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Increasing the stability of Empeltre olive oils by aromatization with rosemary (*Rosmarinus officinalis)* and garlic (*Allium sativum*)

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ABSTRACT

A strategy to increase shelf life of Empeltre olive oils could consist in the incorporation of flavoring agents (rich in antioxidant compounds). The aim of this study was to describe the quality of Empeltre olive oils flavored with rosemary and garlic at different concentrations and methods (maceration and co-processing during malaxation).

The incorporation of garlic during malaxation increased total phenol content and antioxidant capacity.

Aromatization with rosemary (added during malaxation) increased total phenol content more than 50%. Changes in individual phenols were observed after garlic and rosemary aromatization. Slight increases in α-tocopherol were also observed at 5 and 7% concentration. Pigment content increased with rosemary concentration. As a consequence, antioxidant capacity and oxidative stability increased. Aromatization with rosemary by co-processing during malaxation was more effective in increasing antioxidant compounds than the maceration method.

Empeltre olive oils aromatized with garlic achieved a greater sensory acceptance and better scores than with rosemary.

Introduction

A number of previous investigations describe the aromatization of olive oils with different sources of natural bioactive ingredients such as olive leaves, olive pomace, herbs and spices (red pepper, lavender, laurel, chili, garlic, rosemary, basil, thyme, mint, oregano, etc) fruits (lemon, orange, etc), truffles, licopen, seaweed, walnuts, fish oil, etc (Damechki et al., 2001; Bendini et al., 2002; Ayadi et al., 2009; Sousa et al., 2015; Baiano et al., 2016; González et al., 2017; Campo et al., 2018; Kasimoglu et al., 2018). Aromatization methods include traditional maceration, maceration with the application of ultrasound (Assami et al., 2016) or microwaves (Benmousa et al., 2016), extraction of different compounds (by solid-liquid extraction, liquid-liquid extraction, or supercritic extraction) from the material and incorporation of the extract into the oil, and, finally, co-processing during milling or malaxation (Clodoveo et al., 2016; Yilmazer et al., 2016; Sacchi et al., 2017; Sena-Moreno et al., 2018; Issaoui et al., 2019). Reboredo-Rodríguez et al. (2017) have reviewed the current potential of producing virgin olive oils enriched with bioactive compounds, which allow for an optimum intake of phenols in diet. In most cases, the incorporation of different sources of bioactive ingredients leads to an increase of phenols in olive oil, coupled with an increase in oxidative stability and antioxidant activity. Such olive oils can thus be regarded as functional olive oils with a highly interesting potential for added value and market diversification. The consumption of such aromatized olive oils can likewise help avoid certain chronic diseases.

Empeltre is an olive variety mainly cultivated in the northeast regions of Spain. These olives are known to provide yellow-coloured oils with a soft taste, a fruity touch, and a bitterness that is not excessive. This makes them ideal for consumers accustomed to the taste of refined oils obtained by solvent extraction. Empeltre olives are characterized by their low polyphenolic content (Gracia-Gómez et al., 2009), and for this reason oil stability during shelf life is short.

From Empeltre olives it is possible to produce enriched virgin olive oil by adding different sources of natural biologically active substances such as polyphenols. A transfer of bioactive compounds from these materials to the oil takes place in the process. Apart from aromatizing the olive oil from a sensory point of view, these sources also possess antioxidant and antimicrobial properties. They thus respond to the current demand for new healthy foods.

Some previous studies described the aromatization of different olive oil cultivars as Arbequina, Coratina, Peranzana, Ogliarola, Picual,

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Table 1
Experimental design of aromatized Empeltre olive oils with different concentrations of rosemary and garlic, and different methods for aromatization.

NATURAL BIOACTIVE INGREDIENT	AROMATIZATION METHOD					
	Maceration			Added during malaxation		
	1%	5%	7%	1%	5%	7%
Rosemary powder	RI1	RI5	RI7	RM1	RM5	RM7
Garlic powder	GI1	GI5	GI7	GM1	GM5	GM7
Control						

Cobrançosa, Galega (Serrano et al., 2016; Romanielo and Baiano 2018; Sena- Moreno et al., 2018). Nevertheless, the effect of the enrichment of Empeltre olive oils with some of these compounds has not been previously described and this is the novelty of this work.

The aim of this study is to investigate the effect of garlic and rosemary aromatization of Empeltre olive oils on their physico-chemical, nutritional, and sensorial properties, as well as on their oxidative stability. Two different aromatization methods (maceration and co-processing during malaxation) are herein assayed, at three different concentrations.

Material and methods

Plant material

Olive fruits (*Olea Europaea* L.) of the Empeltre variety were supplied from an olive mill near Zaragoza (Spain) in the 2019–2020 crop season. The olives had a 5.45 ± 0.17 maturity index following the method based in the pigmentation levels (Hermoso et al., 1991).

Commercial rosemary dry powder (*Rosmarinus officinalis*) and garlic powder (*Allium sativum*) were used.

Olive oil extraction process

Oil extraction was carried out with Abencor laboratory equipment, following the method described by Martínez et al. (1975). The olives were cleaned and crushed with a mill, and the paste was malaxated at 26 °C ± 1 °C for 30 min and then centrifuged at 3500 rpm for 1 min. After filtration, olive oil samples were stored at −18 °C in darkness using amber glass bottles with nitrogen in the headspace prior to analysis

Aromatization of the olive oils

Aromatization with rosemary and garlic was carried out at three different concentrations (1, 5 and 7% w/w) chosen after review of previous works (Clodoveo et al., 2016), and using two different methods. A control olive oil sample without rosemary or garlic was obtained. The experimental design is shown in Table 1.

In the first method (maceration), the rosemary powder was incorporated into the olive oil by stirring at 200 r.p.m. during 24 h at room temperature. The olive oils were subsequently filtered: first by using Whatman ashness number 40 (fast-medium flow) paper filters and then Whatman ashness number 42 (slow-flow) paper filters. In the second method, the rosemary or garlic powder was added to the olive paste prior to malaxation. After filtering, all olive oils were kept frozen in dark bottles.

Analysis

Evaluations of physico-chemical quality parameters (free acidity, peroxide value, and UV absorption characteristics K_{270} and K_{232}) were performed following the official methods described in Regulation EEC/2568/91 of the Commission of the European Union (EEC, 1991).

α-tocopherol determination

A solution of 1 g oil in 10 mL hexane was analyzed by HPLC Hewlett Packard (Agilent-series 1100) with a Zorbax SB-C18 phase-reverse column (particle size 3.5 μm, 150 mm × 4.6 mm i.d.; Agilent Technologies), eluted with acetonitrile/water (99:1 v/v) at a flow rate of 1 mL/min. The injection volume was 20 μl. A photodiode matrix detector (G1315B, serie 1100) was used. Chromatograms were registered at 295 nm. Results were expressed as mg α-tocopherol/kg oil.

