Effect of infusion of spices into the oil vs combined malaxation of olive paste and spices on quality of naturally flavored virgin olive oils

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Abstract

Olive oil flavoring with aromatic plants and spices is a traditional practice in Mediterranean gastronomy. The aim of this work was to compare the influence of two different flavoring techniques (infusion of spices into the oil vs. combined malaxation of olives paste and spices) on chemical and sensory quality of flavored olive oil. In particular, oxidative and hydrolytic degradation (by routine and non-conventional analyses), phenolic profiles (by HPLC), volatile compounds (by SPME-GC/MS), antioxidant activity, and sensory properties (by a trained panel and by consumers) of the oils were evaluated. The obtained results evidenced that the malaxation method was more effective in extracting the phenolic compounds, with a significantly lower level of hydrolysis of secoiridoids. As a consequence, antioxidant activity was significantly lower in the oils obtained by infusion, which were characterized by a higher extent of the oxidative degradation. The volatile compounds were not significantly influenced by changing the flavoring method, apart for sulfur compounds that were more abundant in the oils obtained by the combined malaxation method. From a sensory point of view, more intense bitter and pungent tastes were perceived when the infusion method was adopted.

Keywords: Extra virgin olive oil, Flavoring method, Infusion method, Malaxation, Spices

1. Introduction

Extra virgin olive oil is a key ingredient of the Mediterranean cuisine and diet. It is appreciated for its nutritional properties, aroma, and taste. Its peculiar fatty acid composition, characterized by high contents of monounsaturated fatty acids (oleic acid), is known to show protective effects against many modern life-style diseases such as cancer (Owen, Haubner, Würtele, Hull, Spiegelhalder, & Bartsch, 2004) and cardiovascular diseases (Covas, 2007). Other compounds of interest, typically contained in olive oil, are the phenolic compounds, whose amount depends on several factors, including cultivar, agronomic techniques, olive ripening stage, fruit pre-storage, extraction technologies, and storage conditions of the oil (Baiano, Terracone, Viggiani, & Del Nobile, 2013; Servili, Selvaggini, Esposto, Taticchi, Montedoro, & Morozzi, 2004). Several studies, in fact, showed the ability of these compounds to reduce oxidative modification of the low-density lipoproteins, associated with atherosclerosis, cancers and Alzheimer's disease (Fito et al., 2000; Middleton, Kandaswami, & Theoharides, 2000; Offord, Guillot, Aeschbach, Loliger, & Pfeifer, 1997; Owen et al., 2000; Panza et al., 2007; Visioli, Bellomo, Montedoro, & Galli, 1995). Moreover, the correlation between phenolics and the oxidative stability of the oil is well known (Caponio, Alloggio, & Gomes, 1999; Cinquanta, Esti, & Di Matteo, 2001; Velasco & Dobarganes, 2002).

A traditional practice in Mediterranean gastronomy is the aromatization of olive oil with aromatic plants and spices, such as oregano, basil, rosemary, lemon, thyme, chilli, or garlic. It is well known that the aromatic plants and spices contain essential oils with antioxidant and antimicrobial properties (Gutierrez, Barry-Ryan, & Bourke, 2008; Reichling, Schnitzler, Suschke, & Saller, 2009).

Many investigations have been carried out with particular regard to the changes of sensory characteristics of flavored oils by comparing aroma type and concentration (Akçar & Gümüşkesen, 2011; Antoun & Tsimidou, 1997; Caporaso, Paduano, Nicoletti, & Sacchi, 2013; Damechki, Sotiropoulou, & Tsimidou, 2001; Gambacorta, Faccia, Pati, Lamacchia, Baiano, & La Notte, 2007). Other authors investigated the evolution of quality indices and stability of flavored virgin olive oil during storage. Baiano, Gambacorta, Terracone, Previtali, Lamacchia, and La Notte (2009) observed that, after 9 months of storage, both the unflavored and flavored oils had quality indices below the limit allowed for the extra-virgin. In addition, they observed that garlic-flavored oil showed the lowest phenolic content and the most marked decrease of antioxidant activity. Sousa, Casal, Malheiro, Lamas, Bento, and Pereira (2015) observed a general decrease of total phenol content in the flavored oils and an improving of the oxidative stability, correlated with the total vitamin E content. Gambacorta et al. (2007) showed that flavorings improved the stability of the olive oils, although conflicting results were present in literature relatively to the capacity of the essential oils to protect olive oil from thermo-oxidative processes (Ayadi, Grati-Kamoun, & Attia, 2009; Issaoui, Flamini, Hajaij, Cioni, & Hammami, 2011).

Different methods are used to flavor olive oils: i) infusion of spices into the oil; ii) ultrasound-assisted maceration; iii) combined malaxation of olives paste and spices during the oil-productive process. In literature, the majority of the authors considered the infusion method (Akçar et al., 2011; Baiano et al. 2009; Caporaso et al., 2013; Damechki et al., 2001). Veillet, Tomao, and Chemat (2010) evaluated the ultrasound-assisted maceration for aromatizing oil with basil and observed that flavoring was achieved in few minutes, whereas conventional maceration required several days. Ultrasound-assisted extraction is a green technique (Li, Fabiano-Tixier, Tomao, Cravotto, & Chemat, 2013) that has been proposed also to recover oleuropein from olive leaves (Achat et al., 2012) and essential oil and aroma from different plant matrices (Chemat, Vian, & Cravotto, 2012; Rombaut, Tixier, Bily, & Chemat, 2014). However, a negative influence of ultrasound-assisted technologies on oil quality is reported, in terms of off flavor development and oxidative oil degradation (Chemat, Grondin, Shum Cheong Sing, & Smadja, 2004; Patrick, Blindt, & Janssen, 2004; Schneider, Zahn, Hofmann, Wecks, & Rohm, 2006). In addition, the implementation of this technique by the oil-productive industries would involve new investments and management costs related to the ultrasound-generating machinery.

