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ORIGINAL PAPER

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EVALUATION OF THE QUALITY AND OXIDATIVE STABILITY OF VIRGIN OLIVE OIL FROM *MOROCCAN PICHOLINE* OLIVES FORTIFIED WITH *ROSMARINUS OFFICINALIS* L. ESSENTIAL OIL DURING STORAGE

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ABSTRACT

Background. Virgin olive oil is highly valued for its sensory attributes and health benefits, primarily due to its rich composition of monounsaturated fats, polyphenols, and tocopherols. However, during storage, oxidative processes can lead to the deterioration of these compounds, resulting in off-flavors, reduced nutritional value, and overall loss of quality.

Material and methods. The aim of this work was to evaluate the impact of the fortification of virgin olive oil, obtained by harvesting olives of the *Moroccan Picholine* variety in a discontinuous system, with *Rosmarinus officinalis* L. essential oil at three different concentrations (OOR1 = 0.025%, OOR2 = 0.05% and OOR3 = 0.1%) on the quality and oxidative stability of fortified olive oil during a year of storage. The quality evaluation (acidity, peroxide value) was carried out monthly. The K_{232}, K_{270}, polyphenol content and color assessments and a sensory evaluation were carried quarterly.

Results. The results show that after adding *Rosmarinus officinalis* L. essential oil, the acidity and peroxide value of the olive oil decreased immediately, especially for OOR3 (0.87% oleic acid and 2.51 mequiv of O_2/kg respectively) compared to the control. Fortification had a significant effect on quality parameters (free fatty acids, peroxide value, K_{232}, K_{270}, color assessment and sensory evaluation). However, polyphenol content did not exhibit significant changes throughout the storage period.

Conclusion. Overall, this study revealed a remarkable improvement in the quality of virgin olive oil after fortification with *Rosmarinus officinalis* L. essential oil.

Keywords: virgin olive oil, *Moroccan Picholine*, *Rosmarinus officinalis* L. essential oil, oxidative stability

INTRODUCTION

The olive tree is among the oldest known cultivated trees worldwide (Vossen, 2007). In Morocco, the land is particularly suitable for its cultivation thanks to the favorable climate and the rich tradition of olive

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growing. These long-standing traditions provide essential competitive advantages for the Moroccan olive industry (Vossen, 2007).

According to data from the National Institute of Agricultural Research (INRA), olive oil production is of great importance and is one of the priority areas in the new "Green Generation 2020–2030" strategy. In recent years, olive cultivation has seen significant improvement. It covers 1.1 million hectares, representing 12.3% of the country's Utilized Agricultural Area (UAA) and 65% of its tree crops by 2019. Between 2016 and 2020, average production reached 1.5 million tons, including 170,000 tons of olive oil and 120,000 tons of table olives. During the same period, Morocco exported an average of 100,000 tons annually, comprising 30,000 tons of olive oil and olive pomace, and 70,000 tons of table olives. However, the quality of the virgin olive oil produced is influenced by various factors, including the method of extraction used (Chimmi, 2001). Actually, there are three different systems of extraction; the more recently developed three-phase and two-phase continuous systems and the traditional discontinuous system (Benyahia and Zein, 2003). In Morocco, the discontinuous system is widely used (Chimmi, 2001). Although it produces a lower quantity of vegetable water than the continuous system, the quality of the olive oil obtained is lower (Benyahia and Zein, 2003). This is because the grinding stage is carried out in the open air and lasts up to 45 minutes, allowing contact of the olive paste with light and oxygen, which initiates the formation of free radicals. These unstable molecules catalyze the autoxidation reaction, which causes damage to cells and is associated with various health issues, including aging and disease (Silenzi et al., 2020; Šarolić et al., 2014).