Total phenol content

The extraction of the total phenols from the olive oil was carried out following the method described by Favati et al. (1994). The phenols were extracted by Solid Phase Extraction (SPE) using Isolute C18 cartridges (6 mL/1g solid phase). The extract was dried at 40 °C in a rotary evaporator, and the residue was dissolved in 5 mL methanol. For the colorimetric determination of total phenols, 2.5 mL of extract were mixed with 1.25 mL of Folin-Ciocalteau reagent, and, after 3 min, 2.5 mL of sodium carbonate 7.5% were added. The absorption of the solution was measured at 725 nm after 1 h of reaction. Results were expressed as mg gallic acid/kg oil.

Individual phenols

Phenolic compounds were extracted from olive oil following the method described by Gutfinger (1981). A HPLC Hewlett Packard (Agilent-series 1100) with a Zorbax SB-C18 phase-reverse column (particle size 3.5 μm, 150 mm × 4.6 mm i.d.; Agilent Technologies) was used. The eluents were 0.2% aqueous acetic acid (pH 3.1) and methanol, the flow rate was 1.5 mL/min, and the injection volume was 20 μl. The total run time was 60 min. The initial composition was 95% aqueous acetic acid and 5% methanol, and the gradient changed as follows: the concentration of methanol was maintained for 2 min, then it was increased to 25% for 8 min, and, finally, the methanol percentage was increased to 40, 50, and 100% for 10 min at each of the percentages. Initial conditions were reached after a total of 15 min. Chromatograms were obtained at 280 nm and 339 nm. Phenolic compounds were identified on the basis of their retention times compared to those of the standard compounds. Individual phenols (tyrosol; vanillic acid; vanillin; coumaric acid; 3.4-DHPEA-AC; 3.4-DHPEA-EDA; *p*-HPEA-EDA; pinoresinol and 3.4-DHPEA-EA) were quantified at 280 nm, lutein and apigenin were identified and quantified at 339 nm. The results were expressed as mg/kg oil.

Oxidative stability

A Rancimat 743 apparatus was used to obtain the oxidation induction time (hours). An oil sample of 3 g was warmed to 120 °C with 20 L/h air flow. The induction time is the time required to reach the breaking point of the curve.

Determination of chlorophyll and carotenoids compounds

Chlorophyll and carotenoid were determined from the absorption spectra of each sample (7.5 g) dissolved in cyclohexane (25 mL) following the method of Minguez-Mosquera et al. (1991). Maximum absorption is related to the chlorophyll fraction at 670 nm and to the carotenoid fraction at 470 nm. The applied values of the coefficients of specific extinction were E0 = 613 for pheophytin (the major component in the chlorophyll fraction) and E0 = 2000 for lutein (the major component in the carotenoid fraction). The concentrations of chlorophyll and carotenoids were expressed as mg pheophytin and lutein/kg oil, respectively.

Antioxidant activity

10 g olive oil samples were dissolved in 50 mL hexane. A 20 mL metanol/water (60/40) solution was added and mixed, and the extract was collected and dried in a rotavapor. The process was repeated three times. Samples were dissolved in 5 mL metanol. Different dilutions with the extracts, metanol, and DPPH 100 μM were prepared, as well as a

Table 2
Physico-chemical parameters of Empeltre olive oils aromatized with rosemary (2a) and garlic (2b)

AROMATIZATION METHOD AND CONCENTRATION OF ROSEMARY	PHYSICOCHEMICAL PARAMETERS			
	Acidity (% oleic cid)	Peroxide value (mEq O_2 active/kg oil)	K_{232} (Abs 232 nm)	K_{270} (Abs 270 nm)
Control	$0.17 \pm 0.01^{A;E}$	$7.33 \pm 0.67^{B;E}$	$1.20 \pm 0.19^{A;E}$	$0.05 \pm 0.01^{A;E}$
Maceration				
1%	$0.16 \pm 0.02^{A;I}$	$3.11 \pm 0.77^{A;I}$	$1.32 \pm 0.02^{AB;I}$	$0.08 \pm 0.01^{B;I}$
5%	$0.30 \pm 0.02^{B;K}$	$6.44 \pm 0.38^{B;K}$	$1.57 \pm 0.02^{BC;K}$	$0.13 \pm 0.02^{C;K}$
7%	$0.30 \pm 0.03^{B;M}$	$7.78 \pm 1.68^{B;M}$	$1.81 \pm 0.07^{C;M}$	$0.17 \pm 0.01^{D;M}$
Added during malaxation				
1%	$0.23 \pm 0.01^{F;J}$	$8.67 \pm 0.67^{E;J}$	$1.48 \pm 0.03^{F;J}$	$0.13 \pm 0.01^{F;J}$
5%	$0.43 \pm 0.02^{G;L}$	$9.11 \pm 1.39^{E;L}$	$1.83 \pm 0.01^{G;L}$	$0.06 \pm 0.02^{E;L}$
7%	$0.52 \pm 0.02^{H;N}$	$9.11 \pm 0.38^{E;M}$	$2.08 \pm 0.01^{H;N}$	$0.07 \pm 0.02^{E;N}$

AROMATIZATION METHOD AND CONCENTRATION OF GARLIC	PHYSICOCHEMICAL PARAMETERS			
	Acidity (% oleic acid)	Peroxide value (mEq O_2 active/kg oil)	K_{232} (Abs 232 nm)	K_{270} (Abs 270 nm)
Control	$0.17 \pm 0.01^{A;E}$	$7.33 \pm 0.67^{C;FG}$	$1.20 \pm 0.19^{A;E}$	$0.05 \pm 0.01^{A;F}$
Maceration				
1%	$0.15 \pm 0.02^{A;I}$	$3.33 \pm 0.67^{B;I}$	$1.30 \pm 0.15^{A;I}$	$0.03 \pm 0.01^{A;I}$
5%	$0.15 \pm 0.03^{A;K}$	$3.11 \pm 0.38^{AB;K}$	$1.18 \pm 0.04^{A;K}$	$0.08 \pm 0.02^{B;K}$
7%	$0.13 \pm 0.02^{A;M}$	$2.00 \pm 0.01^{A;M}$	$1.17 \pm 0.02^{A;M}$	$0.06 \pm 0.01^{AB;M}$
Added during malaxation				
1%	$0.15 \pm 0.02^{E;I}$	$9.33 \pm 1.15^{F;J}$	$1.40 \pm 0.05^{E;I}$	$0.03 \pm 0.01^{E;I}$
5%	$0.17 \pm 0.01^{E;K}$	$6.00 \pm 1.15^{G;L}$	$1.32 \pm 0.03^{E;L}$	$0.03 \pm 0.01^{E;L}$
7%	$0.18 \pm 0.04^{E;M}$	$1.11 \pm 0.38^{E;N}$	$1.19 \pm 0.01^{E;M}$	$0.03 \pm 0.01^{EF;N}$

Values reported are mean values and standard deviations of three replicates. $^{A-D}$For each parameter, different letters for the maceration aromatization method with rosemary indicate statistically differences ($p < 0.05$) among the concentration; $^{E-H}$For each parameter, different letters for the aromatization with rosemary added during malaxation indicate statistically differences ($p < 0.05$) among concentration; $^{I-J}$For each parameter, different letters for the concentration of 1% with rosemary indicate statistically differences ($p < 0.05$) between the aromatization method; $^{K-L}$For each parameter, different letters for the concentration of 5% with rosemary indicate statistically differences ($p < 0.05$) between the aromatization method; $^{M-N}$For each parameter, different letters for the concentration of 7% with rosemary indicate statistically differences ($p < 0.05$) between the aromatization method.

