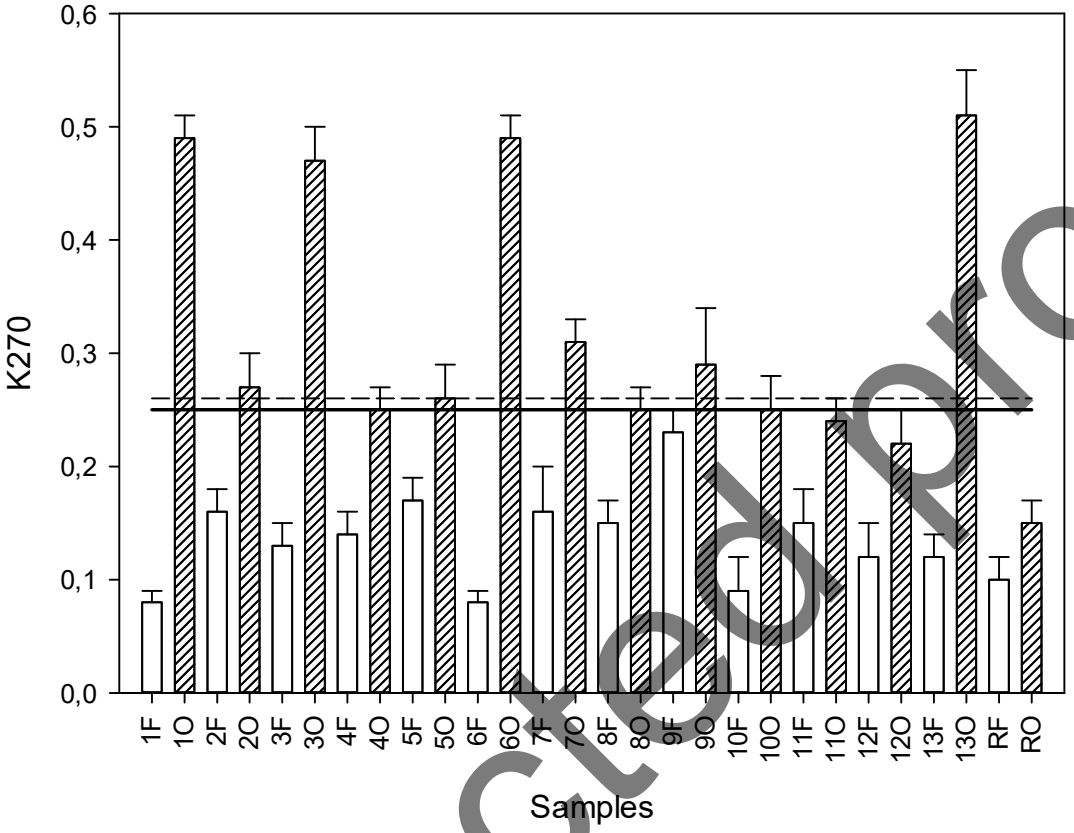
F, O, and R represent 3 month, 6 month and reference samples, respectively. The solid line indicates the upper standard for the peroxide level.

