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Effect of Aromatization by Aromatic Plants on the Physicochemical, Sensorial and Oxidative Stability of Moroccan Virgin Olive Oil

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Abstract

Food oxidation presents one of the major deterioration in food industry. It minimizes food shelf-life, production of undesirable flavor or color. The introduction of synthetic antioxidant is a better solution to stop these reactions; although many studies demonstrated the negative effect of using these antioxidants. The use of natural antioxidants like food additives, actually present a successful strategy to satisfy consumer needs. Currently, consumers demand for healthy food is increasing, also rejecting foods with synthetic antioxidant due to diseases that can be caused by the consumption of these products. In this study, rosemary and laurel fresh leaves were added to virgin olive oil and stored in different modes. Physicochemical, sensory evaluation and oxidation stability were performed to determine the most likely natural aromatic plants that could be used to conserve olive oil. Experimental results showed that the aromatization of olive oils with rosemary or laurel stored in the dark led to increasing the value of total phenolic contents and preserving them against autoxidation. This increase led to show lower free acidity, peroxide and thiobarbituric acid reactive substances. The trained panel preferred olive oil aromatized by rosemary fresh leaves stored in the dark.

Keywords: Virgin olive oil, aromatization, oxidative stability, sensory analysis

1. Introduction

Olive oil production is one of the most traditional agricultural industries in the Mediterranean regions and plays an important role in the rural economy. The Moroccan farms have a total production of around 1,500,000 tons of olives, covering an area of 784,000 hectares [1]. Morocco has become one of the largest producers of olive oil, while the production of olive oil has achieved the value of 160,000 tons [1]. The Moroccan olive grove contains more than 96% of the Moroccan Picholine variety. Olive oil quality is characterized by many factors such as physicochemical and organoleptic parameters, permitted to ranking theses olive oils in different categories [2]. While this quality is affected by many factors such as technical methods of cultivation, extraction methods and storage [3]. Lipid oxidation is one of the famous reactions of the lipid chemistry. Caused by many factors such as; light, oxygen and transition of metal ions. Lipids containing polyunsaturated fatty acids and their esters are exposed to autoxidation by molecular oxygen. Autoxidation of lipids has been recognized as a major deterioration process affecting both sensory and nutritional quality of foods, which limited food shelf life and may be responsible for undesirable rancid taste. Nevertheless, it is well known that antioxidants such as tocopherols, ascorbic

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acid, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) inhibited these biochemical changes [4].

The Mediterranean area is characterized by the frequent cultivation of aromatic plants such as sage, rosemary, thyme, laurel, garlic, hot chili pepper, oregano, etc. Many authors has studied some of these plants for their antioxidant properties and described their suitability to be used as natural antioxidants in food industries as they contain major secondary metabolites [5-7]. Rosemary (*Rosmarinus officinalis* spp) is one of the major aromatic plants cultivated in Morocco, commonly used as natural antioxidant for its health beneficial properties such as antimicrobial, antialgesic, and antirheumatic effects [6, 8, 9]. Furthermore, the laurel (*Laurus nobilis* L) is an evergreen tree (shrub), cultivated in many parts of the world, particularly in the Mediterranean areas [10]. Dried or fresh laurel leaves are commonly used as flavoring material in traditional Moroccan culinary preparations (fish soup and meat preparations). Laurel leaves or their extracts are characterized by their antioxidant activity, antibacterial and antifungal properties. Whereas, laurel leaves or their essential oil increase the gastric fluid secretion and act in contrast to digestive disorders such as flatulent colics [10].

Many researchers and professionals from this sector are looking for alternatives, making it possible to improve olive oil quality by direct introduction of aromatic plants after extraction or mixed with olive fruit before extraction [5, 11]. For this reasons and to satisfy the consumer needs, the aim of this study is to evaluate the effect of aromatization with 5% of rosemary or laurel leaves and their storage in different modes on the physicochemical, oxidative stability and organoleptic parameters of virgin olive oil.