Efforts to improve and preserve the quality of virgin olive oil have led to the exploration of new practices. One such approach is the fortification of olive oil with aromatic plants or essential their oils. Studies have shown that by infusing olive oil with aromatic plants such as oregano, rosemary, thyme, and garlic, both its flavor and antioxidant content are enhanced and its shelf life is extended (Baiano et al., 2005; Gambacorta et al., 2007; Baiano and Terracone, 2009; Hande Akçar and Gümüşkesen, 2011; Caporaso et al., 2013; Chaaben et al., 2018). Essential oils are aromatic compounds extracted from plants which often possess potent biological properties. According to numerous studies, essential oils have powerful antioxidant properties that can effectively capture and neutralize free radicals (Calucci et al., 2003; Amorati et al., 2013; Dorman et al., 2000; Arpiwi et al., 2023; Andrade et al., 2018). In this context, the main objective of this study is to evaluate the quality of virgin olive oil obtained from olives of the *Moroccan Picholine* variety fortified with *Rosmarinus officinalis* L. essential oil. This study will provide a new solution for companies aiming to enhance the value of virgin olive oil and introduce a new product to the market.

MATERIAL AND METHOD

Plant material

In order to choose the virgin olive oil to be flavored, a preliminary study was carried out (Latifi et al., 2024). Five samples of virgin olive oil obtained from olives of the *Picholine marocaine* variety collected on the same day from five areas of the provinces of Béni Mellal, Fquih Ben Salah and Azilal were collected, and their phenol content was measured. These samples were taken from olives crushed in oil mills using the discontinuous press extraction system (Latifi et al., 2024).

In order to maximize the benefit from the polyphenols contained in the *Rosmarinus officinalis* L. virgin olive oil with low polyphenol content was chosen to prepare the fortified oil.

The essential oils of *Rosmarinus officinalis* L. that were used in the study are commercially available in local markets with camphor as major compound.

For the preparation of the fortified virgin olive oil samples, we added *Rosmarinus officinalis* L. at three concentrations (OOR1 = 0.025%, OOR2 = 0.05% and OOR3 = 0.1%), packaged them in opaque glass bottles, and stored them away from light. Unflavored olive oil was used as a control.

Quality parameters

The measurement of the quality indices was carried out according to the method described by the International Olive Council (IOC). The measurements of free acidity FFA (% oleic acid) (IOC, 2017a), and the peroxide index PV (IOC, 2017b) were carried out

monthly, while the measurement of absorbance (COI, 2019a) in the UV was only carried out quarterly.

Extraction of polyphenols

The extraction of phenols was carried out using liquid-liquid extraction with methanol as a solvent, according to the method described by (Ollivier et al., 2004), slightly modified for application to fortified oils. 5 ml of methanol/water (80:20, v/v) was added to 5 g of virgin olive oil and mixed with a Vortex mixer for 10 min. The hydro alcoholic phase containing the phenolic compounds was separated from the oily phase by centrifugation (38,000 rpm, 15 min), and this operation was repeated 3 times. Finally, the hydro alcoholic extracts were put into vials for further analysis.

Polyphenol content

The quantification of total phenolic compounds involved the use of the Folin-Ciocalteau reagent, and the method was adapted from Vita Di et al. (2022). In a test tube, 200 µL of phenolic extract was mixed with the 1 ml of Folin-Ciocalteau reagent and 2 ml of water, and after 4 minutes we added 0.8 ml solution of Na_2CO_3 (7.5%). The mixture was incubated for 2 hours. The total phenolic content was determined colorimetrically at 765 nm. The standard curve was prepared from diluted solutions of gallic acid in a methanol:water solution (70:30, v/v). The total phenolic content was expressed in mg of gallic acid equivalents per kg of oil.

Sensory assessment

In order to evaluate the general acceptability of fortified olive oil to the consumer, we conducted a sensory analysis every 3 months over a period of 9 months. A panel was assembled, consisting of 14 panelists aged between 23 and 40 who were researchers, doctoral students and staff of the laboratory of Agro-food Sciences and Technologies of Tadla, Regional Agronomic Research Center of Tadla, National Institute of Agronomic Research (INRA), Béni Mellal. The sensory evaluation was carried out according to the method of Lawless and Hildegarde (2010). The fortified olive oil samples and the coded control were presented randomly, accompanied by small pieces of bread as well as slices of *Granny Smith* apple and mineral water to rinse the mouth after tasting each sample.

The panelists were asked to smell and taste the samples, then indicate the degree of pleasure or displeasure that they gave by checking the appropriate statement on the form (Fig. 1), which used a scale of 1–9 (1 = extremely pleasant, 9 = extremely unpleasant).