Figure 3: K_{232} level of the samples and the reference



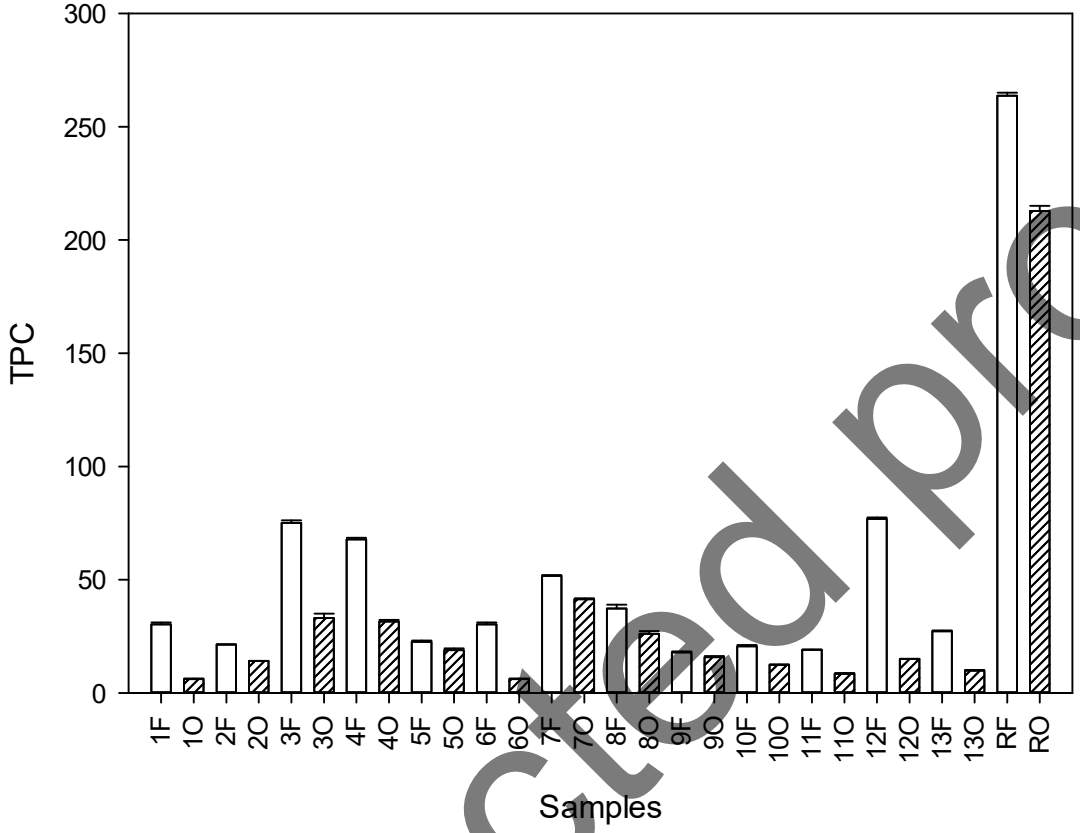
F, O, and R represent 3 month, 6 month and reference samples, respectively. The solid line, and the dashed lines indicate the upper standards respectively for the extra virgin olive oil, and virgin olive oil.

Figure 4: K_{270} level of the samples and the reference



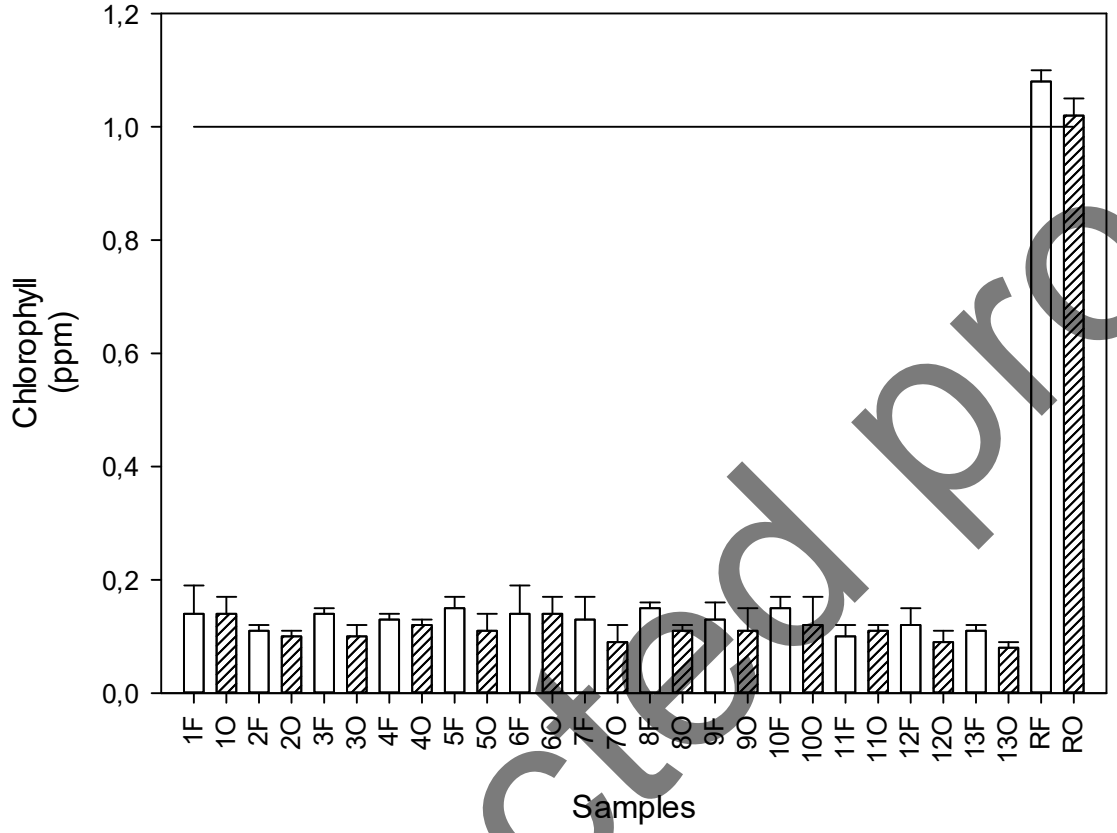
F, O, and R represent 3 month, 6 month and reference samples, respectively. The solid line, and the dashed lines indicate the upper standards respectively for the extra virgin olive oil, and virgin olive oil.