2. Materials and Methods

2.1. Sampling

The study focused on six samples of olive oils extracted from olive fruits obtained from Moroccan Picholine variety in January, 2016 (Béni Mellal region, Morocco). The olive oil samples were preserved in clean transparent bottles with a volume of 1 L in each sample. These samples were divided to two groups; the first group exposed to sunlight in room temperature contain 3 samples (olive oil without aromatization (OL), olive oil aromatized by 5% of fresh rosemary leaves (ORL) and olive oil aromatized by 5% of fresh laurel leaves (OLL)), the second group also designed by same mode of the first group, but samples are stored in dark at room temperature also contains 3 samples (olive oil without aromatization (OD), olive oil aromatized by 5% of fresh rosemary leaves (ORD) and the third sample is olive oil aromatized by 5% of fresh laurel leaves (OLD)) for a period of forty-five (45) days compared with the same sample of olive oil without aromatization in 0 day of storage (control).

2.2. Analytical Methods

2.2.1. Determination of Free Acidity

The free acidity (FA), expressed as a percentage of oleic acid, was determined according to ISO [12]. A 1.0 g of olive oil was dissolved in 50 mL of ethanol. The mixture was titrated with a potassium hydroxide (KOH) solution (0.1 N) in the presence of phenolphthalein as color indicator.

2.2.2. Determination of the Peroxide Value

The peroxide value is the number of hydroperoxide formed in fat in its conservation; it provides information on the state of oxidation. Consequently, olive oils lose their freshness if the peroxide value is higher than 20 mEq O_2/kg of oil [2]. The peroxide value according to ISO [13], about 1.0 g of olive oil was dissolved in a mixture of 12.2 mL acetic acid/chloroform (3:2 v/v), and saturated solution of KI (15 mL) was added to the mixture. The liberation iodine was titrated with sodium thiosulphate solution (0.01N) in the presence of starch as color indicator.

2.2.3. Absorbance of Ultraviolet

Absorbance of UV was used to detect abnormal oxidized compounds in virgin olive oil determined according to norms established by IOC [2]. A 0.1 g of the sample was dissolved in 10 mL cyclohexane. After homogenization, the absorbance was measured at 232 nm (K_{232}) and 270 nm (K_{270}) using M501 single beam scanning UV/visible (Spectronic Camspec Ltd Leeds, West Yorkshire, LS25 1DX, England, United Kingdom).

2.2.4. Determination of Total Phenolic Content

The total phenolic content was determined by the Folin-Ciocalteau method described by Capannesi *et al*. [14]. The quantification was obtained from a calibration curve using the Gallic acid (Sigma, Morocco) as a standard phenol (r^2=0·996) obtained by the use of 7 points for the calibration (0-0.12 mg of Gallic acid/mL) measured by M501 single beam scanning UV/visible at 750 nm.

Results were expressed as the equivalence of milligrams of Gallic acid per kilogram of oil (mg GAE/kg of oil). Samples were extracted by methanol-water 80: 20 (v / v).

2.2.5. Reactive Substances with 2-thiobarbituric Acid (TBARs)

Oxidation level was measured by TBARs (Thiobarbituric acid reactive substances assay) as described by Diaz *et al*. [15], 49.0 mL H_2O and 1.0 mL BHA (7% in ethanol) reagent were added to 10.0 g of oil, after homogenization, an aliquot of the suspension was mixed with 5.0 mL of 4.0 N HCl and 45.0 mL of distilled H_2O, the mixture was distilled at 130°C, after recovery of distillate, a 5.0 mL of aliquot was mixed with 5.0 mL of thiobarbituric acid (TBA). The mixtures were placed in a water bath at 100°C for 35 min. Orange-red color was obtained and the absorbance at 532 nm was measured by M501 single beam scanning UV/visible. The results were expressed as mg of malondialdehyde (MDA) per 1 kg oil.

2.2.6. Sensory Analysis of Olive Oils

The sensory analysis of olive oils was carried out according to standard of IOC [2]. The sensory evaluation was performed using a descriptive quantitative analysis; a trained panel was used in this study. This study was carried out to determine the existence of significant differences between the control olive oil and those aromatized by rosemary or laurel for 45 days of storage. The sensory analysis was performed by asking panel for their opinion of the odor intensity, taste of rancid taste, residual taste and overall acceptance of olive oils using a 5-point hedonic scale: 1 point was given to the least accepted sample, 3 points to the samples which produced a neutral opinion and 5 points to the most acceptable sample. The panel was composed of an average of 10 panelists.