Values reported are mean values and standard deviations of three replicates. $^{A-D}$For each parameter, different letters for the maceration aromatization method with garlic indicate statistically differences ($p < 0.05$) among the concentration; $^{E-H}$For each parameter, different letters for the aromatization with garlic added during malaxation indicate statistically differences ($p < 0.05$) among concentration; $^{I-J}$For each parameter, different letters for the concentration of 1% with garlic indicate statistically differences ($p < 0.05$) between the aromatization method; $^{K-L}$For each parameter, different letters for the concentration of 5% with garlic indicate statistically differences ($p < 0.05$) between the aromatization method; $^{M-N}$For each parameter, different letters for the concentration of 7% with garlic indicate statistically differences ($p < 0.05$) between the aromatization method.

calibration curve with Trolox 100 μM. After keeping the dilutions 30 min in the dark, their absorbance at 515 nm was monitored in the UV–Visible spectrophotometer (Unicam UV 500).

Sensory analysis

A sensory analysis of the samples was carried out by 10 selected and trained panelists. They evaluated the samples by ascribing them with positive (fruity, bitter, and pungent) and negative (fusty, winey/vinegary, musty, muddy, rancid, metallic, and other) attributes based on EU Regulations EEC/2568/91 (EEC, 1991). Other attributes (rosemary and garlic taste and smell, total taste and smell, color intensity, turbidity and particles) were likewise included. The assessment of turbidity and the presence of particles was carried out visually. The equilibrium was assessed considering all the attributes together.

Statistical analysis

Statistical analysis was performed using GraphPad Prism, Version 5.0 (GraphPad Software, Inc., USA). Results were expressed as mean ± standard deviation of three experiments and as least squares mean ± 95% confidence interval.

Significant differences between different concentrations of garlic and rosemary and different aromatization methods (maceration, or co-processing during malaxation) were determined by analysis of variance (one-way ANOVA) and Multiple Range Test.

Sensory analysis data were analyzed by PCA (Principal Component Analysis) using PanelCheck software (Norway).

Results and discussion

Different aromatization methods and flavoring agents have an influence on the quality parameters of Empeltre olive oil.

The physico-chemical quality parameters of the aromatized olive oils are shown in Table 2a and Table 2b in comparison with the control olive oil. We compared the results with the European regulations for extra virgin olive oils (EEC, 1991). In all cases, the values lay below the limits established for extra virgin olive oil (acidity $\leq$ 0.8% oleic acid, peroxide index $\leq$ 20 meq O_2/kg oil, $K_{232} \leq 2.5$ and $K_{270} \leq 0.22$). Anyway these aromatized olive oils are not included in this commercial classification and must be considered as a condiment. In this way, following Spanish Agency for Food Safety and Nutrition these products should follow spanish regulations for condiments.

No significant differences were observed for acidity in olive oils aromatized with garlic. Results were lower than the values described by Sousa et al. (2015). The peroxide index decreased in garlic-aromatized olive oils prepared by maceration. In garlic-aromatized olive oils, significative differences in K_{232} were not observed with respect to control. The values decreased when garlic concentration increased. K_{270} was lower than control when malaxation was used to incorporate the garlic to the olive oil. When maceration was used, the coefficient was higher at 5 and 7% garlic concentration.

Acidity increased with rosemary concentration, especially when rosemary was incorporated before malaxation. Significant differences were observed in comparison with control. Similar trends have been observed in previous studies (Ayadi et al., 2009). Nevertheless, these values were lower than those previously described by Ait et al. (2016) and Karacabey et al. (2016), who reported values around 0.6–0.7% for 1–1.5% rosemary concentration and 1–1.26% for 5% rosemary concentration, respectively. The peroxide index increased when rosemary was added before malaxation, and it was higher than control. This was an unexpected result since due to the antioxidant content of the rosemary would protect the olive oil from peroxidation. The values in the olive oils aromatized by maceration were lower than the 8 meqO_2/kg olive oil reported by Damechki et al. (2001) in olive oils aromatized by maceration with 5% rosemary. K_{232} increased with rosemary concentration added in both methods. Nevertheless, the values were lower than

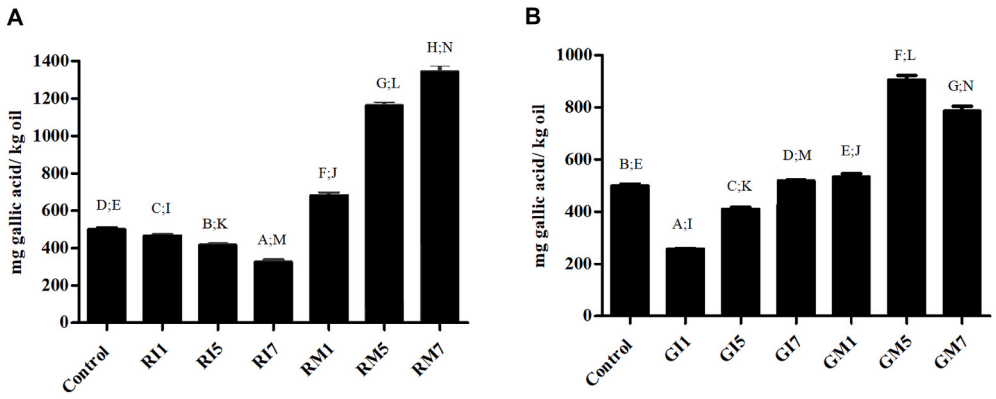

Fig. 1. Total phenol content of Empeltre olive oils aromatized with rosemary (1a) and garlic (1b).

Table 3
Individual phenols of Empeltre olive oils aromatized with rosemary (3a) and garlic (3b)

