Research Article

Screening of physico-chemical parameters of commercial Extra Virgin Olive Oils to verify their compliance with EU Regulation

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Running tittle: Quality parameters of EVOOs along their "best-before" date.

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Keywords: extra virgin olive oil / oxidation / peroxide value / acidity / extinction coefficient / regulation / compliance

Abbreviations: **EVOO**, Extra Virgin Olive Oil, **PV**, Peroxide value, **K**, extinction coefficient, **CV**, Coefficient of Variation, **VOO,** Virgin Olive Oil, **LOO,** Lampante Olive Oil

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Screening of physico-chemical parameters of commercial Extra Virgin Olive Oils to verify their compliance with EU Regulation

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Abstract: The objective of this project is to evaluate whether Extra Virgin Olive Oil (EVOO) for sale comply or not with the regulation by determining three physico-chemical parameters (acidity, peroxide value and extinction coefficients at 232 and 268 nm). In this research article, we focus on analysing the EVOO quality parameters during the shelf-life until 1 month to the “best-before” date. The results obtained during the study were compared with the limit values of current EU regulation, to verify if they complied or not. At last, a 14.6% non-compliance with the regulation was obtained. These non-compliances are due to oxidative deterioration, since the acidity conforms for all the EVOOs analysed.

Practical application: Knowing compliance of marketed Extra Virgin Olive Oils with regulation can be helpful for packaging industries when setting a “best-before” date.

Keywords: extra virgin olive oil / oxidation / peroxide value / acidity / extinction coefficient/ compliance / regulation

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1 Introduction

Virgin olive oil is obtained from the fruits of *Olea europaea* only by mechanical procedures, and among them Extra Virgin Olive Oil (EVOO) is the highest quality oil, with an impeccable taste and odor and without defects.[1] This type of oil is an important product of the Mediterranean diet and highly appreciated for its beneficial health effects due to the presence of mono-unsaturated fatty acids and for its antioxidant properties. Chemically, it is divided into a saponifiable and an unsaponifiable fraction. The saponifiable fraction is the main part, accounting for about 98-99% of the oil, and it mainly consists of monoglycerides, diglycerides and triglycerides. Monoglycerides and diglycerides come both from incomplete triglycerides synthesis and triglycerides hydrolysis, which gives rise to monoglycerides, diglycerides and free fatty acids. The unsaponifiable fraction accounts for approximately the 2% of the oil, and contains compounds with antioxidant properties such as polar and apolar phenols (mainly secoiridoids and lignans and tocopherols, respectively), as well as pigments (chlorophylls, pheophytins, carotenoids), sterols and squalene, among others.[2]

The quality of virgin olive oils is defined by different parameters, such as acidity, peroxide value (PV), extinction coefficients (K_{232}, K_{268} and ΔK) and sensory evaluation. Ks and PV evaluate the grade of oxidative deterioration, while the acidity evaluates the hydrolytic alteration. According to these parameters, virgin olive oils are classified into different commercial categories, two edible (EVOO and Virgin Olive Oil - VOO) and one inedible (Lampante Olive Oil - LOO):[3]

- EVOO: with an excellent flavour, acidity in oleic acid ≤0.8 gram per 100 grams, $K_{232} \leq 2.50$, $K_{268} \leq 0.22$, PV ≤ 20 mEq O_2/kg.
- VOO: similar conditions of EVOO, except for the tolerance for slight sensory defects, the acidity in oleic acid, which must be a maximum of 2 grams per 100 grams, $K_{232} \leq 2.60$, $K_{268} \leq 0.25$, PV ≤ 20 mEq O_2/kg.
- LOO: with defective flavour or the physico-chemical parameters exceeding limits fixed for VOO.