2.3. Statistical Analysis

The statistical analysis was carried out using the statistical package for Windows (version 8.0, Analytical Software, USA) and Minitab software (version 17, Minitab Inc. USA). A one-way multivariate ANOVA and a post hoc Tukey's test with a 0.05 significant level were carried out for the data analysis.

3. Results and Discussions

3.1. Effect of Aromatization by Aromatic Plants and Storage Mode on Physicochemical Composition of Olive Oils

Table 1 detailed the physicochemical values of olive oils aromatized by rosemary and laurel fresh leaves stored in different modes. Aromatization and mode of storage significantly affect the physicochemical parameters of olive oils studied.

Table 1. Physicochemical Values of Olive Oils Aromatized by Rosemary and Laurel Fresh Leaves

Samples	Free acidity (% oleic acid)	Peroxide value meq O_2 /Kg oil	K_{270}	K_{232}	ΔK
Control	0.84 ± 0.11^{c}	15.00 ± 1.40^{c}	0.18 ± 0.003^{c}	1.12 ± 0.006^{c}	0.01 ± 0.0003^{ab}
OD	1.17 ± 0.10^{abc}	23.43 ± 0.69^{ab}	0.23 ± 0.007^{ab}	2.15 ± 0.043^{ab}	0.01 ± 0.006^{ab}
OL	1.42 ± 0.02^{a}	26.85 ± 2.77^{a}	0.24 ± 0.003^{a}	2.16 ± 0.037^{a}	0.01 ± 0.002^{ab}
ORL	1.26 ± 0.03^{ab}	21.37 ± 0.72^{abc}	0.24 ± 0.002^{a}	2.03 ± 0.012^{b}	0.008 ± 0.001^{ab}
OLL	1.02 ± 0.10^{bc}	19.41 ± 0.64^{bc}	0.21 ± 0.006^{b}	1.19 ± 0.006^{c}	0.001 ± 0.0003^{b}
ORD	1.00 ± 0.12^{bc}	19.48 ± 0.70^{bc}	0.19 ± 0.003^{c}	1.14 ± 0.046^{c}	0.01 ± 0.005^{a}
OLD	0.86 ± 0.09^{c}	15.94 ± 1.40^{c}	0.19 ± 0.003^{c}	1.13 ± 0.006^{c}	0.01 ± 0.0004^{ab}
SEM	0.08	1.19	0.004	0.031	0.003

SEM: Standard error of means; a,b,c: letters used in Tukeys' test ($P < 0.05$), indicate significant statistical differences when exist in the same column

In relation with FA, the values are expressed as a percentage of oleic acid. FA was affected by aromatization and storage mode. The highest values of FA was found in olive oils exposed to sunlight for 45 days of storage while the lower values were obtained for the control and the olive oils aromatized by rosemary and laurel fresh leaves stored in the dark. The increase in FA values in our study might be related to an increase in enzymatic activity that promotes lipolytic reactions which can be explained by the effect of light and oxygen on the oxidative stability of the olive oil, on the other hand, the laurel aromatization decreased significantly the values of FA of olive oil compared with rosemary aromatization.

The peroxide values of olive oils were also affected by aromatization and storage mode. The highest peroxide values were obtained for olive oils exposed to light, however, the lower values were obtained for those aromatized and stored in dark. The addition of rosemary and laurel fresh leaves to olive oils led to the preservation of these oils against oxidation. While olive oils exposed to light have peroxide values exceeding the limit established by the International Olive Oil Council for virgin olive oil and extra virgin (≤ 20). This can be explained by the oxidation of fatty acids by the action of light [5].

The values of ultraviolet absorbance K_{270} for those samples aromatized by aromatic plants and stored in dark do not exceed the limit established by the International Olive Council for the extra virgin olive oil, which is less than or equal to 0.22,

the values of K_{232} for all samples do not exceed the limit established by the IOC [2], which is less than or equal to 2.6. It should be noted that oils with high values of K_{232} and K_{270}, presented higher peroxide values.

3.2. Total Phenolic Content and TBARs of Olive Oils Aromatized by Rosemary and Laurel Fresh Leaves Stored in Different Modes

Table 2 detailed the total phenolic concentration of olive oils aromatized by rosemary and laurel leaves stored in different modes. The results obtained show that the highest values are recorded for samples containing rosemary and laurel, the phenolic content can be explained by the transfer of these compounds in oil during the storage period and the lower values showed for samples without aromatization exposed or not exposed to light.