No studies are present in literature about the direct malaxation of the olive paste with spices. This technique is easy to carry out and is faster than infusion. The latter, indeed, requires variable contact times depending on the spice used. Moreover, both flavoring techniques are green conventional processes that no require the use of any organic solvents (Chemat, Fabiano-Tixier, Vian, Allaf, & Vorobiev, 2015). In this framework, the aim of this paper was to evaluate the influence of the productive process on quality of flavored oils, by comparing the infusion method with the combined malaxation of olive paste and spices. Basil, chilli, and chilli plus garlic were used and the influence of the type of spice on flavored oil quality was also investigated.

2. Material and methods

2.1 Samples

An amount of blended olives from *Ogliarola*, *Coratina* and *Peranzana cv.* (50%, 30%, 20%, w/w/w) accounting for about 5,000 kg was utilized for the experimental trials, that were carried out at the Olearia Clemente industry (Manfredonia, Italy) in the crop season 2014-2015. In particular, the olives were processed by a continuous process. The obtained oil showed low level of oxidative and hydrolytic degradation and the good results of panel test allowed to classify the oil as extra virgin (free fatty acid, 0.42 g/100 g; peroxide value, 6.64 meq O_2/kg; K_{232}, 1.86; K_{270}, 0.23; triglyceride oligopolymers, traces; oxidized triglycerides, 0.32 g/100 g; diglycerides, 1.62 g/100 g; fruity, 6.8; defects, zero). The levels of PCs confirmed the results of the routine analyses, with values typical of high quality oils (Caponio, Bilancia, Pasqualone, Sikorska, & Gomes, 2005), whereas the total content of phenolics corresponded to those typical of the chosen cultivars (Baiano et al., 2013; Caponio, Gomes, & Pasqualone, 2001).

Dried basil (B), dried chilli (C), and a combination of dried chilli and garlic (C&G) were utilized for flavoring olive oil both by adding them to the olives during malaxation (M) and by infusion method (In), as showed in figure 1. On the whole, six different flavored oils were produced: M-B, M-C, M-C&G, In-B, In-C, and In-C&G. The spices were chosen among the most commonly used for aromatizing olive oil, whereas parameters such as oil/spice ratio, time, and processing temperature were set up on the basis of the studies reported in literature (Ayadi et al., 2009; Caporaso et al., 2013). Three independent trials were carried out for each flavoring method, starting from the same olive lot.

2.2 Routine analyses

Free fatty acid (FAA), peroxide value (PV), and spectrophotometric indices (K_{232}, K_{270}, and ΔK) were determined following the analytical methods described by the EEC Regulation 2568/91.

2.3 Extraction and determination of phenols

The extraction of phenolic compounds was carried out according to the procedure described by Baiano et al. (2009). The extracts prepared for HPLC analysis were obtained according to the same procedure, but with the addition of 0.5 mL of gallic acid solution, as internal standard, at the concentration of 100 mg/L in a methanol/water (70:30, v/v) solution. The total phenolic content was determined using the Folin-Ciocalteu reagent according to Di Stefano, Cravero, and Genilizzi (1989). The standard curve was prepared using diluted solutions of gallic acid in a methanol:water (70:30, v/v) solution. The total phenolic content was expressed as milligrams of gallic acid equivalents per kg of oil. The HPLC analysis of the phenolic extracts was carried out according to Gambacorta, Previtali, Pati, Baiano, and La Notte (2006) as described in Baiano et al. (2009).

2.4 Evaluation of the antioxidant activity

The antioxidant activity of the oil phenolic extracts was evaluated on the basis of the scavenging activity of the ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999) and DPPH (1, 1- diphenyl 2-picrylhyorazyl) free radical, (Brand-Williams, Cuvelier, & Berset, 1995) stable radical, as described in Baiano et al. (2013). These assays are based on the abilities of the antioxidants present into the extracts to scavenge the radical in comparison with that of a standard antioxidant (Trolox, (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)).

2.5 Volatile compounds determination

For the volatile compounds determination, the flavored oils (0.5 ± 0.005 g) were weighed into 20-mL vials, sealed with a screw top aluminium cap and pierceable butyl rubber septa, and submitted to the (SPME/GC-MS) in the conditions reported by Caponio, Summo, Paradiso, and Pasqualone (2014).

2.6 Determination of polar compounds

Flavored oils were submitted to separation of polar compounds (PCs) by silica gel column chromatography, according to the AOAC method no. 982. 27 (AOAC. Method 982.27). The efficacy of separation was checked by TLC as recommended by the same method. Then the PC, recovered in tetrahydrofuran (THF), were analysed by means of high-performance size-exclusion chromatography (HPSEC) in order to separate the compound classes constituting them in the conditions reported in previous papers (Gomes, 1992; Gomes & Caponio, 1999).