The sensory analysis session was repeated on two consecutive days, so the overall score for each sample was the average of 28 scores.

Color measurement

The color assessment was carried out by measuring the coordinates (L^*, a^* and b^*) with a Chroma meter (CR-410, KONICA MINOLTA, INC, made in Japan). It was conducted using 30 mL samples in Petri dishes against a background of white tiles (Markovic et al., 2013).

Statistical analysis

All tests were done in triplicate. All the results are expressed as means ±standard deviation (SD). GraphPad PRISM 8.0.1 software was used to generate graphs and carry out statistical analysis. Quantitative differences were interpreted by ANOVA followed by Tukey's test, and *p*-values of 0.05 or less were considered statistically significant.

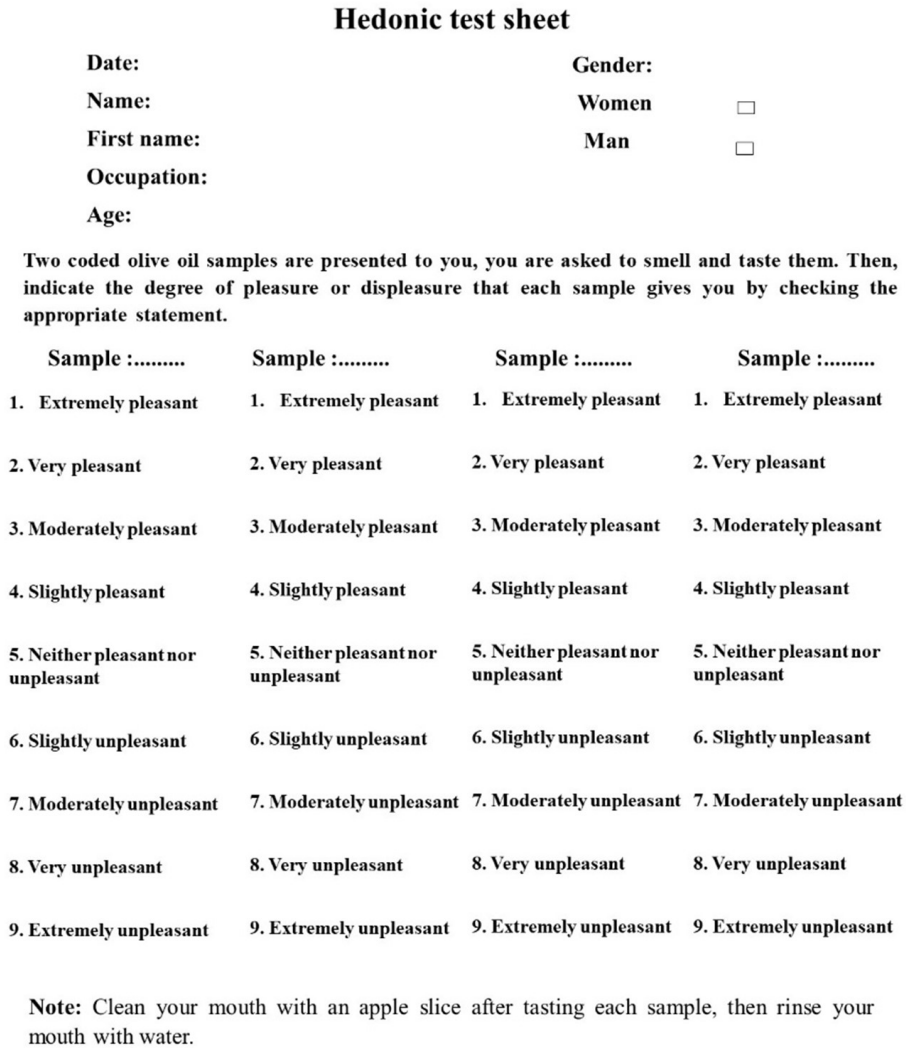
RESULTS AND DISCUSSION

Impact of fortification on the quality indices

Free acidity FFA (% oleic acid) gives an idea of the free fatty acids in olive oil, and consequently it indicates the state of oxidation and can be used to help evaluate the quality of an olive oil.

