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Analysis of the volatile fraction of "Pesto Genovese" by headspace sorptive extraction (HSSE)

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Abstract

This paper describes the development and application of a new analytical method for the analysis of the volatile fractions of *pesto*, a typical Italian basil-based pasta sauce, and its ingredients. The extraction of the volatile fraction was realized by headspace sorptive extraction (HSSE) and the extracted volatiles were then thermodesorbed, cryoconcentrated in a cooled injector and analysed by gas chromatography-mass spectrometry.

This study shows that despite the great variability detected in the composition of the volatile fractions of several samples of "Pesto Genovese", the ingredients traditionally used in *pesto* preparation (basil, pine nuts, walnuts, cheese, extra-virgin olive oil and garlic) give a significant and distinctive contribution to the *pesto* volatile fraction. Thus, the HSSE analysis of "Pesto Genovese" could be very useful for a careful control of the composition of this valuable niche product.

Keywords: Headspace sorptive extraction; Volatile fraction; Pesto genovese; Pasta sauce; Basil; Traditional ingredients

1. Introduction

Pesto is a typical Italian basil-based pasta sauce which is becoming well known all over the world for its enjoyable taste and its very pleasant aroma. Traditional *pesto* origins in Liguria, a North Italian region that stretches along a wide tract of the Mediterranean coast. Some years ago traditional *pesto* was not commonly known out of Liguria, owing to its short shelf-life. The development of mild preservation technologies that do not involve heat processing has now make it available all over the world.

Despite several unsuccessful imitation attempts, the Ligurian *pesto* still has peculiar organoleptic features, resulting from the distinctive sensorial character of the PDO (Protected Designation of Origin) basil "Basilico Genovese" (*Ocimum basilicum L.*) (EC Regulation 1623/2005), which has a particularly "fresh" aroma without mint flavour. Nevertheless, *pesto* is a very complex sauce, and the other traditional ingredients are cheese, extra-virgin olive oil, pine nuts and/or walnuts and garlic. They significantly affect its taste and flavour although only their balanced contribution guarantees its quality. In order to obtain a traditional high quality *pesto*, PDO Parmigiano Reggiano or Grana Padano or Pecorino should be used, and Italian pine nuts should be preferred to walnuts because of their sweet and balsamic taste and their much pleasant effect on the texture of the finished product.

The influence of cultivar, growing method and climatic conditions on the composition of basil essential oil has been extensively studied. Several authors also considered the changes induced by bleaching, drying and other technological processes to its composition.

On the contrary, *pesto* has not yet been studied as extensively and only technological aspects have been considered. For example, in order to extend the shelf-life of *pesto*, Fabiano, Perego, Pastorino, and Del Borghi (2000) proposed a combination of modified atmosphere packaging and refrigeration, which should avoid the significant changes in its composition and aroma caused by its

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pasteurization or sterilization (Vicini & Previdi, 1992). Lately, Antonelli et al. (2004) studied the application of multivariate image analysis to the evaluation of the influence of both technological variables and storage on *pesto* appearance. Nevertheless, up to day the aroma of this palatable and fragrant sauce has not yet been studied. Thus, in sight of a possible application to European Union for the PDO "Pesto Genovese" a deeper knowledge of the composition of *pesto* aroma might contribute to single out the different ingredients and their amounts, to verify its organoleptic quality and to trace back its geographical origin.