2.7 Panel and consumer tests

The oils were submitted to both panel and consumer test. Before being tested, the oils were kept in the dark and stored at constant temperature (15 °C) and humidity (65%) in hermetically sealed 750 mL-dark glass bottles.

The sensory analysis was performed by a trained panel, composed of 8 judges. The evaluation of the samples was carried out under the conditions described in EC Regulation 640/2008, by using the profile sheet for virgin olive oil modified to include specific attributes related to flavoring. Each panelist evaluated visual, olfactory, and gustatory characteristics on a continuous unstructured line scale of 10 cm, ranging from low to high intensity. Both the defects (smells of fusty/muddy sediment, musty-humid-earthy, winey-vinegary/acid-sour, metallic, and rancid) and positive attributes were evaluated. The latter were: fruity (intended as olive flavour, evaluated both in olfactory and gustatory phase), bitter and pungent (only in gustatory phase). Color was evaluated with reference to natural colour of extra-virgin olive oils (green/yellow).

The consumer test was performed by 100 tasters (age 20-53 years, 60 females and 40 males). Before tasting the samples, they were introduced to the test through a concise description of basic oil quality attributes. The participants were then asked to rank the intensity of color, fruity taste, sweet taste, bitter taste, and pungent taste on a 5-point scale (1: “very weak”; 5: “very strong”). Subsequently, the participants were asked to evaluate overall pleasantness as well as the pleasantness of smell, taste, and color attributes on a 9-point hedonic scale (1: “dislike extremely”; 9: “like extremely”).

The samples (14-16 mL) were served to panelists and consumers at room temperature (25 °C), in glasses covered with watch-glasses, and in a randomized order codified by a 3-digit number. Each assessor was also provided with a glass of about 200 mL of water at room temperature, as a palate cleanser between tastings. Samples were prepared immediately before being served.

2.8 Statistical Analysis

Each analysis was replicated at least three times. The averages and the standard deviations were calculated by using Excel software V. 11.5.1 (Microsoft, Redmond, WA). Analysis of variance (two-way ANOVA) and Dunnett test were carried out on the experimental data by the XLStat software (Addinsoft SARL, New York, NY, USA).

3. Results and discussion

Table 1 includes the results of the analyses carried out on the flavored oils whereas in Table 2 are reported the results of Dunnett test regarding the antioxidant activity, phenolic compounds, and volatile compounds. The unflavored oil (control) was compared to the single flavored oils. The productive process influenced the level of oxidation of the oils, with significantly higher levels of PV and K_{232} in the oils obtained by infusion, probably due to longer processing times. Values of ΔK were all negative and remained below the maximum allowed for extra virgin olive oils (data not shown). Among the spices, the aromatization with basil determined the highest oxidative degradation level, in agreement with the findings of other authors (Ayadi et al., 2009; Baiano et al., 2009; Sousa et al., 2015). In fact, the basil-flavored oils showed lower phenolic compound contents than the other oils. These compounds, such as diglycerides (DAG) and PCs were significantly more abundant in the oils flavored by malaxing olive paste and spices at the same time. The malaxation method, indeed, appeared more effective in extracting the phenolic compounds, although the differences with the other oils were statistically significant only when garlic was used, probably due to its well-known strong antioxidant properties able to protect oil phenolics (Banerjee, Mukherjee, & maulik, 2003; Bozin, Mimica-Dukic, Samojlik, Goran, & Igic, 2008; Cho & Xu, 2000; Wang et al., 1996). In addition, garlic is rich of flavonoids (Harborne & Williams, 1996) and anthocyanins (Fossen & Andersen, 1997), which make it appreciated for its healthy features (Cho et al., 2000; Wang et al., 1996). The total antioxidant activity was in accordance with the phenolic levels, with higher values in the oils obtained by malaxation method, although with significant differences only when the DPPH assay was applied.

The results of Dunnett test (Table 2) did not show significant differences in the antioxidant activity and total phenols among control and flavored oils. Regards the single phenolic compounds, instead, the results showed a general increase of their amounts, attributable to the spices, with significant differences for *p*-vanillic acid, *p*-vanillin, and *p*-coumaric acid (the latter only for C and C&G flavored oils); as well as for hydroxytyrosol, tyrosol, and 1-acetoxypinoresinol in B oil and 3,4 DHPEA-AC, 3,4 DHPEA-EDA, and 3,4 DHPEA-EA in C&G oil.

Table 3 reports the the results of the two-way ANOVA, followed by Tukey HSD test for multiple comparison) of the single phenolic compounds of the flavored oils, determined by HPLC-DAD.