The quality index of the control at the beginning of the experiment allowed it to be classified as a virgin olive oil (FFA = 1.17 % oleic acid, PV = 5.68 meq O_2/kg, K_{232} = 2.0 and K_{270} = 0.135). As presented in Table 1, after adding the essential oil, FFA values (expressed as % oleic acid) decreased. The same trend was observed in the study conducted by Chahdoura et al. (2023) using a traditional method that consists of extracting the natural aroma of *Rosmarinus officinalis* L. by infusing it into the oil.

Fortified olive oil at a concentration of 0.1% had the lowest acidity value (0.87%) followed by OOR2 (0.91%), OOR1 (1.07%), and finally the control sample (1.17%). Although flavored olive oil is not yet



Hedonic test sheet

Date: **Gender:**

Name: **Women** ☐

First name: **Man** ☐

Occupation:

Age:

Two coded olive oil samples are presented to you, you are asked to smell and taste them. Then, indicate the degree of pleasure or displeasure that each sample gives you by checking the appropriate statement.

Sample :.....	Sample :.....	Sample :.....	Sample :.....
1. Extremely pleasant	1. Extremely pleasant	1. Extremely pleasant	1. Extremely pleasant
2. Very pleasant	2. Very pleasant	2. Very pleasant	2. Very pleasant
3. Moderately pleasant	3. Moderately pleasant	3. Moderately pleasant	3. Moderately pleasant
4. Slightly pleasant	4. Slightly pleasant	4. Slightly pleasant	4. Slightly pleasant
5. Neither pleasant nor unpleasant	5. Neither pleasant nor unpleasant	5. Neither pleasant nor unpleasant	5. Neither pleasant nor unpleasant
6. Slightly unpleasant	6. Slightly unpleasant	6. Slightly unpleasant	6. Slightly unpleasant
7. Moderately unpleasant	7. Moderately unpleasant	7. Moderately unpleasant	7. Moderately unpleasant
8. Very unpleasant	8. Very unpleasant	8. Very unpleasant	8. Very unpleasant
9. Extremely unpleasant	9. Extremely unpleasant	9. Extremely unpleasant	9. Extremely unpleasant

Note: Clean your mouth with an apple slice after tasting each sample, then rinse your mouth with water.

Fig. 1. Hedonic test sheet used by the panelists

Table 1. Quality parameters of the control and fortified virgin olive oil at the beginning of the experiment (T_0)

Quality indices	Control	OOR1	OOR2	OOR3	IOC Standards	
					Extra virgin olive oil	Virgin olive oil
FFA % oleic acid	1.172 ±0.301	1,253 ±0.289	0.913 ±0.024	0,873 ±0.092	≤0.8	≤2.0
Peroxide value méq O_2/kg	5.527 ±0.499	1,894 ±0.514	2.784 ±0.279	2.445 ±0,510	≤20	≤20
E232	1.957 ±0.038	1.107 ±0.038	1.135 ±0.004	1.275 ±0.039	≤2.5	≤2.6
E270	0.135 ±0.022	0.142 ±0.054	0.154 ±0.002	0.225 ±0.049	≤0.22	≤0.25

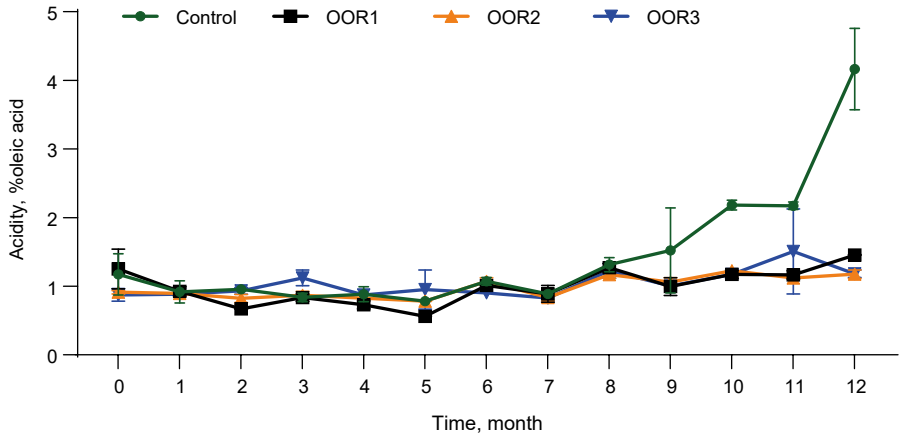


Fig. 2. Evolution of FFA (% oleic acid) during a year of storage

included in the standards set by the IOC, we employed these criteria to help us categorize the flavored virgin olive oil that is subject of our research.