The peroxide value is a quality parameter that determines the primary oxidation, allowing to determine whether EVOOs have undergone any oxidation reactions.[4] Peroxides are formed when an oxygen molecule reacts with a lipid radical coming from unsaturated fatty acids. According to the EU regulation, this parameter must be lower than or equal to 20 meq O_2/kg of oil for VOO and EVOO.[3]

UV absorbance at given wavelengths (232 and 268 nm) indicates the presence of oxidation compounds in oils. Specifically, the levels of oxidation compounds are measured by the specific extinction at these wavelengths, expressed as K_{232} and K_{268}.
In particular, the value of K_{232} indicates the presence of conjugated dienes, which can come from primary oxidation of polyunsaturated fatty acids and is usually correlated with the amount of peroxides present in the oil.[5,6,7] EU regulation establishes that for EVOOs this value must be less than or equal to 2.5.[3] The value of K_{268} indicates the presence of conjugated trienes and of carbonylic compounds in olives with conjugated double bonds (aldehydes and ketones, coming from secondary oxidation).[8,9] EU regulation establishes that for EVOOs must be less than or equal to 0.22.[3]

The oxidation reactions are highly influenced by the presence of oxygen, light, heat, and the presence of free metals. Therefore, the greater the exposure to these factors, the higher the oxidation and the value of these oxidation parameters.[4]

Acidity is caused by hydrolytic alterations leading to the rupture of bonds between glycerol and the fatty acids that compose the glycerides. This alteration is caused by low phytosanitary conditions of olives, by damage of olives during the collection and the storage before milling or inappropriate conditions during oil storage.[10] Acidity measures the amount of free fatty acids in the oil, expressed as percentage of oleic acid. Low acidity values point out that the oil has been produced properly, from healthy and fresh olives.[10,11] According to the EU regulation, in EVOO this parameter must be less than or equal to 0.8%.[3]

Previous studies carried out by consumers' associations[12,13] warned about the commercialisation of EVOOs that do not comply with the requirements for this category. These studies claimed frauds, although the intentionality in the labelling disconformity is not demonstrable. It is important to stand out that during the conservation the oils' quality can decrease with the consequent modification of their qualitative parameters over time, which may cause non-compliances with the current regulations. The principal alteration of oils during storage is due to oxidative deterioration.[14] So, changes in the physico-chemical parameters and unwanted flavours may appear during storage due to the incidence of different factors, as oxygen, temperature and light.[15,16,17] This fact must be taken into account by the packager or producer when fixing the "best-before" date. Until the expiration of this date, the company guarantees the quality of the EVOO and its compliance with the current regulations, as long as it has been stored under the recommended conditions. The foresight of the oil's shelf-life is usually carried out taking into account its compositional characteristics, which affect the resistance of the oil to oxidation, and usual shelf storage conditions.[18] Improper storage conditions or incorrect shelf-life estimation may cause the quality parameter limits set for the EVOO category to be exceeded before the "best-before" date, giving rise to non-compliant samples.

The aim of this project is to evaluate whether EVOOs present in the market comply with the current EU regulation[3] by determining physico-chemical quality parameters at distinct periods (1, 2-3, 4-6, and 7-8 months) before the "best-before" date declared on the label.

2 Materials and methods

2.1 Materials

2.1.1 Samples

For this study, 41 EVOOs were purchased in supermarkets (Barcelona, Spain) and analysed according to their "best-before" date. In some cases, more bottles of the same oil and batch were purchased and stored at simulated shelf conditions (room temperature, artificial light by day, darkness by night) to be analysed at different dates before their "best-before" date. It is indispensable to open a new oil bottle for each analysis time to avoid any external factor that can influence the product quality.

The dates of analysis for each oil are reported in Table S1 of the Supplementary material, where we can observed that 55 bottles of oil were analysed.

Samples included supermarket own brands as well as large and small producers. Eight own brands from supermarket chains with the highest market share in Spain were taken. The market share of these supermarkets was calculated by their turnover in 2018, and it was estimated to cover around 60% of the Spanish market share.[19] All of these oils were purchased during October 2019.

At each analysis time, a bottle was opened and three oil aliquots were introduced in amber glass vials flushed with a nitrogen stream, and stored at -20°C until analysis. Just before the analysis, the samples were thawed in a water bath at 18ºC and at room temperature until a complete homogenization was achieved.

2.1.2 Reagents

To determine the PV, chloroform for analysis, ACS grade, glacial acetic acid for analysis, potassium iodide, for analysis, ACS grade, starch, 1% w/v solution and sodium thiosulfate, 0.1 N standardized solution were purchased at Scharlab (Barcelona, Spain).