Considering the *flavoring method* variable, hydroxytyrosol and tyrosol were significant higher in flavored oils obtained by infusion whereas on the contrary 3,4 DHPEA-EDA, *p*-HPEA-AC, *p*-HPEA-EDA, 1-acetoxypinoresinol, pinoresinol, and 3,4 DHPEA-EA were significant higher in flavored oils obtained by malaxing olives and spices, especially when garlic was used (with the exception of 1-acetoxypinoresinol, that was more related to the use of basil). As regards *spices* variable, only *p*-vanillin, ferulic acid, and pinoresinol did not show significant differences. Hydroxytyrosol, tyrosol, and 1-acetoxypinoresinol were significantly higher in B oil; *p*-vanillic acid was significantly higher in C oil; 3,4 DHPEA-AC, 3,4 DHPEA-EDA, *p*-HPEA-AC, *p*-HPEA-EDA, and 3,4 DHPEA-EA were significantly higher in C&G oil. Moreover, the two flavoring methods did not induce significant differences in the single phenolics when chilli was used, with the exception of *p*-HPEA-EDA, which was more abundant in M-C oil. The In-B oil showed significantly higher hydroxytyrosol and tyrosol levels than the other oils, whereas the malaxation method led to significantly more abundant tyrosol in case of chilli alone, or chilli and garlic. These results paralleled the antioxidant activity evaluated both by DPPH and ABTS test, in accordance with Baldioli, Servili, Perretti, and Montedoro (1996) that evidenced a significant positive correlation between the content of 3,4 DHPEA-EDA and 3,4 DHPEA-EA and the total antioxidant activity. The first order interaction showed that also *p*-HPEA-EA was significantly influenced by the flavoring method, with higher values in In-B and M-C&G trials than in the others. This opposite trend did not allow to point out a significant difference for the variable “flavoring method”. The results obtained for the single phenolic compounds, in accordance with the total phenolics ascertained by Folin-Ciocalteu method, would indicate a greater hydrolysis level of phenolics in In-B oil. In fact, it is known that hydroxytyrosol and tyrosol increase during olive oil storage due to a progressive hydrolysis of more complex phenolics (Bendini et al., 2007; Cinquanta, Esti, & La Notte, 1997; Montedoro, Servili, Baldioli, & Miniati, 1992).

Table 4 reports the volatile compounds of the flavored oils, grouped according to their chemical class. The volatile profile was not significantly influenced by the flavoring method, apart for sulfur compounds, detected only when garlic was used (Banerjee et al., 2003; Bozin et al., 2008; Cho et al., 2000; Wang et al., 1996), that were more represented in the oils obtained by combined malaxation of olive and spices than in the infused oils. The esters were significantly more abundant in C and C&G oils obtained by infusion, due to the higher amounts of methyl and ethyl acetate (data not shown). Terpens were abundant in basil-flavored oils, which were rich in eucalyptol, linalool, camphor, and estragole (data not shown), in agreement with other authors (Klimankova, Holadova, Hajslova, Cajca, Poutka, & Koudela, 2008; Lee, Umano, Shibamoto, & Lee, 2005), with significantly higher amounts in oils obtained by malaxation method. Ketones and carboxylic acids were abundant in basil- and chilli-flavored oils, containing higher levels of 3-pentanon and acetic acid (data not shown), respectively. This could be due to the oxidative level and antioxidant activity of the flavored oils; in fact ketones and

acids derive from homolytic cleavage of the hydroperoxide group (Angerosa, 2002). Moreover, acetic acid, as well as ethyl acetate, could be originated from sugar alcoholic fermentation by LOX pathway (Angerosa, Lanza, & Marsilio, 1996). The results of Dunnett test (Table 2) showed the general increase of the volatile fraction of the flavored oils than control, due to contribution by the typical volatiles of the different spices.

Figure 2 shows the results of panel test carried out on the flavored oils. The sensory profiles of flavored oils obtained by using the two different flavoring methods largely overlapped and all of them were devoid of defects (data not shown). The classical fruity odorous and taste notes disappeared in both In-C&G and M-C&G oils, whereas pungent and bitter taste were marked in the same oils. Pungent taste was also marked in chilli-flavored oils and bitter taste in basil-flavored oils, that maintained the typical color of olive oil. The most evident differences due to flavoring method were observed in bitter taste intensity, higher in the oils obtained by infusion, and in fruity smell that, on the contrary, was more evident in the oils obtained by malaxing olive and spices together. The chilli-flavored oils showed milder fruity smell and stronger pungent taste when obtained by infusion.

Table 5 reports the results of the consumer test carried out on the flavored oils. Considering the *flavored method* variable, only for bitter, pungent, and color intensity were find significant differences with higher values for oils obtained by infusion. With regards the *spices* variable, the greatest differences were observed in pungent and color intensity, with values significantly decreasing in the order: C oils (that were more appreciated for their olfactory features) > C&G oils > B oils. The latter, on the contrary, showed sweet intensity and color pleasantness significantly higher than the other oils, while C&G oils were significantly more bitter, probably due to higher content of phenolics. Overall, the differences observed among different spices were similar to literature data (Antoun et al., 1997; Gambacorta et al., 2007).

4. Conclusions

The obtained results showed that the flavoring technique significantly influenced the chemical and sensory quality of the flavored oils. In particular, the infusion of oils with spices caused a greater oxidative degradation due to lower content of total phenols. On the other hand, the oils obtained by combined malaxation of olives and spices were less bitter. The profile of volatile compounds was not significantly influenced by the method of flavoring, with the exception of sulfur compounds that were greater in oils obtained by malaxation. The practice of olive oil flavoring, leading to the so called "gourmet oils", could increase the use of olive oil among non-traditional consumers and, at the same time, add further value to this precious agricultural product.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Figure captions

Figure 1. Flowchart of the productive process of flavored olive oils.

Figure 2. Results of the panel test carried out on the flavored olive oils obtained by infusion (In) or by adding the species during olive paste malaxation (M). B, basil; C, chilli; C&G, combination of chilli and garlic.