During a year of storage (Fig. 2), the FFA value for the flavored samples remained within the guidelines set by the IOC for virgin olive oil, which is 2% oleic acid (COI, 2019b). Nevertheless, a significant divergence between the control sample and the fortified olive oil was observed after the eighth month of storage. Specifically, the FFA of the control sample increased rapidly after the eighth month of storage. From the tenth month onwards, the acidity of the control (2.183%) exceeded 2%, but it was still suitable for human consumption. However, after a year of storage the FFA (4.164%) exceeded the limit for virgin olive oil suitable for human consumption. Also, it was higher than the FFA of the fortified samples by 65%, 71.8%, and 71.6% for OOR1, OOR2, and OOR3, respectively. This confirms the impact of fortification with rosemary essential oil on the acidity value of virgin olive oil.

The peroxide values suggest that primary oxidation products are formed. When the experiment first began, these values were 1.94 mequiv O_2/kg for OOR1, 2.51 mequiv O_2/kg for OOR3, 2.88 mequiv O_2/kg for OOR2, and 5.68 mequiv O_2/kg for the control. After the addition of rosemary essential oil, the peroxide value decreased, so that OOR1 had the lowest value (1.94 mequiv O_2/kg), as illustrated in Table 1.

This index gives an idea of the onset of oxidation of the oil. The higher it is, the more the oil is oxidized. After reaching a peak, it decreases with the advancing oxidation state (Fig. 3). The evaluation of the results relating to the peroxide index during storage showed that the control sample reached its peak faster than the fortified oil. In fact, we observed an increase up to the peak of 8.37 mequiv O_2/kg for the control during the 6th month, whereas for the flavored oil the peak was in the 7th month (HR2 = 11.73 mequiv O_2/ kg, HR1 = 11.22 mequiv O_2/kg, HR3 = 10.03 mequiv O_2/kg). Then, the peroxide index values regressed for all samples. This evolution can be explained by the conditions of storage, which was at room temperature. The more the temperature increases, the more primary oxidation of the samples occurs.

Early stages of the oxidation of virgin olive oil result in the formation of undesirable compounds and a decrease in quality. The K_{270} value is associated with the content of certain compounds, such as conjugated dienes and trienes, which are formed during the oxidation of unsaturated fatty acids in the oil. The K_{232} value represents the absorbance at 232 nm, which is an indicator of the quality and freshness of olive oil. As illustrated in Table 1, after adding rosemary essential oil, the K_{232} values decreased, whereas the K_{270} values slightly increased. Nevertheless, all values adhered to the standards set by the IOC.

Polyphenol content

Polyphenols are compounds that help neutralize free radicals in the body (Ortega-García et al., 2008). The latter are molecules that can cause damage to cells