INDIVIDUAL PHENOLS	*Control*	*Maceration method*			*Added during malaxation*		
		1%	5%	7%	1%	5%	7%
Hydroxytyrosol	$1.30 \pm 0.11^{A;E}$	$2.33 \pm 0.25^{B;I}$	$4.12 \pm 0.11^{C;K}$	$2.62 \pm 0.23^{B;M}$	$1.54 \pm 0.06^{E;J}$	$2.55 \pm 0.02^{F;L}$	$6.61 \pm 0.17^{G;N}$
Tyrosol	$1.83 \pm 0.05^{C;E}$	$2.33 \pm 0.04^{D;I}$	$1.03 \pm 0.10^{A;K}$	$1.40 \pm 0.03^{B;M}$	$2.40 \pm 0.07^{F;I}$	$2.57 \pm 0.01^{G;L}$	$3.17 \pm 0.10^{H;N}$
Vanilic acid	$0.15 \pm 0.01^{A;E}$	$0.48 \pm 0.03^{B;I}$	$0.35 \pm 0.01^{C;K}$	$0.79 \pm 0.03^{D;M}$	$0.31 \pm 0.02^{F;J}$	$0.75 \pm 0.03^{G;L}$	$0.61 \pm 0.02^{H;N}$
Vanillin	$1.11 \pm 0.02^{A;E}$	$1.47 \pm 0.01^{B;I}$	$1.21 \pm 0.05^{C;K}$	$1.44 \pm 0.01^{B;M}$	$1.51 \pm 0.01^{F;J}$	$1.88 \pm 0.01^{G;L}$	$1.99 \pm 0.01^{H;N}$
Coumaric acid	$0.65 \pm 0.01^{B;G}$	$0.38 \pm 0.02^{A;I}$	$0.50 \pm 0.01^{C;K}$	$0.65 \pm 0.02^{B;M}$	$0.50 \pm 0.01^{E;J}$	$0.60 \pm 0.01^{F;L}$	$0.60 \pm 0.02^{F;N}$
3.4-DHPEA-AC	$18.03 \pm 0.19^{A;E}$	$22.54 \pm 0.14^{B;I}$	$16.50 \pm 0.06^{C;K}$	$23.44 \pm 0.38^{D;M}$	$21.43 \pm 0.73^{G;I}$	$19.56 \pm 0.24^{F;L}$	$17.26 \pm 0.10^{E;N}$
3.4-DHPEA-EDA	$214.24 \pm 9.60^{AB;E}$	$364.95 \pm 1.78^{C;I}$	$197.34 \pm 10.00^{A;K}$	$225.00 \pm 2.77^{B;M}$	$372.72 \pm 5.66^{F;I}$	$365.53 \pm 4.64^{F;L}$	$298.32 \pm 5.16^{G;N}$
p-HPEA-EDA	$88.99 \pm 2.47^{B;G}$	$84.04 \pm 0.50^{B;I}$	$62.29 \pm 4.97^{A;K}$	$57.56 \pm 3.85^{A;M}$	$99.20 \pm 2.20^{F;J}$	$81.25 \pm 1.52^{E;L}$	$80.50 \pm 2.00^{E;N}$
Pinoresinol	$33.62 \pm 1.19^{C;E}$	$42.70 \pm 0.18^{B;I}$	$23.47 \pm 1.15^{A;K}$	$40.97 \pm 3.47^{B;M}$	$54.31 \pm 4.10^{G;I}$	$32.90 \pm 1.43^{F;L}$	$38.50 \pm 0.87^{F;M}$
3.4-DHPEA-EA	$27.37 \pm 0.35^{C;H}$	$21.19 \pm 0.42^{B;I}$	$18.09 \pm 0.10^{A;K}$	$18.03 \pm 0.17^{A;M}$	$29.11 \pm 0.04^{G;J}$	$22.11 \pm 0.17^{E;L}$	$22.76 \pm 0.27^{F;N}$
Luteolin	$1.59 \pm 0.06^{B;G}$	$1.37 \pm 0.02^{C;I}$	$1.19 \pm 0.03^{A;K}$	$1.67 \pm 0.10^{B;M}$	$1.76 \pm 0.03^{F;J}$	$1.42 \pm 0.02^{E;L}$	$1.73 \pm 0.05^{F;M}$
Apigenin	$0.94 \pm 0.01^{A;E}$	$2.38 \pm 0.01^{B;I}$	$1.17 \pm 0.01^{C;K}$	$1.38 \pm 0.02^{D;M}$	$1.12 \pm 0.01^{F;J}$	$1.08 \pm 0.01^{G;L}$	$1.19 \pm 0.02^{H;N}$
INDIVIDUAL PHENOLS	*Control*	*Maceration method*			*Added during malaxation*		
		1%	5%	7%	1%	5%	7%
Hydroxytyrosol	$1.30 \pm 0.11^{A;E}$	$1.53 \pm 0.06^{A;I}$	$1.94 \pm 0.13^{B;K}$	$2.27 \pm 0.06^{C;M}$	$1.70 \pm 0.07^{F;J}$	$3.1 \pm 0.02^{G;I}$	$4.11 \pm 0.07^{H;N}$
Tyrosol	$1.83 \pm 0.05^{C;E}$	$1.68 \pm 0.01^{A;I}$	$2.94 \pm 0.10^{B;K}$	$1.57 \pm 0.05^{A;M}$	$1.91 \pm 0.02^{E;J}$	$2.45 \pm 0.03^{F;L}$	$2.50 \pm 0.08^{F;N}$
Vanilic acid	$0.15 \pm 0.01^{C;E}$	$0.26 \pm 0.01^{B;I}$	$0.26 \pm 0.02^{B;K}$	$0.11 \pm 0.01^{A;M}$	$0.16 \pm 0.01^{E;J}$	$0.36 \pm 0.01^{F;L}$	$0.41 \pm 0.02^{G;N}$
Vanillin	$1.11 \pm 0.02^{A;E}$	$1.21 \pm 0.01^{B;I}$	$1.23 \pm 0.01^{B;K}$	$1.11 \pm 0.01^{A;M}$	$1.13 \pm 0.01^{E;J}$	$1.31 \pm 0.02^{F;L}$	$1.33 \pm 0.01^{F;N}$
Coumaric acid	$0.65 \pm 0.01^{B;G}$	$0.55 \pm 0.01^{A;I}$	$0.81 \pm 0.02^{C;K}$	$1.05 \pm 0.01^{D;M}$	$0.53 \pm 0.01^{F;I}$	$0.53 \pm 0.01^{F;L}$	$0.50 \pm 0.01^{E;N}$
3.4-DHPEA-AC	$18.03 \pm 0.19^{A;F}$	$28.13 \pm 0.20^{B;I}$	$25.32 \pm 0.81^{C;K}$	$27.86 \pm 0.41^{B;M}$	$16.24 \pm 0.09^{E;J}$	$18.78 \pm 0.38^{G;L}$	$20.58 \pm 0.07^{H;N}$
3.4-DHPEA-EDA	$214.24 \pm 9.60^{BC;F}$	$225.44 \pm 0.68^{B;I}$	$216.29 \pm 0.47^{B;K}$	$200.74 \pm 4.62^{AC;M}$	$198.71 \pm 3.86^{F;J}$	$174.83 \pm 7.44^{E;L}$	$194.4 \pm 9.06^{EF;M}$
p-HPEA-EDA	$88.99 \pm 2.47^{BA;G}$	$93.35 \pm 1.16^{B;I}$	$87.54 \pm 1.11^{A;K}$	$73.72 \pm 2.30^{C;M}$	$72.71 \pm 0.05^{F;J}$	$64.95 \pm 1.60^{E;L}$	$66.98 \pm 2.23^{E;N}$
Pinoresinol	$33.62 \pm 1.19^{A;F}$	$35.08 \pm 0.76^{AB;I}$	$37.6 \pm 1.28^{BC;K}$	$40.23 \pm 0.76^{C;M}$	$35.34 \pm 0.25^{F;I}$	$30.16 \pm 0.48^{E;L}$	$33.63 \pm 1.59^{F;N}$
3.4-DHPEA-EA	$27.37 \pm 0.35^{B;F}$	$20.54 \pm 0.04^{C;I}$	$19.33 \pm 0.25^{A;K}$	$27.64 \pm 0.33^{B;M}$	$27.67 \pm 0.24^{F;J}$	$22.16 \pm 0.15^{E;L}$	$37.72 \pm 0.52^{G;N}$
Luteolin	$1.59 \pm 0.06^{B;F}$	$1.10 \pm 0.03^{A;I}$	$1.08 \pm 0.01^{A;K}$	$1.15 \pm 0.01^{A;M}$	$1.50 \pm 0.02^{E;J}$	$1.47 \pm 0.01^{E;L}$	$1.49 \pm 0.02^{E;N}$
Apigenin	$0.94 \pm 0.01^{A;F}$	$1.08 \pm 0.01^{B;I}$	$1.08 \pm 0.01^{B;K}$	$1.13 \pm 0.01^{C;M}$	$1.01 \pm 0.01^{F;J}$	$0.98 \pm 0.01^{G;L}$	$1.00 \pm 0.01^{FG;N}$