Table 2. Phenolic Total Content and TBARs of Olive Oils Aromatized by Rosemary and Laurel Leaves

Samples	Phenolic total content GAE/Kg oil	TBARS mgMDA/Kg oil
Control	320.44 ± 2.60^{c}	0.45 ± 0.01^{d}
OD	275.64 ± 3.85^{d}	1.31± 0.03^{a}
OL	260.56 ± 9.9^{d}	1.58 ± 0.05^{b}
ORL	378.2 ± 7.6^{b}	0.65 ± 0.03^{c}
OLL	396.71 ± 3.43^{b}	0.60 ± 0.05^{cd}
ORD	405.4 ± 8.3^{b}	0.53 ± 0.01^{d}
OLD	447.9 ± 9.4^{a}	0.50 ± 0.01^{d}
SEM	6.44	0.03

a,b,c,d: letters used in Tukeys' test ($P < 0.05$), indicate significant statistical differences when exist in the same column

The results presented in Table 2 showed a negative correlation well established between the phenolic contents in olive oils with that of TBARs which gives an indication about the oxidation of the oil by malondiadéhyde production, observing that the samples having a high content of phenolic compounds submit to less oxidation which results in low levels of malondialdehyde production. The transfer of phenolic compounds from laurel and rosemary to olive oil during storage led to increase the concentration of these phenolic compounds and increasing their protection versus the autoxidation.

3.3. Sensory Analysis of Olive Oils Aromatized by Rosemary and Laurel Fresh Leaves Stored in Different Modes

The results of sensory evaluation of the various samples are shown in Figure 1. Statistical analysis shows that significant differences are obtained between the control olive oil and those aromatized by aromatic plants.

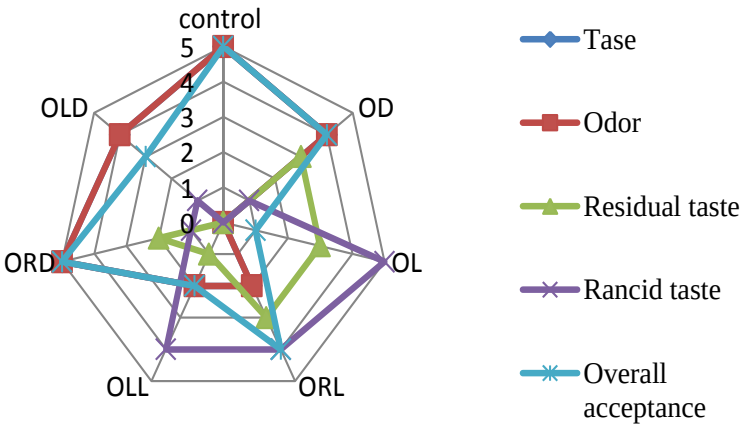

Figure 1. Sensory Analysis of Olive Oils Aromatized by Rosemary and Laurel Leaves

Samples exposed to sunlight aromatized or not have a rancid taste and a bad odor, and are not preferred by trained panel. The aromatized olive oils stored in the dark present a suitable odor and taste. Nevertheless, samples aromatized by rosemary present a residual taste compared with the control and those aromatized by laurel fresh leaves. Based on the overall acceptance of olive oils studied in this work, the trained panel preferred the olive oil aromatized with rosemary fresh leaves stored in the dark.

4. Conclusions

The use of aromatic plants (rosemary and laurel) as additive to olive oil led to improve physicochemical and organoleptic quality. In the present work, the results obtained show that the parameters studied (peroxide value, free acidity, the ex-

tinction coefficients, and malondialdehyde) presented a slight increase as a function of storage period for all samples, with more resistance to autoxidation for those oils containing rosemary and laurel fresh leaves as additive. The phenolic compound concentration is higher in aromatized oils, with a maximum value for the oil that contains laurel. This study demonstrated a negative correlation between the total phenolic compounds and the FA, peroxide value and the concentration of malondialdehyde, while the increase in phenolic content led to decrease of the values of these parameters. The olive oil preferred by the trained panel is the olive oil aromatized by rosemary fresh leaves stored in the dark.

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