In this study, headspace sorptive extraction (HSSE) coupled with gas chromatography–mass spectrometry (GC–MS) was applied to the analysis of the volatile fractions of *pesto* and its ingredients. This analytical technique, proposed by Tienpont, David, Bicchi, and Sandra (2000), was used for the extraction of volatiles and semivolatiles from coffee (Bicchi, Iori, Rubiolo, & Sandra, 2002), virgin olive oils (Cavalli, Fernandez, Lizzani-Cuvelier, & Loiseau, 2003), essential oils (Kreck, Scharrer, Bilke, & Mosandl, 2002) and for the detection of off-flavour compounds in drinking water (Ochiai et al., 2001). HSSE was developed for the enrichment of solutes from a gaseous matrix, i.e., the headspace of the analysed samples. The headspace compounds are extracted using stir bars coated with 30–300 μl of polidimethylsyloxane (PDMS), commercialized under the name of Twister®(Gerstel, Mulheim an der Ruhr, Germany). The equilibrium concentration of solutes in the PDMS is dependent on their PDMS-air distribution coefficient. Thus, HSSE can be considered as a modification of the classic headspace-solid phase microextraction (HS-SPME) technique, but HSSE relies on a greater amount of stationary phase and leads to higher recoveries of analytes (Plutowska & Wardencki, 2007). Moreover, both HSSE and HS-SPME do not require the use of high temperature or solvents, they prevent the introduction of water into the GC–MS system and allow isolating and enriching volatile components without interference from other matrix components or thermal degradation products (Plutowska & Wardencki, 2007). A conclusive matter in the choice of HSSE for the analysis of the aroma of *pesto* and of its ingredients was a study that showed that HSSE had better performances when compared to both PDMS and multicomponent fibers HS-SPME (Bicchi, Cordero, Iori, & Rubiolo, 2000) on the analysis of the volatile fraction of medicinal and aromatic plants.

2. Materials and methods

2.1. Instruments

GC–MS analysis was performed by an Agilent 6890 GC equipped with an Agilent 5973 MS quadrupole detector (Agilent Technologies, Palo Alto, CA, USA). The injector was a Gerstel CIS4 programmed temperature injector and was coupled with an external thermal desorption unit (TDS2, Gerstel). A capillary transfer line connected TDS2 to CIS4, which could be cooled down to −150 °C (with liquid nitrogen) and heated up to 350 °C. Helium flowed through TDS2 and transfer line to CIS4 injector.

2.2. HSSE sampling

PDMS stir bars (film thickness 0.5 mm, length 10 mm) were supplied by Gerstel. For HSSE sampling 10 ml headspace glass vials with PTFE/Red Rubber septa were used. A self-made holder for Twister® was prepared by winding a short zinc-plated iron wire round an Allen screw. Then a small hole was drilled in a septum, thus allowing inserting the holder and fixing it in the septum. In a headspace vial isobutyl acetate (1 μl, internal standard) was added to a 4.00 g *pesto* sample; then, the Twister® was suspended by the holder in the headspace of the sample and the crimped vial was incubated at 25 °C for 2 h. As far as the analyses of single ingredients are concerned, 1.00 g of extra-virgin olive oils or ground walnuts, pine nuts and cheese, 0.10–0.30 g of ground basil leafs and 0.01 g of ground garlic were weighed. The stir bars were used many times after reconditioning at 300 °C for 120 min in a flow of helium to avoid "carryover" effects. On the contrary, each holder was used only once.

2.3. Stir bar thermodesorption (TD)

After HSSE extraction, the stir bar was removed by forceps and immediately placed in an empty glass tube, which was introduced in the TDS2, set at 20 °C, with a −120 °C CIS4 temperature. The extracted compounds were desorbed by programming the TDS2 from 20 to 40 °C at 30 °C/min (held for 20 min). A Helium flow favoured the stripping of volatiles, which were then trapped and focused on the cooled surface of the injector liner and cryo-focalized in the CIS4 at −120 °C.

2.4. GC–MS analysis

At the injection time, the temperature of the CIS4 injector was raised up to 300 °C (held for 5 min) at a ramp rate of 12 °C/s. In this step the carrier pressure (Helium) was 8.76 psi, the column flow 1.2 ml/min and the split vent flow 5 ml/min.