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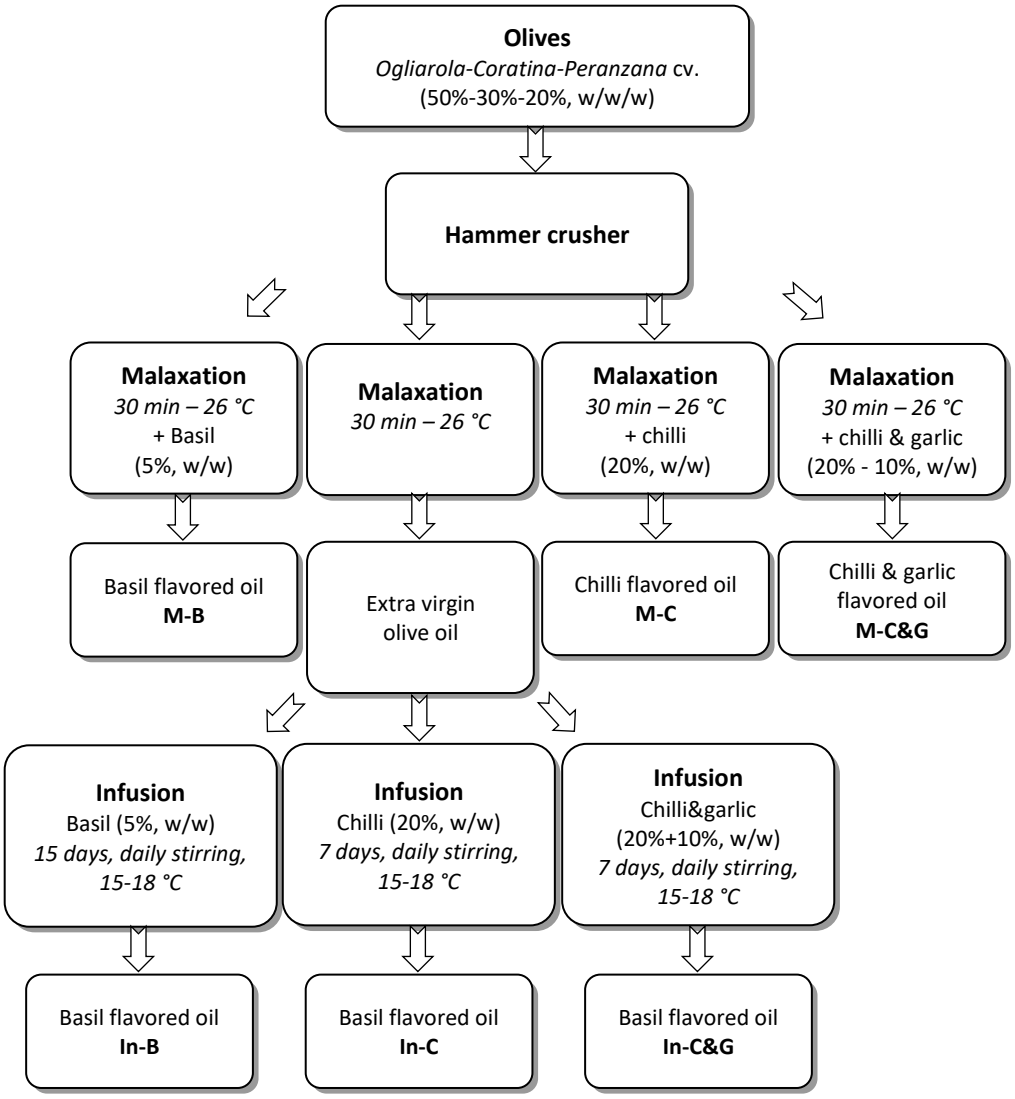

Figure 1. Caponio et al.