Figure 5: Total phenol content of the samples and the reference



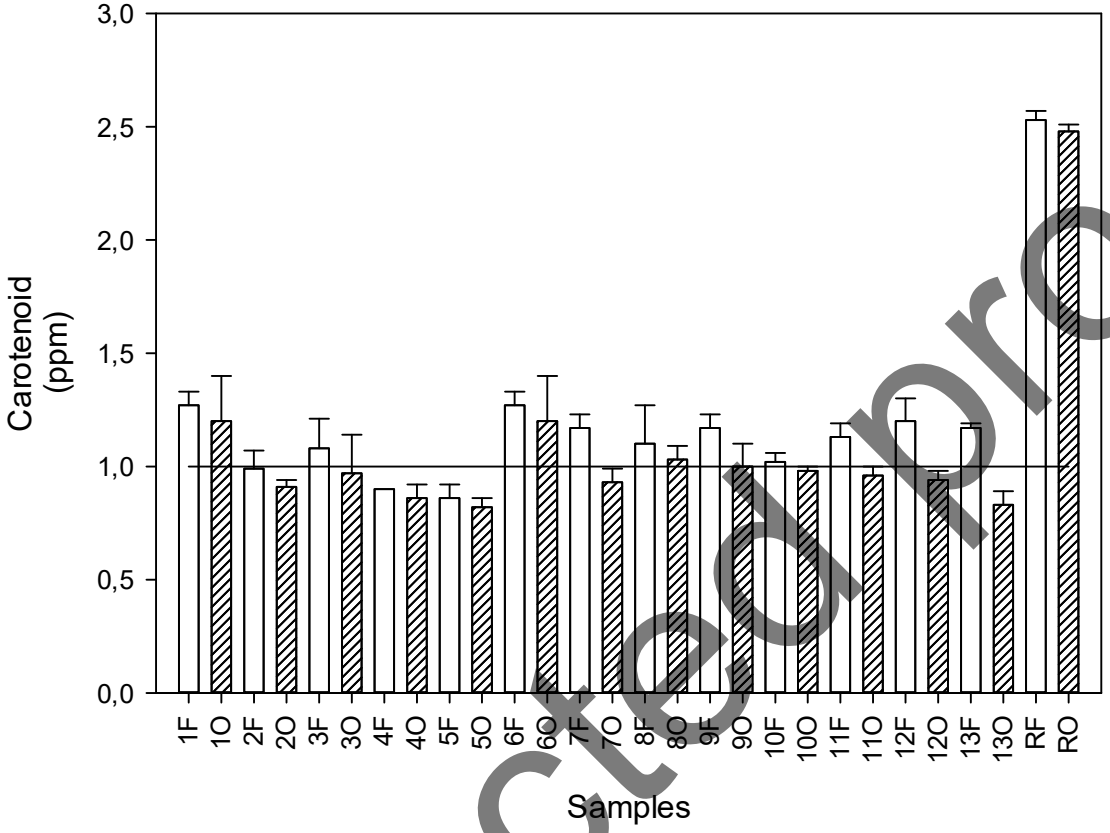
F, O, and R represent 3 month, 6 month and reference samples, respectively.