For the gas chromatographic analysis a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB-5MS fused-silica capillary column (J&W, Folsom, CA, USA) was used at a Helium flow rate of 1.2 ml/min. The oven temperature was kept at 40 °C for 5 min and then programmed at 5 °C/min to 160 °C, kept for 10 min, under flow-controlled conditions (constant flow 1.2 ml/min). A 10 min post-run at 300 °C was then performed. The mass-spectrometer interface temperature was set at 250 °C. The temperature of the ion source was 230 °C, electron energy 70 eV and quadrupole temperature 150 °C, with a 2 min solvent delay.

The chromatographic plots were obtained by total ion current (TIC) mode with a mass range between 40 and

250 amu (Fig. 1). The identification of compounds was performed by their retention index (RI) and by comparison of their mass spectra with the spectra of their pure standards and/or with the spectra reported for each compound in Wiley 275 Mass Spectra Library or other libraries freely available on Internet (http://www.flavornet.org;http://

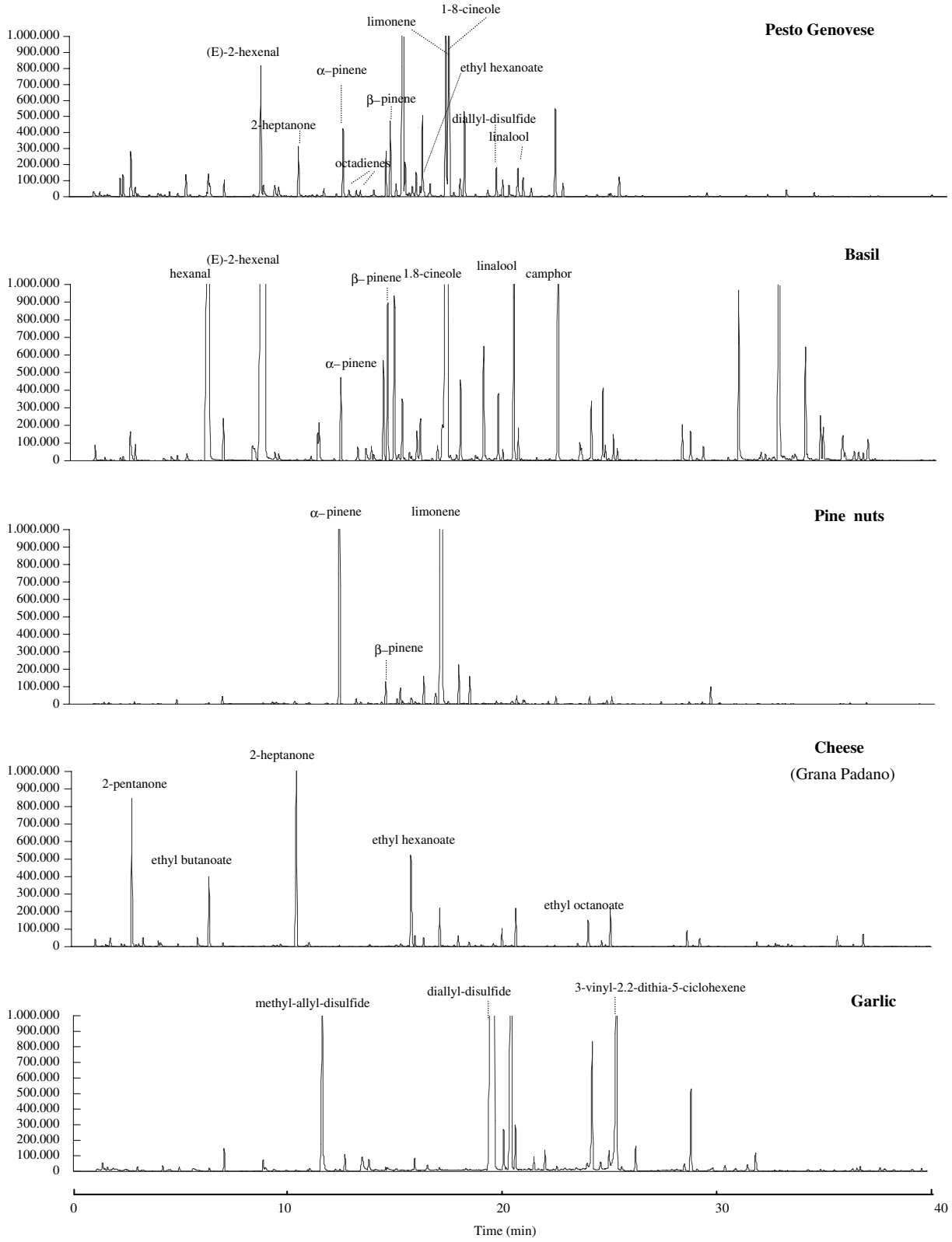

Fig. 1. Representative GC–MS plots of the volatile fractions extracted by HSSE from "Pesto Genovese", basil, pine nuts, cheese and garlic.

www.pherobase.com;http://www.aist.go.jp/RIODB/SDBS/cgi-bin/cre_index.cgi). More than 250 compounds were detected in the volatile fraction of *pesto* and/or of its ingredients. Table 1 reports 112 compounds that were not only detected, but also identified, in the analysed samples of *pesto*. Some of these compounds clearly originated from

Table 1
Identified volatile compounds of "Pesto Genovese" samples

Compound	Identification method[a]	Ingredient[b]	Compound	Identification method[a]	Ingredient[b]
Butanal	GC–MS		δ-Carene	GC–MS	b
Hexane	GC–MS		(*E,E*)-2,4-heptadienal	MS	
Ethyl acetate	GC–MS		α-Terpinene	GC–MS	b
3-Methyl-1-butanal	GC–MS	b, c	Limonene	GC–MS	b, pn
2-Methyl-1-butanal	GC–MS	b, c	1,8-Cineole	GC–MS	b
3-Pentenol	GC–MS	b, c	Benzyl alcohol	GC–MS	
2-Pentanone	GC–MS	c	(*Z*)-α-ocimene	MS	b
Heptane	GC–MS	o, pn	Phenylacetaldehyde	GC–MS	
3-Pentanone	GC–MS		(*E*)-β-ocimene	GC–MS	b