To determine the Ks, isooctane, for analysis, ACS grade, was purchased at Scharlab.

To determine the acidity, diethyl ether for analysis and phenolphthalein were purchased at Scharlab, ethanol 96% (v / v), ACS grade at PANREAC (Barcelona, Spain) and Potassium hydroxide standardized solution in ethanol 0.1 N at Quimivita (Barcelona, Spain).

2.2 Methods

All determinations were carried out in duplicate.

2.2.1 Extinction coefficient

The extinction coefficients (K_{232}, K_{268} and ΔK) were determined according to the ECC method.[3]

2.2.2 Peroxide Value

The PV was determined according to the ECC method.[3] To avoid the oxidation of the sample during the analysis, it was carried out under attenuated light.

2.2.3 Acidity

The acidity was determined according to the ECC method.[3]

In order to guarantee the quality of the results obtained, before the analyses the analyst was trained until a satisfactory intra-laboratory repeatability was achieved: RSD=0.8% for PV (mean value = 8.76; n = 6), RSD=1.6% for K_{232}, (mean value = 1.752; n = 7) RSD=1.7% for K_{268} (mean value = 0.126; n = 8) and RSD=2.0% for acidity (mean value = 0.149; n= 8). These values fulfil the specifications stated in the ISO official methods for these determinations.[20,21,22]

3 Results and discussion

The Ks, PV and acidity values obtained for the 41 EVOOs analysed at different periods before their "best-before" date are represented in different boxplots (Figures 1-5). Samples are grouped into 4 sets according to the time remaining to the "best-before" date: 1 month (n = 15); 2-3 months (n = 10); 4-6 months (n = 24); and 7-8 months (n = 6). With this representation, we can graphically observe whether EVOOs comply with the regulation according to proximity of the declared shelf-life end.

The data used to make the different boxplots can be found in Table S2 of the Supplementary material.

3.1 K_{232}, K_{268} and ΔK

Figure 1 shows that there is one non-compliance for the K_{232} parameter, accounting for the 2.4% of the EVOOs analysed. The non-compliance is at 3 months remaining to the "best-before" date, which represents the 10% of the 2-3 months sample set. Regarding K_{268} (Figure 2), we can see that there are three non-compliances for this parameter, accounting for the 7.3% of the EVOOs analysed. The non-compliances are at 1 month and 6 months remaining to the "best-before" date, which represents the 13.33% and 4.17% of oils from each set, respectively.

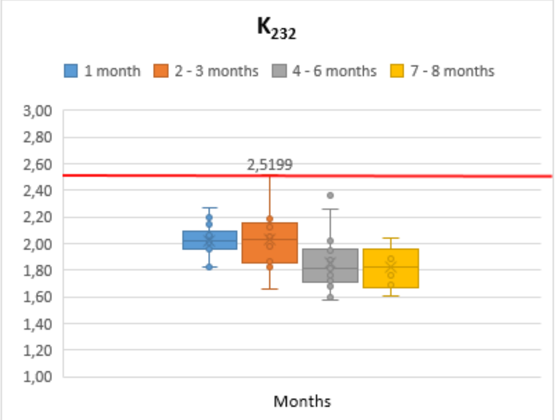

Figure 1. Distribution of K_{232} values according to the time remaining to the "best-before" date. The red line marks the maximum limit allowed by EU Regulation for EVOOs[3]

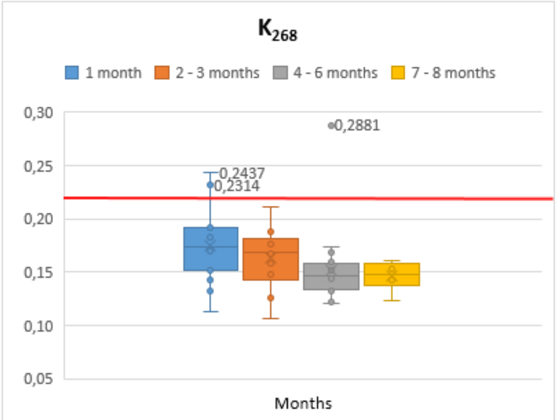

Figure 2. Distribution of K_{268} values according to the time remaining to the "best-before" date. The red line marks the maximum limit allowed by EU Regulation for EVOOs[3]

As expected, K_{232} and K_{268} values tend to increase in oils, as they get closer to the "best-before" date. For both parameters, the medians of the boxplots at 1 month and 2-3 months to the "best-before" date are quite similar and higher than those of boxplots at 4-6 months and 7-8 months to the "best-before" date, which are also quite similar among them. In addition, there is a greater variability of K_{232} values at 2-3 months before the "best-before" date, while for K_{268} the variability increases as the samples get closer to the "best-before" date.