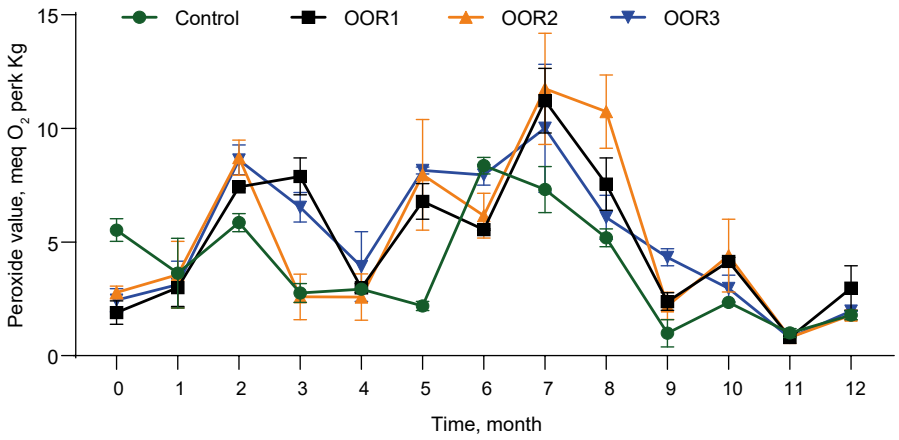


Fig. 3. Evolution of peroxide value during a year of storage

and contribute to various diseases (Moskovitz et al., 2002). The polyphenol content of the control and fortified oils is presented in Figure 4. After fortification, the polyphenol content increased slightly with no significant difference ($p < 0.05$). The polyphenol content of the fortified olive oil OOR3 (at 0.1% concentration) was the highest at 500.90 mg GAE/kg, whereas the control sample had the lowest polyphenol content at 380.7 mg GAE/kg. This rise might be explained by the enrichment brought about by the addition of rosemary essential oil, which contains a significant amount of polyphenols, as previously reported (Haida et al., 2015). Statistical analysis showed no significant difference ($p < 0.05$) between the control and the fortified olive oils except in the 12th month of storage, when there was a difference between the control and OOR3.

Throughout the storage period, the polyphenol content increased for all samples, with a slight decrease after a year of storage. This upward trend could be due to the hydrolysis of certain phenolic compounds, such as oleuropein, which breaks down into hydroxytyrosol, and may be found in free, bound, or esterified forms (Ortega-García et al., 2008) (Chahdoura et al., 2023; Díaz-Montaña et al., 2022). Thereafter, it decreased as a consequence of decomposition caused by antioxidant action (Chahdoura et al., 2023).

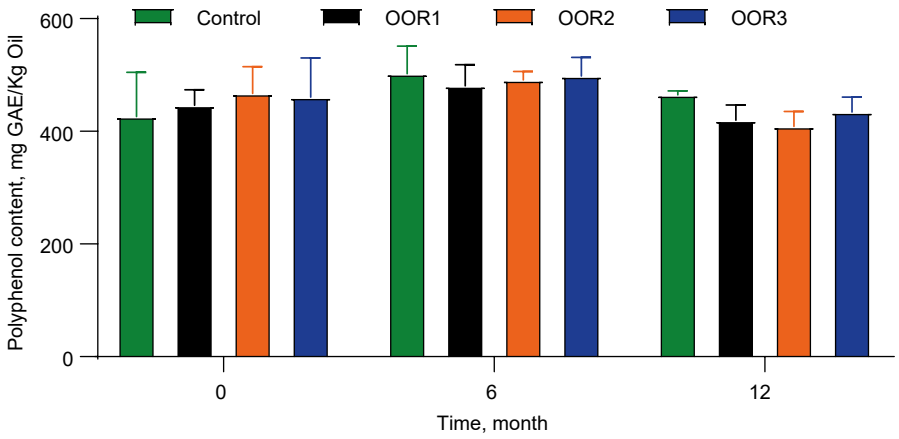


Fig. 4. Evolution of polyphenol content of control and fortified olive oil during a year of storage

Table 2. Sensory evaluation scores of control and samples of olive oil fortified with rosemary essential oil

	0	3rd month	6th month	9th month
Control	3	3	4	4
OOR1	4	4	5	4
OOR2	4	4	5	4
OOR3	5	5	5	4

Sensory test

The results (Table 2) of the sensory analysis show that at the start of the experiment the fortified oils OOR1 and OOR2 had a score of 4 (slightly pleasant) and OOR3 had a score of 5 (neither pleasant nor unpleasant). The control had a score of 3 (moderately pleasant). At the end of the experiment, sensory test indicated that the overall acceptability was equally good for all samples. These results are compatible with those of Benmoussa et al. (2017), who studied the overall acceptability of olive oil flavored by *Rosmarinus officinalis* L. leaves by conventional maceration for 12 h and by microwave-assisted maceration (10 min and 400 W). That study similarly indicated that all samples were equally acceptable to the general consumer.

However, further evaluation of the fortified virgin olive oil by trained panels would be needed for product development.