Methyl butanoate	GC–MS	c	γ-Terpinene	GC–MS	b
3-Methyl-1-butanol	GC–MS		*n*-Octanol	GC–MS	b
2-Methyl-1-butanol	GC–MS		Diallyl-disulfide	GC–MS	g
Dimethyl-disulfide	GC–MS	g	Nonenone	GC–MS	c
Toluene	GC–MS	pn	α-Terpinolene	GC–MS	b, pn
cis-2-Pentenol	MS		2-Nonanone	GC–MS	c
Octane	GC–MS		Allyl-propyl-disulfide	MS	g
Hexanal	GC–MS	b, o, wn	Linalool	GC–MS	b, pn
Ethyl butanoate	GC–MS	c	*n*-Nonanal	GC–MS	b, c, o, pn
2,4-Dimethyl-heptane	GC–MS		2-Phenyl-ethyl-alcohol	GC–MS	
(*E*)-2-hexenal	GC–MS	b, o	4,8-Dimethyl-1,3,7-nonatriene	MS	o
(*Z*)-3-hexenol	GC–MS	b, o	Methyl octanoate	GC–MS	c
4-Methyl-octane	MS		Camphor	GC–MS	b, pn
(*E*)-2-hexenol	GC–MS	b, o, pn	δ-Terpineol	GC–MS	b
1,3-Dimethylbenzene	GC–MS		Borneol	GC–MS	b
1,4-Dimethylbenzene	GC–MS		Naphtalene	GC–MS	
1-Hexanol	GC–MS	b, o, wn	3-Vinyl-2,2-dithia-5-ciclohexene	MS	g
1,2-Dimethylbenzene	GC–MS		α-Terpineol	GC–MS	b, pn
2-Heptanone	GC–MS	c	Ethyl octanoate	GC–MS	c
Nonane	GC–MS	pn	Estragole	GC–MS	b, pn
Ethyl valerate	GC–MS		Decanal	GC–MS	b, o, pn
Heptanal	GC–MS		1,3-Cycloctane	GC	b
3-Methyl-thiopropanal	GC–MS	c	2-Vinyl-4 H-1,3-dithine	GC	g
(*E,E*)-2,4-Hexadienal	GC–MS		2-Decenal	GC–MS	c
Methyl-allyl-disulfide	MS	g	Bornyl acetate	GC–MS	b
Methyl exanoate	GC–MS	c	Thymol	GC–MS	b, pn
α-Thujene	GC–MS	b	Carvacrol	GC–MS	b, pn
α-Pinene	GC–MS	b, pn	Diallyl-trisulfide	MS	g
2,6-Dimethyl-octane	MS	pn	2-Methyl-naphtalene	MS	pn
3-Ethyl-1,5-octadiene	MS	o	δ-Elemene	GC–MS	
3-Ethyl-1,5-octadiene	MS	o	Eugenol	GC–MS	b
Camphene	GC–MS	b, pn	α-Copaene	GC–MS	b, o, pn
(*E*)-2-Heptenal	GC–MS		Biphenyl	GC–MS	
Benzaldehyde	GC–MS		β-Cububene	GC–MS	b
Sabinene	GC–MS	b, pn	β-Elemene	GC–MS	
β-Pinene	GC–MS	b, pn	Ethyl-naphtalene	MS	
1-Octen-3-ol	GC–MS	b	Methyl eugenol	GC–MS	b
3-Octanone	GC–MS	c	β-Caryophyllene	GC–MS	b, pn
Myrcene	GC–MS	b, pn	α-Bergamotene	GC–MS	b
3,5-Dimethyl-1,6-octadiene	MS	o	α-Guaiene	MS	b
3-Ethyl-1,5-octadiene	MS	o	α-Cadinene	MS	b
3-Octanol	MS	b	(E)-β-farnesene	GC–MS	b
Decane	GC–MS	pn	α-Caryophyllene	GC–MS	b
Ethyl exanoate	GC–MS	c	Germacrene	GC–MS	b
Octanal	GC–MS	c, b	Bicyclogermacrene	GC–MS	b
α-Phellandrene	GC–MS	b	α-Farnesene	GC–MS	b
Hexenol acetate	GC–MS	o, b	γ-Cadinene	GC–MS	b