Values reported are mean values and standard deviations of three replicates. 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, dialdehydric form of elenolic acid linked to hydroxytyrosol; p-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycone.
$^{A-D}$For each parameter, different letters for the maceration aromatization method with rosemary indicate statistically differences ($p < 0.05$) among the concentration; $^{E-H}$For each parameter, different letters for the aromatization with rosemary added during malaxation indicate statistically differences ($p < 0.05$) among concentration; $^{I-J}$For each parameter, different letters for the concentration of 1% with rosemary indicate statistically differences ($p < 0.05$) between the aromatization method; $^{K-L}$For each parameter, different letters for the concentration of 5% with rosemary indicate statistically differences ($p < 0.05$) between the aromatization method; $^{M-N}$For each parameter, different letters for the concentration of 7% with rosemary indicate statistically differences ($p < 0.05$) between the aromatization method.

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$^{A-D}$For each parameter, different letters for the maceration aromatization method with garlic indicate statistically differences ($p < 0.05$) among the concentration; $^{E-H}$For each parameter, different letters for the aromatization with garlic added during malaxation indicate statistically differences ($p < 0.05$) among concentration; $^{I-J}$For each parameter, different letters for the concentration of 1% with garlic indicate statistically differences ($p < 0.05$) between the aromatization method; $^{K-L}$For each parameter, different letters for the concentration of 5% with garlic indicate statistically differences ($p < 0.05$) between the aromatization method; $^{M-N}$For each parameter, different letters for the concentration of 7% with garlic indicate statistically differences ($p < 0.05$) between the aromatization method.

those described by Baiano et al. (2009) and higher than the values cited by Karacabey et al. (2016) in olive oils with 1% rosemary. K_{270} showed significative differences with respect to control when the maceration method was used, and increased in tandem with rosemary concentration.

Fig. 1a and Fig. 1b show the total phenol content in the aromatized olive oils. During maceration or malaxation, garlic and rosemary phenols migrate to the olive oil.

For olive oils aromatized with garlic, this value increased with addition in malaxation at 5 and 7% concentration. It decreased in comparison with control when the maceration method was used, and when the rosemary concentration increased. Significant differences

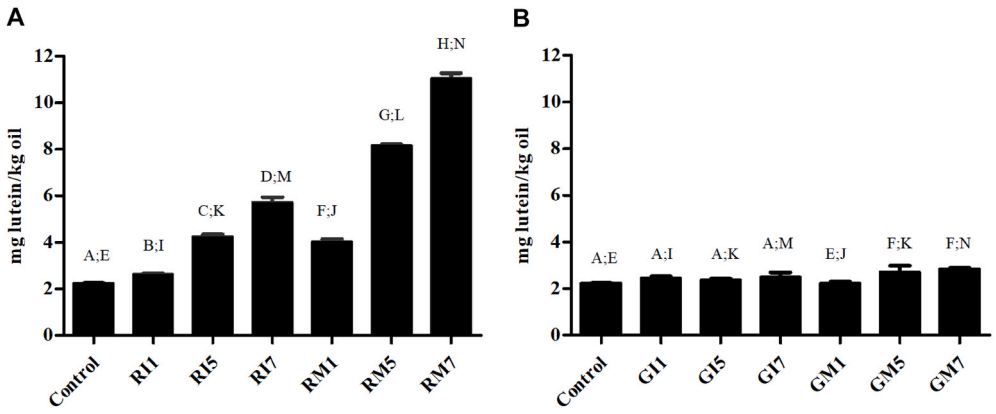


Fig. 2. Carotenoid content of Empeltre olive oils aromatized with rosemary (2a) and garlic (2b).

were observed. Nevertheless, an increase was observed when rosemary was added before malaxation. At 5% and 7% rosemary concentration phenol content was more than twice that of control olive oil. In both flavored olive oils, malaxation was more effective than maceration for increasing total phenol content. This behavior has been described in previous studies by Caponio et al. (2016) and Clodoveo et al. (2016), who indicate that vegetation water in the olive paste can act as a solvent for the extraction of polar compounds from herbs.

Regarding individual phenols (Table 3a and Table 3b), certain changes were observed after aromatization with both garlic and rosemary. In previous studies this behavior has also been described. The aromatization method affects the individual phenol profile (Caponio et al., 2016). The health claim for olive oil polyphenols as approved by European regulations ("Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress. The claim may be used only for olive oil, containing at least 5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil") leads to increased general interest in these individual phenols (EU, 2012). In our study, in control oil and rosemary and garlic aromatized olive oils the most abundant individual phenol was 3, 4-DHPEA-EDA (at any concentration and method). This value increased when rosemary was incorporated during malaxation, and decreased when garlic was incorporated with the same method. For rosemary-flavored olive oils, Baiano et al. (2009) found that p-HPEA-EDA was the most abundant compound. In our study, hydroxytyrosol increased in all the aromatized olive oils (with both garlic and rosemary) in comparison with control. Anyway this compound is not present in garlic and rosemary. This individual phenol plays an important role in the antioxidant stability and antioxidant activity of extra virgin olive oils (Baiano et al., 2016). Tyrosol increased during malaxation of garlic and rosemary.

Chromatograms of the aromatized olive oils (results not shown) were different in comparison with those of the control olive oils due to the presence (especially in rosemary-flavored oils) of unidentified peaks in the final part of the chromatogram, probably due to the flavoring agents. In rosemary and garlic some different individual phenols in comparison with olive oil are observed (Vallverdú- Queralt et al., 2014; Liu et al., 2018). In rosemary coumaric acid and apigenin like in olive oil are observed. Also other compounds are observed: rosmarinic acid, carnosol, rosmanol, p-hydroxybenzoic acid, caffeic acid, syringic acid, In garlic coumaric acid, luteolin and apigenin like in olive oil are observed. Another phenols compounds in garlic are ferulic acid, p-coumaric acid, naringenin, protocatechuic acid, isorhamnetin, phthalic acid and quercetin.