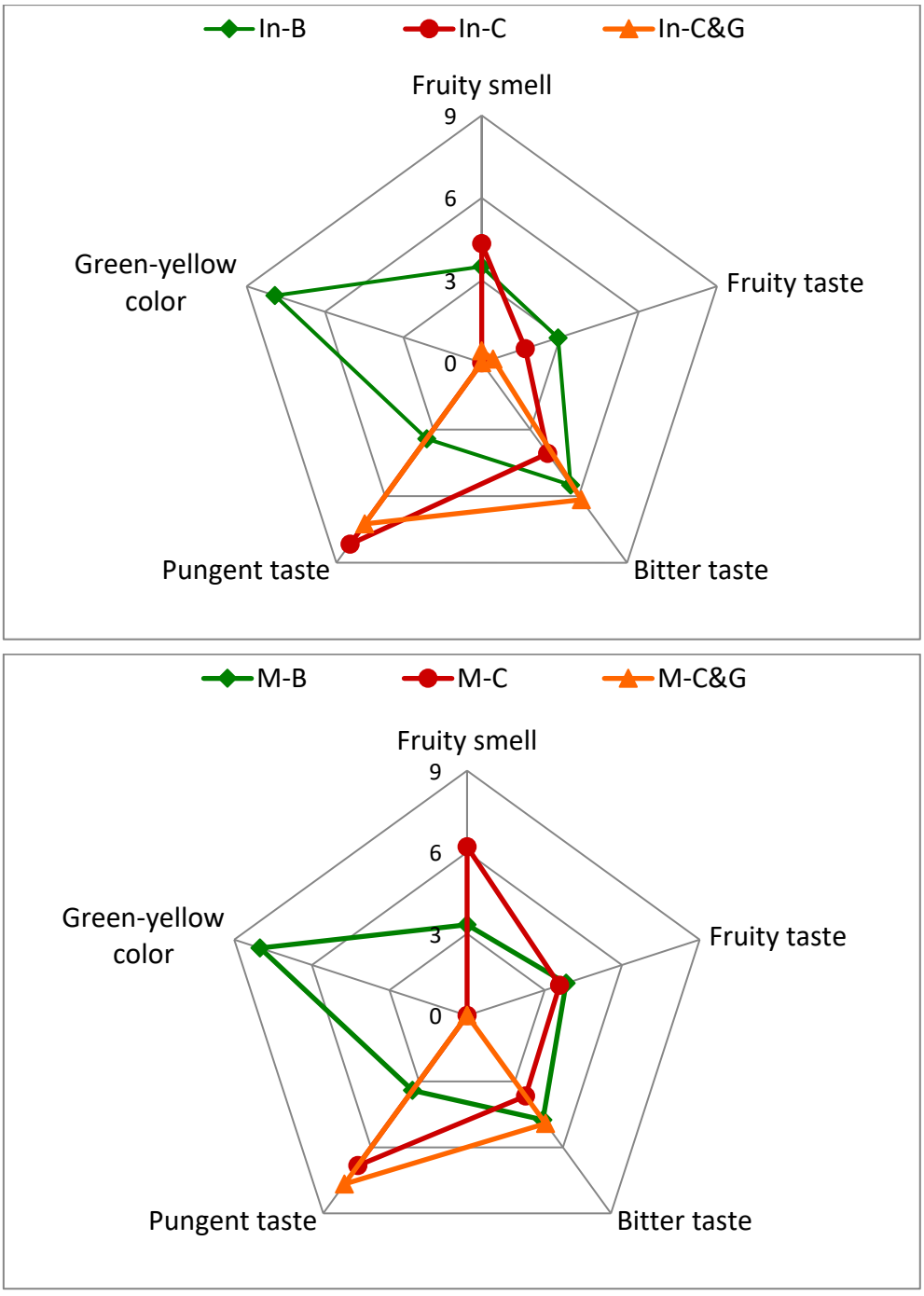

Figure 2. Caponio et al.

Table 1. Mean values, standard deviation, and results of statistical analysis (two-way ANOVA followed by Tukey HSD test for multiple comparison) of the analyses carried out on the flavored oils.

	FFA	PV	K_{232}	K_{270}	TAGP	ox-TAG	DAG	PCs	Phenols	ABTS test	DPPH test
					Flavoring method						
In	a	a	a	a	-	a	b	b	b	a	b
M	a	b	b	a	-	a	a	a	a	a	a
					Spice						
B	a	a	a	a	-	a	c	b	b	b	b
C	a	c	c	b	-	a	a	a	b	ab	b
C&G	a	b	b	b	-	a	b	b	a	a	a
					*Flavoring method*Spice*						
In-B	0.41±0.04 a	8.68±0.12 a	2.67±0.08 a	0.34±0.08 a	tr	0.32±0.01 a	1.61±0.01 c	2.33±0.04 c	373±8 b	0.82±0.05 b	0.71±0.04 b
In-C	0.44±0.02 a	6.53±0.09 bc	1.95±0.02 c	0.22±0.01 b	tr	0.33±0.01 a	1.70±0.06 bc	2.48±0.08 abc	391±8 b	1.02±0.14 ab	0.81±0.02 b
In-C&G	0.43±0.02 a	6.86±0.08 bc	2.10±0.04 b	0.24±0.01 b	tr	0.32±0.01 a	1.62±0.04 c	2.37±0.04 bc	380±14 b	0.96±0.19 ab	0.76±0.05 b
M-B	0.43±0.03 a	8.44±0.11 a	2.59±0.03 a	0.34±0.02 a	tr	0.33±0.01 a	1.63±0.02 c	2.42±0.01 bc	396±19 b	0.85±0.02 b	0.79±0.06 b
M-C	0.45±0.04 a	6.38±0.17 c	1.95±0.03 c	0.23±0.02 b	tr	0.31±0.02 a	1.82±0.03 a	2.59±0.07 a	393±6 b	0.84±0.01 b	0.78±0.02 b
M-C&G	0.45±0.04 a	6.75±0.19 b	2.02±0.03 bc	0.22±0.02 b	tr	0.32±0.01 a	1.74±0.03 ab	2.52±0.03 ab	489±7 a	1.28±0.21 a	1.05±0.04 a

In, infusion method; M, malaxation method; B, basil; C, chilli; C&G, combination of chilli and garlic; tr, traces.

FAA, free fatty acids (g/100 g); PV, peroxide value (meq O_2/kg); K_{232}, specific absorption at 232; K_{270}, specific absorption at 270 nm; TAGP, triacylglycerol oligopolymers (g/100 g); ox-TAG, oxidized triacylglycerols (g/100 g); DAG, diacylglycerols (g/100 g); PCs, polar compounds (g/100 g); ABTS, 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (mmoli Trolox eq/kg); DPPH, 2,2-diphenyl-1-picrylhydrazyl (mmoli Trolox eq/kg).