ΔK measures the variation of the absolute value of the extinction at a given wavelength. This parameter may increase in the presence of both refined oils and highly oxidised oils.

In the samples analysed in the present study, this parameter exceeds the limit fixed by EU regulation for edible virgin olive oils in three samples, at 2-3 and 4-6 months to their "best-before" date (Figure 3), accounting for the 7.3% of the EVOOs analysed, which represents the 10% and 8.33% of each sample set, respectively. This EVOO is one of those exceeded the K_{268} limit; which indicates an oxidative deterioration.

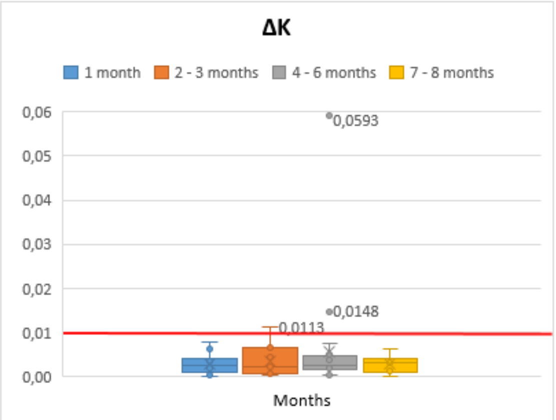

Figure 3. Distribution of ΔK values according to the time remaining to the "best-before" date. The red line marks the maximum limit allowed by EU Regulation for EVOOs[3]

The median value of ΔK is quite similar from 7-8 months to 1 month before the end of the shelf-life, while the variability is slightly higher at 2-3 months.

3.2 Peroxide Value

In Figure 4, we can see that the oils are in general far below the limit established for edible virgin olive oils, excepting for one sample, which accounts for the 2.4% of the EVOOs analysed. This non-compliance is at 3 months to the "best-before" date, which represents the 10% of the 2-3 months set. This EVOO is one of those exceeded the K_{232} limit; which is closely related because this extinction coefficient indicates a primary oxidation of polyunsaturated fatty acids and is usually correlated with the amount of peroxides present in EVOO.

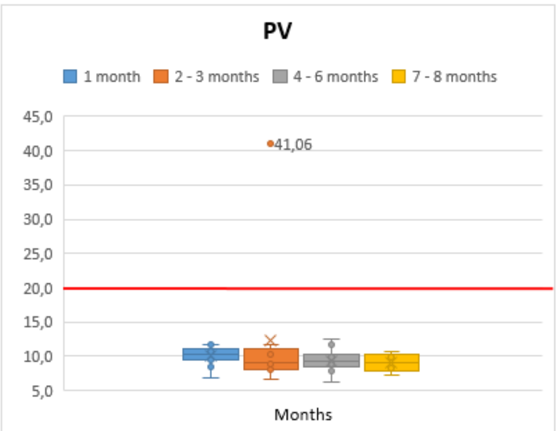

Figure 4. Distribution of PV values according to the time remaining to the "best-before" date. The red line marks the maximum limit allowed by EU Regulation for EVOOs[3]

As we can see, the medians of all boxplots are quite similar except for the 1-month boxplot, which is slightly greater. In relation to the variability of values, boxplots at 1 month and 2-3 months remaining to the "best-before" date are similar compared to the other boxplots, which are greater for 4-6 months boxplot and lower for 7-8 boxplot. On the other hand, although the results obtained at different times are very similar, a slight increase can be observed as they get closer to the "best-before" date.

3.3 Acidity

The results obtained are from 41 commercial EVOOs analysed at 1 month (n=7); 2-3 months (n = 10); 4-6 months (n = 18); and 7-8 months (n = 6) to the "best-before" date. For this parameter, the number of bottles analysed are lower than in the case of oxidation parameters due to the exceptional situation caused by COVID-19 crisis.