Chlorophylls and carotenoids are pigments responsible for color in olive oil. The carotenoid content of the garlic and rosemary flavored olive oils is shown in Fig. 2a and Fig. 2b. When garlic was added by maceration, no significant differences were observed in comparison with control olive oil. When garlic was added during malaxation, significant differences were observed at 5 and 7% concentration. In the case of rosemary incorporation, carotenoid content increased with concentration using both methods of aromatization, especially with malaxation. In all cases, significant differences were observed, and the influence on this content was greater when compared with garlic flavoring.

Chlorophyll content did not change significantly with garlic concentration and aromatization method. Nevertheless, in olive oils aromatized with rosemary, significant differences were observed for both methods in comparison with control (Fig. 3a and Fig. 3b). Chlorophyll content increased with rosemary concentration, and was about one hundred times higher than in garlic-flavored olive oils (Ayadi et al., 2009).

In addition to polyphenols, chlorophylls and carotenoids also contribute to the oxidative stability of olive oils, thanks to their

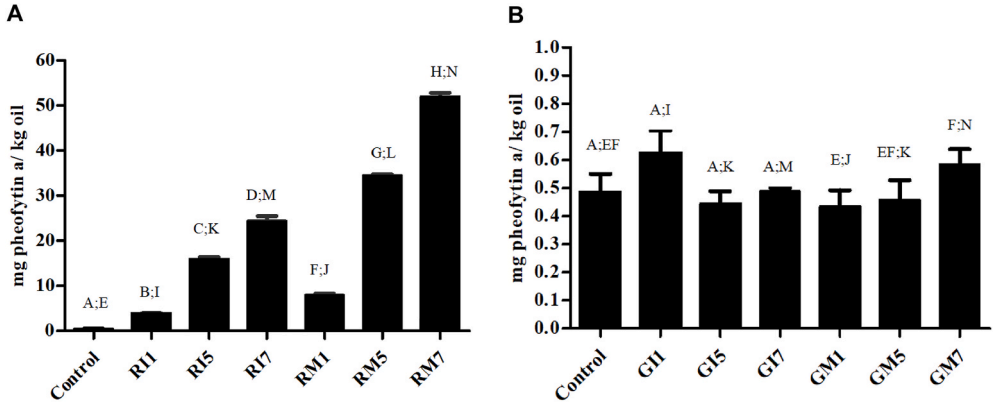


Fig. 3. Chlorophyll content of Empeltre olive oils aromatized with rosemary (3a) and garlic (3b).

Table 4
α-tocopherol, oxidative stability, and antioxidant capacity of Empeltre olive oils aromatized with rosemary (4a) and garlic (4b)

AROMATIZATION METHOD AND CONCENTRATION OF ROSEMARY	PARAMETERS		
	α-Tocopherol (mg/kg oil)	Oxidative stability (hours)	Antioxidant capacity (μmol TE/100 g oil)
Control	296.57 ± 4.90$^{B;E}$	10.58 ± 0.38$^{A;E}$	3.83$^{B;E}$
Maceration			
1%	276.02 ± 2.63$^{A;I}$	18.10 ± 0.24$^{C;I}$	3.78$^{C;I}$
5%	299.60 ± 6.69$^{B;K}$	20.11 ± 0.61$^{B;K}$	3.86$^{B;K}$
7%	313.08 ± 9.82$^{B;M}$	21.74 ± 0.50$^{B;M}$	3.69$^{A;M}$
Added during malaxation			
1%	302.31 ± 11.02$^{EF;J}$	15.31 ± 0.08$^{F;J}$	7.75$^{F;J}$
5%	340.97 ± 5.93$^{G;L}$	23.26 ± 0.06$^{G;L}$	15.55$^{G;L}$
7%	329.68 ± 16.25$^{FG;M}$	22.45 ± 0.07$^{H;M}$	14.75$^{H;N}$
AROMATIZATION METHOD AND CONCENTRATION OF GARLIC	PARAMETERS		
	α-Tocopherol (mg/kg oil)	Oxidative stability (hours)	Antioxidant capacity (μmol TE/100 g oil)
Control	296.57 ± 4.90$^{A;E}$	10.58 ± 0.38$^{A;E}$	3.83$^{C;E}$
Maceration			
1%	312.22 ± 3.60$^{B;I}$	12.90 ± 0.13$^{B;I}$	2.38$^{A;I}$
5%	301.16 ± 2.05$^{A;K}$	12.79 ± 0.41$^{B;K}$	2.36$^{A;K}$
7%	313.72 ± 3.83$^{B;M}$	12.59 ± 0.64$^{B;M}$	4.77$^{B;M}$
Added during malaxation			
1%	290.67 ± 9.48$^{E;J}$	9.95 ± 0.51$^{E;J}$	4.87$^{F;J}$
5%	289.78 ± 1.87$^{E;L}$	9.98 ± 0.30$^{E;L}$	9.42$^{G;L}$
7%	313.68 ± 3.74$^{F;M}$	11.96 ± 0.19$^{F;M}$	9.39$^{G;N}$

Values reported are mean values and standard deviations of three replicates. $^{A-D}$For each parameter, different letters for the maceration aromatization method with rosemary indicate statistically differences ($p < 0.05$) among the concentration; $^{E-H}$For each parameter, different letters for the aromatization with rosemary added during malaxation indicate statistically differences ($p < 0.05$) among concentration; $^{I-J}$For each parameter, different letters for the concentration of 1% with rosemary indicate statistically differences ($p < 0.05$) between the aromatization method; $^{K-L}$For each parameter, different letters for the concentration of 5% with rosemary indicate statistically differences ($p < 0.05$) between the aromatization method; $^{M-N}$For each parameter, different letters for the concentration of 7% with rosemary indicate statistically differences ($p < 0.05$) between the aromatization method.
Values reported are mean values and standard deviations of three replicates. $^{A-D}$For each parameter, different letters for the maceration aromatization method with garlic indicate statistically differences ($p<0.05$) among the concentration; $^{E-H}$For each parameter, different letters for the aromatization with garlic added during malaxation indicate statistically differences ($p<0.05$) among concentration; $^{I-J}$For each parameter, different letters for the concentration of 1% with garlic indicate statistically differences ($p<0.05$) between the aromatization method; $^{K-L}$For each parameter, different letters for the concentration of 5% with garlic indicate statistically differences ($p<0.05$) between the aromatization method; $^{M-N}$For each parameter, different letters for the concentration of 7% with garlic indicate statistically differences ($p<0.05$) between the aromatization method.

antioxidant properties in the dark and prooxidant activity in the light.

The α-tocopherol content for the aromatized olive oils is shown in Table 4a and Table 4b. This value likewise contributes to oxidative stability. When garlic was incorporated, significant differences with control olive oil were described at 7% concentration for both methods. This is may be due to the migration of the garlic α-tocopherol to the aromatized olive oil. When rosemary was added, the highest content was observed at 5 and 7% concentration incorporated during malaxation.