Different letters indicate significant differences at $p < 0.05$.

Table 2. Mean value and results of Dunnett test of the antioxidant activity, phenolic compounds, and volatile compounds carried out on the unflavored and flavored oils.

Parameters	Co	B		C		C&G	
ABTS test	0.87	0.84	ns	0.93	ns	1.21	ns
DPPH test	0.82	0.75	ns	0.80	ns	0.91	ns
Phenols	395	384	ns	392	ns	435	ns
Hydroxytyrosol	2.83	6.58	*	2.71	ns	2.97	ns
Tyrosol	5.18	6.87	*	4.72	ns	5.12	ns
p-Vanillic acid	0.47	0.39	ns	0.72	*	0.60	*
p-Vanillin	0.41	0.57	*	0.62	*	0.55	*
p-Coumaric acid	0.37	0.54	*	0.65	*	0.67	*
3,4 DHPEA-AC	0.18	0.29	ns	0.24	ns	0.36	*
Ferulic acid	0.34	0.49	ns	0.49	ns	0.49	ns
3,4 DHPEA-EDA	13.82	14.88	ns	18.49	ns	20.43	*
p-HPEA-AC	1.83	1.63	ns	1.77	ns	2.20	ns
p-HPEA-EDA	16.77	16.15	ns	18.35	ns	20.82	ns
1-Acetoxypinoresinol	1.45	2.99	*	1.57	ns	1.67	ns
Pinoresinol	15.35	14.91	ns	13.89	ns	15.38	ns
3,4 DHPEA-EA	2.97	3.06	ns	3.21	ns	3.77	*
p-HPEA-EA	3.19	3.67	ns	3.26	ns	3.56	ns
Esters	4.34E+06	6.05E+06	*	6.57E+06	*	6.64E+06	*
Alcohols	3.09E+07	3.71E+07	*	3.57E+07	ns	3.34E+07	ns
Ketones	1.42E+07	1.74E+07	*	2.61E+07	*	1.53E+07	ns
Terpenes	7.73E+05	1.96E+08	*	3.93E+06	ns	2.58E+06	ns
Sulfur compounds	-	-		-		1.10E+08	*
Aldehydes	3.98E+07	5.69E+07	*	6.81E+07	*	5.66E+07	*
Acids	1.95E+07	2.65E+07	*	2.67E+07	*	1.83E+07	ns
Others	7.77E+05	1.80E+06	*	1.90E+06	*	5.29E+05	ns

Co, control (unflavored oil); In, infusion method; M, malaxation method; B, basil; C, chilli; C&G, combination of chilli and garlic. ABTS, 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (mmoli Trolox eq/kg); DPPH, 2,2-diphenyl-1-picrylhydrazyl (mmoli Trolox eq/kg).

* Significant differences compared to control oil at $p < 0.05$; ns, not significant.

Table 3. Mean values, standard deviation, and results of statistical analysis (two-way ANOVA followed by Tukey HSD test for multiple comparison) of the single phenolic compounds of the flavored oils.

Compounds	*Flavoring method*		*Spice*			*Flavoring method*Spice*					
	In	**M**	**B**	**C**	**C&G**	**In-B**	**In-C**	**In-C&G**	**M-B**	**M-C**	**M-C&G**
Hydroxytyrosol	a	b	a	b	b	10.54±0.37 a	2.70±0.01 b	2.91±0.15 b	2.62±0.11 b	2.71±0.05 b	3.02±0.22 b
Tyrosol	a	b	a	c	b	9.45±0.09 a	4.59±0.03 cd	4.58±0.12 cd	4.30±0.06 d	4.85±0.12 c	5.66±0.16 b
p-Vanillic acid	a	a	c	a	b	0.42±0.03 d	0.75±0.05 a	0.55±0.03 bc	0.36±0.03 d	0.68±0.01 ab	0.64±0.04 bc
p-Vanillin	a	a	a	a	a	0.62±0.06 a	0.63±0.01 a	0.50±0.01 a	0.52±0.08 a	0.61±0.06 a	0.60±0.06 a
p-Coumaric acid	a	a	b	a	a	0.56±0.13 ab	0.67±0.06 ab	0.60±0.02 ab	0.52±0.02 b	0.62±0.01 ab	0.73±0.05 a
3,4 DHPEA-AC	a	a	ab	b	a	0.28±0.02 a	0.28±0.04 a	0.30±0.05 a	0.30±0.05 a	0.19±0.17 a	0.36±0.02 a
Ferulic acid	a	a	a	a	a	0.53±0.08 a	0.48±0.09 a	0.49±0.06 a	0.45±0.03 a	0.49±0.07 a	0.50±0.43 a
3,4 DHPEA-EDA	b	a	c	b	a	11.05±0.04 d	18.46±0.06 bc	17.96±0.12 c	18.70±0.26 b	18.53±0.35 b	22.90±0.06 a
p-HPEA-AC	b	a	b	b	a	1.51±0.12 c	1.75±0.05 bc	1.91±0.09 b	1.74±0.13 bc	1.80±0.08 bc	2.49±0.16 a
p-HPEA-EDA	b	a	c	b	a	13.53±0.14 d	17.77±0.08 c	18.14±0.19 bc	18.77±0.39 bc	18.92±0.66 b	23.50±0.58 a
1-Acetoxypinoresinol	b	a	a	b	b	2.73±0.01 b	1.38±0.04 d	1.50±0.03 cd	3.25±0.11 a	1.76±0.18 cd	1.84±0.28 c
Pinoresinol	b	a	a	a	a	13.97±0.26 a	13.76±0.18 a	14.12±0.21 a	15.83±3.11 a	14.01±0.17 a	16.64±0.13 a
3,4 DHPEA-EA	b	a	b	b	a	3.04±0.17 b	3.17±0.09 b	3.45±0.22 b	3.07±0.25 b	3.25±0.24 b	4.08±0.33 a
p-HPEA-EA	a	a	a	b	a	3.82±0.17 a	3.22±0.06 c	3.28±0.10 bc	3.51±0.10 b	3.29±0.06 bc	3.83±0.04 a