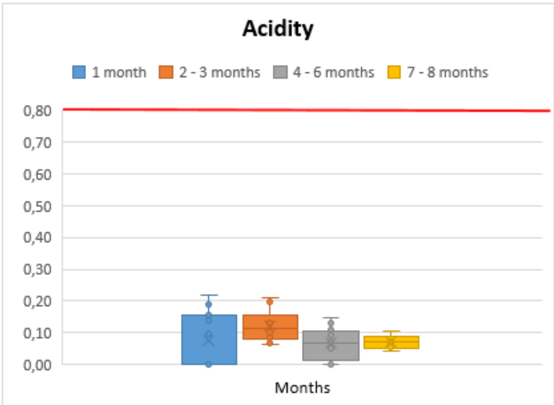

Figure 5. Distribution of Acidity values according to the time remaining to the "best-before" date. The red line marks the maximum limit allowed by EU Regulation for EVOOs.[3]

Unlike the oxidation parameters, in Figure 5 all the EVOOS comply with the regulation. Although a slight increase over time can be observed, the acidity values of all the EVOOs analysed are quite far from the limit set by the regulation for this category. On the other hand, it can be seen the variability of values is different in each boxplot.

4 Conclusions

Based on the results obtained, we can conclude that there are 6 EVOOs out of the 41 analysed that do not comply, which represents the 14.6% non-compliance with respect to the total. Moreover, it should highlighted that two of these non-compliant EVOOs exceed the limits of two parameters at the same time.

Therefore, we demonstrate that 2.4% of EVOOs do not comply for K_{232}, 7.3% do not comply for K_{268}, 7.3% do not comply for ΔK, and 2.4% do not comply for PV. On the other hand, we can also deduce that non-compliance with the acidity grade is unlikely because the values are far from the limit values.

Finally, we evidence that the non-compliance of the EVOO samples is due to the different oxidative parameters analysed, confirming the importance of keeping the product in optimal storage conditions.

5 Acknowledgement

This work has been developed within the Libifood research group. I would like to thank my supervisors Stefania Vichi and Francesc Guardiola.

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Supplementary material

Table S1. Sampling points of EVOOs depending on the "best-before" date.

OIL	SAMPLING POINTS (MONTHS BEFORE "BEST-BEFORE" DATE)			
	7-8	4-6	2-3	1
Oil 1				X
Oil 2				X
Oil 3				X
Oil 4				X
Oil 5				X
Oil 6				X
Oil 7				X
Oil 8			X	X
Oil 9			X	X
Oil 10			X	
Oil 11			X	X
Oil 12			X	X
Oil 13			X	X
Oil 14			X	X
Oil 15			X	X
Oil 16			X	X
Oil 17			X	
Oil 18		X		

Oil 19		X
Oil 20		X
Oil 21		X
Oil 22		X
Oil 23		X
Oil 24		X
Oil 25		X
Oil 26		X
Oil 27		X
Oil 28		X
Oil 29		X
Oil 30		X
Oil 31		X
Oil 32		X
Oil 33		X
Oil 34		X
Oil 35		X
Oil 36	X	X
Oil 37	X	X
Oil 38	X	X
Oil 39	X	X
Oil 40	X	X
Oil 41	X	X

Table S1. Extinction coefficients, peroxide values and acidity of EVOO samples.

Sample	$\overline{K}_{232}$ (≤ 2.50)	$\overline{K}_{268}$ (≤ 0.22)	$\Delta \overline{K}$ (≤ 0.01)	$\overline{IP}$ (≤ 20 meq O_2/kg of oil)	$\bar{A}$ (≤ 0.80% oleic)
Oil 1-1	2.0448	0.1423	0.0010	11.19	0.1722
Oil 2-1	1.8253	0.1710	0.0024	11.21	0.2194
Oil 3-1	2.0083	0.2437	0.0079	9.14	0.1466
Oil 4-1	1.9580	0.1731	0.0020	9.47	0.1886
Oil 5-1	2.0113	0.1823	0.0042	6.95	0.1578
Oil 6-1	2.0887	0.1924	0.0000	10.16	0.1409
Oil 7-1	2.0998	0.1825	0.0017	10.66	0.0985
Oil 8-2	2.1819	0.1762	0.0022	10.38	0.1408
Oil 8-1	2.1985	0.1939	0.0038	10.88	-
Oil 9-2	2.0012	0.2116	0.0065	8.87	0.1296
Oil 9-1	2.1432	0.2314	0.0036	11.66	-
Oil 10-2	1.9770	0.1878	0.0113	6.64	0.0845
Oil 11-3	1.8643	0.1264	0.0043	8.84	0.2105
Oil 11-1	1.9594	0.1328	0.0043	10.05	-