Color assessment

The color of the control and fortified olive oils was analyzed by colorimetric CIEL**a***b** without the oils being filtered or clarified (Cairone et al., 2021). In this coordinate system, the *L** value is a measure of brightness, ranging from 0 (black) to 100 (white), the *a** value ranges from –100 (green) to +100 (redness), and the *b** value ranges from –100 (blue) to +100 (yellow) (Benmoussa et al., 2017). In our study, all samples (Table 3) had a negative value for the chromatic ordinate *a**, classifying them in the green zone, a positive value for the chromatic ordinate *b**, corresponding to the yellow zone, and *L** values ranging from 74.12 (for the control) to 57.18 (for OOR3). As shown in Table 3, the luminosity values (*L**) slightly decreased after fortification, probably as a consequence of changes in pigment content (Benmoussa et al., 2016). After 12 months of storage, there was no significant change in the chromatic ordinate *L**, while there was a significant decrease in the *a** values for the control (–10.04) and OOR3 (–9.76).

Table 3. Values of *L**, *a** and *b** of control and samples of olive oil fortified with rosemary essential oil

		0	3rd month	6th month	9th month	12th month
Control	*L**	74.12 ±28.20^{ab}	71.87 ±3.395^{ab}	69.37 ±0.9404^{a}	57.26 ±0.9322^{a}	63.35 ±1.081^{b}
	*a**	–8.067 ±1.73^{ac}	–10.32 ±0.36^{ac}	–8.243 ±0.21abc	–8.623 ±0.31^{bc}	–10.04 ±0.15^{c}
	*b**	41.88 ±23.10^{cd}	67.63 ±0.89^{ac}	40.59 ±1.46^{d}	38.96 ±0.89^{d}	42.80 ±0.94^{bcd}
OOR1	*L**	62.80 ±2.82^{ab}	66.39 ±8.09^{ab}	69.81 ±1.29^{a}	64.72 ±5.06^{ab}	59.58 ±0.89^{b}
	*a**	–9.01 ±0.50^{a}	–7.46 ±1.36^{a}	–8.84 ±0.19^{a}	–9.34 ±0.26^{a}	–9.00 ±0.17^{a}
	*b**	43.78 ±21.74^{a}	62.29 ±5.35^{a}	46.99 ±0.90^{a}	44.11 ±6.78^{a}	40.69 ±0.88^{a}
OOR2	*L**	61.21 ±2.79^{b}	69.82 ±4.25^{a}	70.56 ±0.89^{b}	61.70 ±1.70^{a}	62.46 ±2.32^{b}
	*a**	–9.32 ±2.00^{abc}	–7.08 ±0.22^{c}	–8.00 ±0.12^{bc}	–9.07 ±0.27^{ab}	–9.45 ±0.40^{a}
	*b**	38.26 ±13.58^{c}	88.42 ±3.96^{ac}	39.29 ±2.27^{bc}	40.03 ±2.42^{bc}	42.06 ±1.12^{bc}
OOR3	*L**	57.18 ±4.60^{a}	62.87 ±4.17^{a}	70.61 ±3.62^{a}	61.65 ±2.13^{a}	63.20 ±1.52^{a}
	*a**	–7.77 ±1.15^{c}	–5.19 ±1.93^{c}	–8.17 ±0.25^{bc}	–9.06 ±0.21^{abc}	–9.76 ±0.13^{ac}
	*b**	40.63 ±15.44^{c}	78.09 ±4.25^{ac}	41.64 ±4.07^{bcd}	39.79 ±2.09^{d}	41.11 ±2.67^{bcd}

CONCLUSION

In conclusion, adding *Rosmarinus officinalis* L. essential oil to virgin olive oil for a year of storage reduced the FFA value by 65%, 71.8% and 71.6% for OOR1, OOR2, and OOR3, respectively. This is a good indication for the quality of the olive oil. Fortified olive oil at a concentration of 0.1% had the lowest acidity value, namely 0.87%.

The polyphenol content did not show a significant difference during the storage period. It increased for all samples, with a slight decrease after a year of storage.

The results of this study suggest that fortification could be a viable option for improving the shelf life and quality of virgin olive oil. Further research could explore the use of other methods of fortification or other types of essential oils and their effects on the oxidative stability of olive oil.

DECLARATIONS

Data statement
All data supporting this study has been included in this manuscript.

Ethical Approval
Not applicable.

Competing Interests
The authors declare that they have no conflicts of interest.

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