In, infusion method; M, malaxation method; B, basil; C, chilli; C&G, combination of chilli and garlic.
Different letters indicate significant differences at $p < 0.05$.

Table 4. Mean values and results of statistical analysis (two-way ANOVA followed by Tukey HSD test for multiple comparison) of volatile compounds of the flavored oils.

	Esters		**Alcohols**		**Ketones**		**Terpenes**		**Sulfur compounds**		**Aldehydes**		**Acids**		**Others**	
Flavoring method																
In	a		a		a		a		b		a		a		a	
M	a		a		a		a		a		a		a		a	
Spice																
B	b		a		a		a		b		a		a		a	
C	a		a		a		b		b		a		a		a	
C&G	a		a		b		b		a		a		b		b	
*Flavoring method*Spice*																
In-B	6.46E+06	ab	3.36E+07	a	1.56E+07	bc	1.82E+08	b	0.00E+00	c	5.23E+07	a	2.83E+07	a	1.62E+06	a
In-C	6.87E+06	a	3.67E+07	a	2.70E+07	a	5.10E+06	c	0.00E+00	c	6.69E+07	a	2.58E+07	a	1.86E+06	a
In-C&G	6.78E+06	a	3.34E+07	a	1.63E+07	bc	3.04E+06	c	9.25E+07	b	5.68E+07	a	2.03E+07	ab	7.20E+05	b
M-B	5.64E+06	b	4.05E+07	a	1.93E+07	b	2.10E+08	a	0.00E+00	c	6.14E+07	a	2.47E+07	ab	1.99E+06	a
M-C	6.28E+06	ab	3.48E+07	a	2.53E+07	a	2.77E+06	c	0.00E+00	c	6.94E+07	a	2.75E+07	a	1.94E+06	a
M-C&G	6.49E+06	ab	3.40E+07	a	1.44E+07	c	2.13E+06	c	1.28E+08	a	5.63E+07	a	1.62E+07	b	3.37E+05	b

In, infusion method; M, malaxation method; B, basil; C, chilli; C&G, combination of chilli and garlic.
Different letters indicate significant differences at $p < 0.05$.

Table 5. Mean values, standard deviation, and results of statistical analysis (two-way ANOVA followed by Tukey HSD test for multiple comparison) of the consumer test of the flavored oils.

	Fruity intensity	**Sweet intensity**	**Bitter intensity**	**Pungent intensity**	**Color intensity**	**Smell pleasantness**	**Taste pleasantness**	**Color pleasantness**	**Overall pleasantness**
					Flavoring method				
In	a	a	a	a	a	a	a	a	a
M	a	a	b	b	b	a	a	a	a
					Spice				
B	a	a	b	c	c	ab	a	a	a
C	a	b	b	a	a	a	a	b	a
C&G	a	b	a	b	b	b	a	b	a
					*Flavoring method*Spice*				
In-B	2.6±1.0 a	2.3±0.9 a	2.6±1.1 ab	2.1±0.9 c	3.3±0.9 d	5.9±1.5 a	5.1±1.4 a	6.7±1.2 a	5.5±1.6 a
In-C	2.8±1.1 a	1.8±0.9 b	2.6±1.1 ab	4.2±0.9 a	4.3±1.0 a	7.0±1.4 a	5. 0±1.8 a	5.8±1.9 b	5.6±2.0 a
In-C&G	2.7±1.3 a	1. 9±0.9 ab	3.1±1.1 a	3.7±1.1 ab	4.0±0.9 ab	5.2±1.9 a	5.1±2.0 a	5.7±1.8 b	5.1±2.0 a
M-B	2.7±1.0 a	2.3±0.9 a	2.3±0.9 b	2.1±1.1 c	3.4±0.9 cd	5.9±1.2 a	5.4±1.8 a	6.7±1.3 a	5.6±1.7 a
M-C	2.5±1.1 a	1.7±0.8 b	2.5±0.9 b	3.8±1.1 ab	3.7±1.0 bc	6.1±1.4 a	5.6±1.7 a	5.8±1.7 b	5.7±1.6 a
M-C&G	2.6±1.1 a	2.0±0.9 ab	2.7±1.0 ab	3.4±1.1 b	3.5±0.9 cd	5.3±1.9 a	5.3±2.0 a	5.6±1.8 b	5.2±2.0 a

In, infusion method; M, malaxation method; B, basil; C, chilli; C&G, combination of chilli and garlic.
Different letters indicate significant differences at $p < 0.05$.