Oil 12-3	1.8228	0.1701	0.0023	8.06	0.1032
Oil 12-1	1.8441	0.1569	0.0035	8.43	-
Oil 13-3	2.0553	0.1484	0.0007	9.37	0.0676
Oil 13-1	2.0240	0.1521	0.0005	9.69	-
Oil 14-3	2.1195	0.1790	0.0012	11.74	0.1183
Oil 14-1	2.0866	0.1917	0.0025	11.75	-
Oil 15-3	2.1524	0.1580	0.0007	10.85	0.1120
Oil 15-1	2.2649	0.1692	0.0005	11.08	-
Oil 16-3	1.6547	0.1067	0.0064	8.06	0.0620
Oil 16-1	1.8228	0.1134	0.0063	10.23	-
Oil 17-3	2.5199	0.1670	0.0002	41.06	0.1973
Oil 18-4	1.7675	0.1214	0.0037	8.23	0.1353
Oil 19-4	1.6880	0.1741	0.0007	8.60	0.1291
Oil 20-4	1.9564	0.1680	0.0076	6.17	0.0846
Oil 21-5	2.0241	0.1703	0.0027	10.54	0.1093
Oil 22-5	1.8031	0.1543	0.0019	8.89	0.1466

Oil 23-5	1.8055	0.1226	0.0027	8.22	0.1127
Oil 24-5	1.7098	0.1547	0.0007	10.26	0.0724
Oil 25-5	1.6761	0.1481	0.0007	9.01	0.0613
Oil 26-5	2.0373	0.1439	0.0017	10.58	0.0674
Oil 27-5	1.8757	0.1703	0.0029	8.44	0.0507
Oil 28-6	1.8144	0.1373	0.0051	9.84	0.0617
Oil 29-6	1.9157	0.1370	0.0024	9.47	0.0562
Oil 30-6	1.8245	0.1322	0.0053	9.05	0.0789
Oil 31-6	1.7197	0.1362	0.0015	7.86	0.1040
Oil 32-6	1.5988	0.1322	0.0593	9.54	0.0917
Oil 33-6	1.8753	0.1541	0.0005	9.85	0.0845
Oil 34-6	1.5812	0.1201	0.0025	8.94	0.0564
Oil 35-6	2.3583	0.2881	0.0148	12.62	0.1071
Oil 36-7	1.8914	0.1233	0.0032	9.80	0.1041
Oil 36-6	1.6922	0.1265	0.0042	10.09	-

Oil 37-7	1.9301	0.1565	0.0014	8.19	0.0733
Oil 37-6	1.9461	0.1368	0.0020	9.37	-
Oil 38-7	1.7609	0.1527	0.0000	7.33	0.0846
Oil 38-6	1.8031	0.1549	0.0002	8.51	-
Oil 39-7	1.6928	0.1426	0.0033	10.07	0.0449
Oil 39-6	1.7481	0.1362	0.0039	10.48	-
Oil 40-7	2.0398	0.1611	0.0031	10.63	0.0676
Oil 40-6	2.0611	0.1595	0.0024	11.63	-
Oil 41-8	1.6123	0.1431	0.0064	8.52	0.0534
Oil 41-6	2.2552	0.1522	0.0054	8.48	-

The alphanumeric code before the dash refers to the oil, while the number after the dash refers to the months remaining to the “best-before” date. The mean values in red color exceed the limits established by the EU regulation.[3] The samples analysed for acidity are less due to the exceptional situation caused by COVID-19 crisis.