Tables 4a and 4b also show the aromatized olive oils' oxidative stability and antioxidant activity. Oxidative stability in olive oils aromatized with garlic was higher than in control, when it was incorporated by maceration. When garlic was incorporated during malaxation this behavior was only observed at 7% concentration. Significant differences were observed. The increase in oxidative stability was particularly significant when rosemary was added using both methods. The value in the olive oils aromatized with rosemary increased more than 50% in comparison with control olive oil. Similar results were described in previous studies (Damechki et al., 2001). This is perhaps due to these oils' higher total phenol content and chlorophylls since these compounds are antioxidant. Considering that Empeltre olive oil has low oxidative stability, aromatization with rosemary could help extend its shelf life.

Along with oxidative stability, antioxidant activity increased with garlic incorporation during malaxation. When rosemary was added using this method, this value was the highest, probably due to carnosic acid (the most powerful terpene antioxidant), carnosol, and a series of phenols in rosemary that have synergistic effects (Hernández-Hernández et al., 2009). Baiano et al. (2009) found higher antioxidant activity in olive oils aromatized with rosemary in comparison with control. Nevertheless, this olive oil displayed a higher antioxidant activity than olive oils aromatized with garlic.

The results from sensorial analysis and Principal Component Analysis are shown in Fig. 4a and Figs. 4b, 5a and 5b. In comparison with control, the samples with garlic added by maceration showed higher color intensity and particles. Clodoveo et al. (2016) indicate that filtration of aromatized olive oils is not necessary when the herbs or spices are incorporated during malaxation. Perhaps for this reason, some particles are observed in olive oils aromatized via maceration. When garlic was incorporated during malaxation, the most important attributes were smell and garlic taste. When rosemary was incorporated by maceration, a certain amount of turbidity was described, along with many particles. When rosemary was added during malaxation, the color intensity increased, as well as rosemary taste and smell. In both aromatized olive oils an increase in total smell and taste was described especially when the flavoring agent was added at higher concentration during malaxation. This could be very interesting since the consumers expected different taste, smell, etc in aromatized olive oils. In general, garlic-flavored olive oils achieved a better score than the olive oils aromatized with rosemary. For the latter, a stronger rosemary taste, pungency, and bitter taste were described by the assessors. The acceptance in garlic olive oils at 5% added during malaxation was similar to the observed for the control.

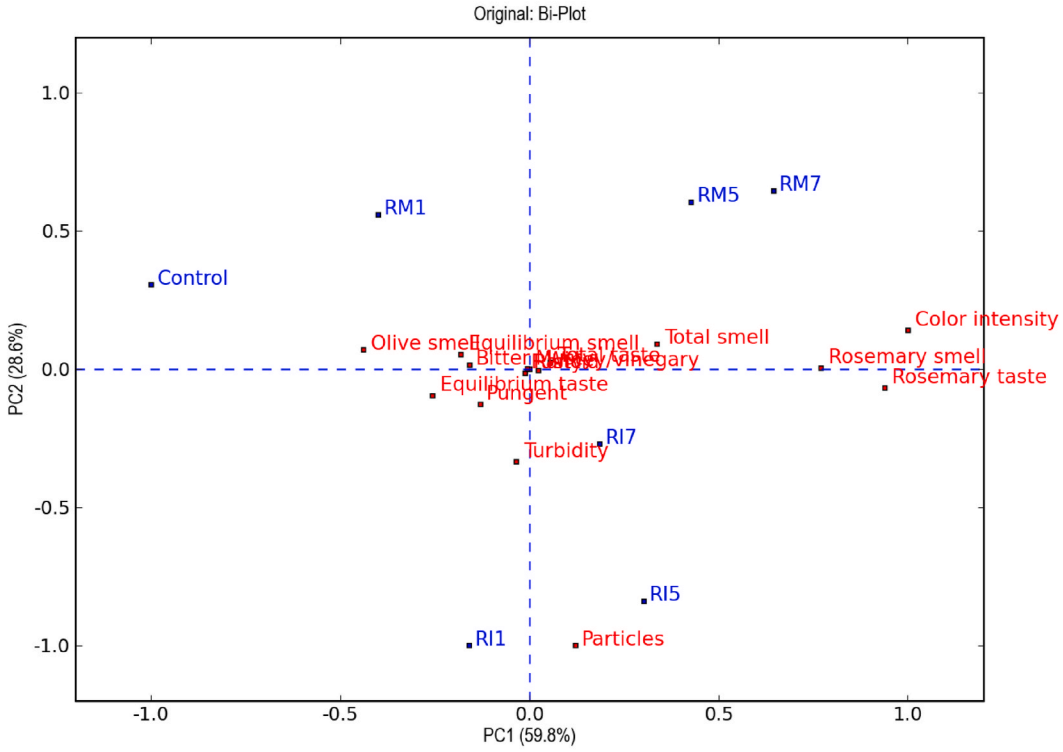
Conclusions

Aromatization of Empeltre olive oils with garlic and rosemary increased antioxidant compounds, antioxidant activity, and oxidative stability, especially when incorporation was carried out during malaxation. This could lead to a strategy to improve the shelf life of Empeltre olive oils.

Rosemary is more efficient than garlic in increasing phenols, α-tocopherols, and pigments. From a sensory point of view, Empeltre olive oils aromatized with garlic achieved a better score.

Because of their special sensorial and nutritional properties, these flavored olive oils could be used as condiments for gourmet products and functional foods, leading to further markets and new customers.

a)



b)