Figure 6: Chlorophyll content of the samples and the reference



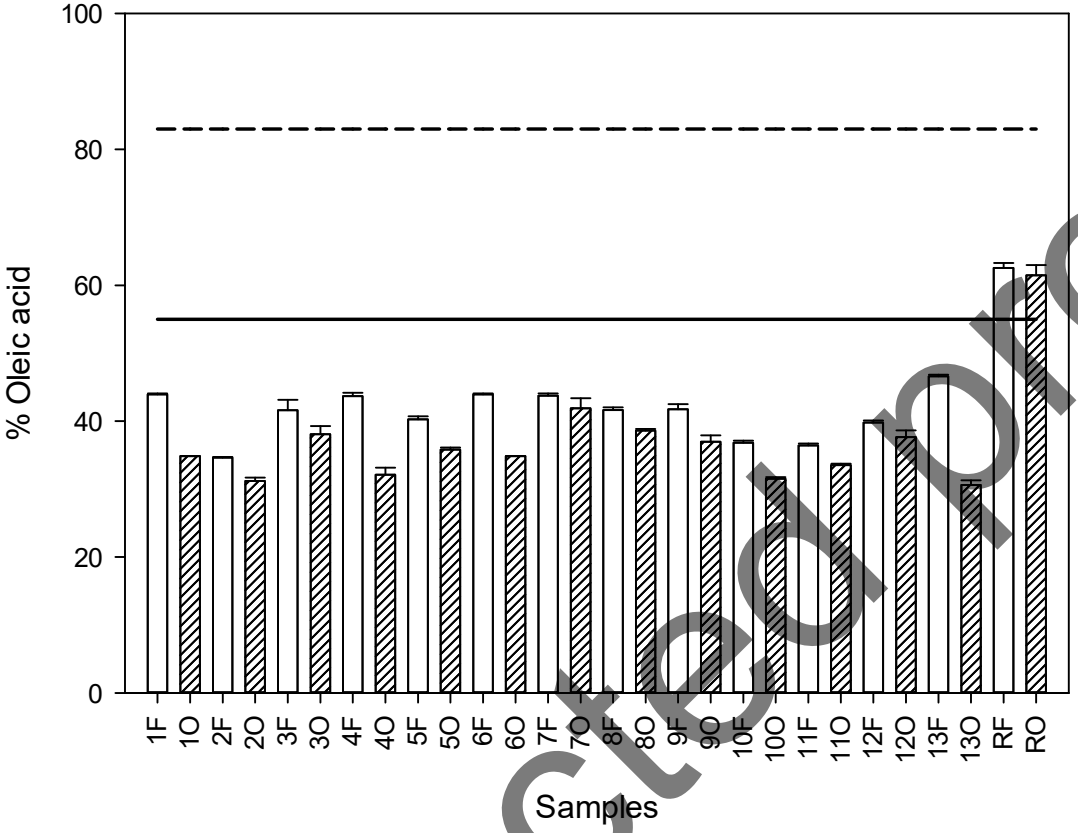
F, O, and R represent 3 month, 6 month and reference samples, respectively. The solid line indicates the expected lowest standard for the extra virgin olive oil.

Figure 7: Carotenoid content of the samples and the reference



F, O, and R represent 3 month, 6 month and reference samples, respectively. The solid line indicates the expected lowest standard for the extra virgin olive oil.

Figure 8: Percent oleic acid of the samples and the reference



F, O, and R represent 3 month, 6 month and reference samples, respectively. The solid line, and the dashed lines indicate the standard range for the extra virgin olive oil.