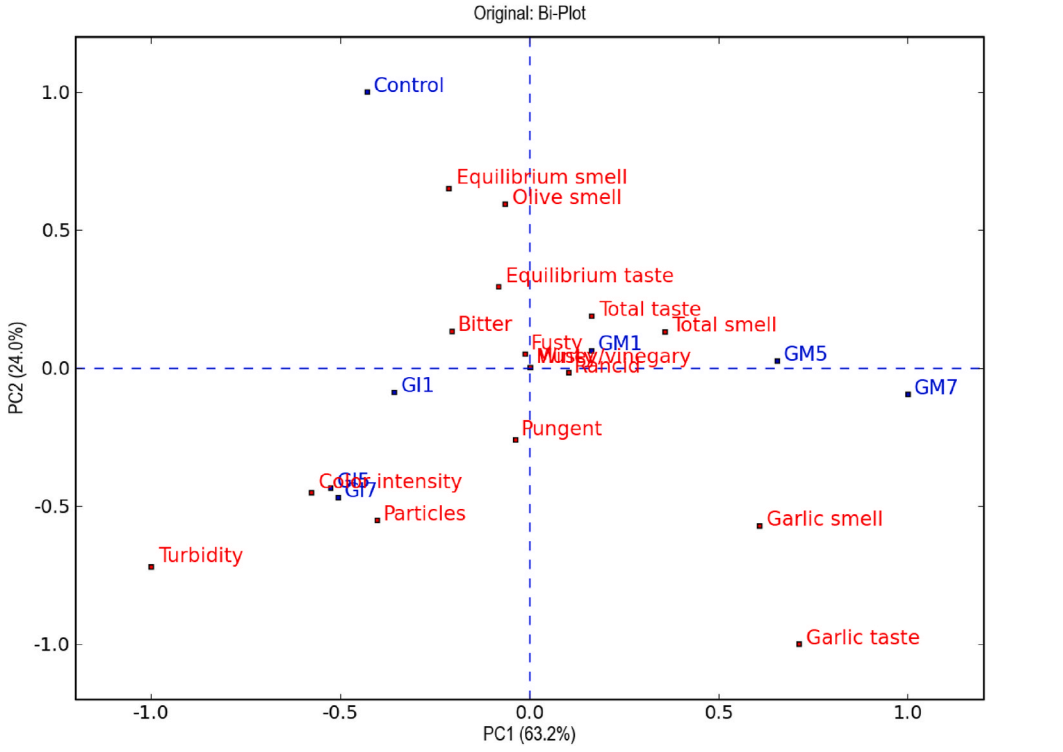
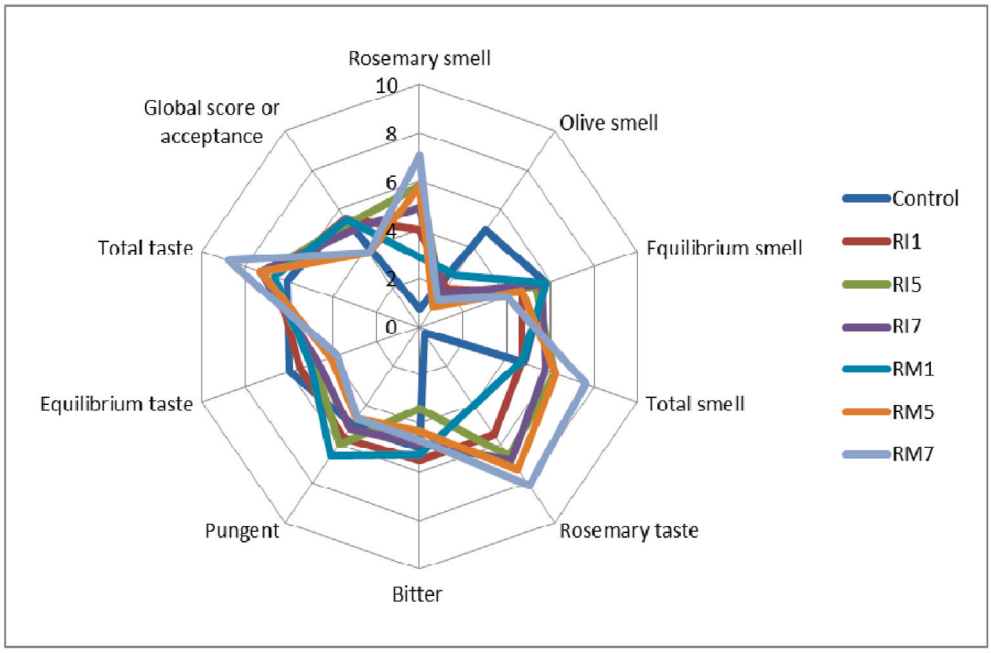


Fig. 4. Sensorial analysis and Principal Component Analysis of Empeltre olive oils aromatized with rosemary (4a) and garlic (4b).

a)



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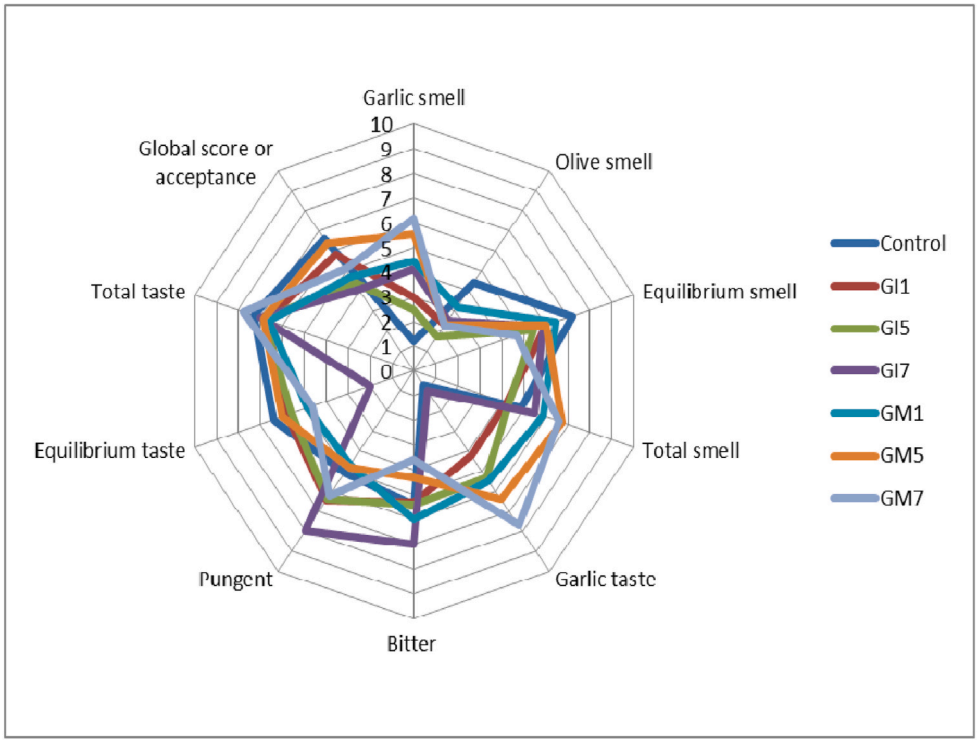

Fig. 5. Spider plot of the sensorial analysis of Empeltre olive oils aromatized with rosemary (5a) and garlic (5b).

It is especially important to obtain high phenol content in olive oil, since a corresponding health claim has been approved by European regulations.

Further work shall be necessary in order to select the best flavoring agent, concentration and method to obtain a long shelf life for Empeltre olive oil, and also in order to diversify products while increasing added value. Good sensory properties are likewise required, since these olive oils need to be accepted by consumers.

For industrial applications the aromatization of Empeltre olive oils with garlic and rosemary during malaxation is possible and would increase stability of these olive oils.

Implications for gastronomy

In this paper rosemary and garlic aromatized olive oils are obtained by different methods and by using different concentrations.

The aromatization increase antioxidant properties and oxidative stability but of course sensorial properties.

By this way some olive oils with special organoleptic properties are obtained: special smell and taste, special color, etc. These olive oils could be used to increase taste and smell in some dishes. For example garlic aromatized olive oil could be used in some salads or purees, or some green beans. In the other side rosemary aromatized olive oils could be used in some roast meats and also to give more taste and color in some boiled fish.

CRediT authorship contribution statement

María Abenoza: Investigation, Formal analysis, Visualization, Writing – review & editing. **Ana Cristina Sánchez-Gimeno:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijgfs.2021.100333.

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