[a] GC–MS: identification based on R.I. and mass spectra; MS: tentatively identification on the basis of library searches.
[b] b, basil; c, cheese; o, extra-virgin olive oil; pn, pine nuts; wn, walnuts.

one ingredient, since they were always detected in significant amounts in the analyses of the volatile fractions of the pure ingredient, and they were characteristic of this ingredient (Table 1). Several identified compounds were always found in more than one ingredient and thus, cannot be considered as markers of the actual presence of one single ingredient. On the contrary, some volatile compounds did not constantly appear in the pure ingredients, and their origin was thus uncertain.

The relative amounts of each compound were calculated by internal normalization. Moreover, isobutyl acetate was used as internal standard in order to compare the contents of each volatile compound and of the total volatile fractions, expressed as MS integrated peaks, regardless of tuning parameters. The overall HSSE-TD-GC–MS repeatability was obtained by analyzing one representative *Pesto* sample three times and relative standard deviation (RSDs) $\ll$10%, and often $\leqslant$5% were obtained for most identified volatiles.

2.5. Samples

Two groups of non-heat processed commercial samples were purchased in local stores and analyzed: IPG (Industrial "Pesto Genovese"), 10 food industries manufactured samples, whose labels reported the exact wording "Pesto Genovese"; SPG, (Shop "Pesto Genovese"), 10 shops manufactured samples that were loose sold as "Pesto Genovese" not later than three days after manufacturing. Most IPG samples listed ascorbic acid among ingredients and could be sold refrigerated in sealed glass or plastic packages for a two-month period.

In order to single out the contribution of each ingredient to the whole aroma of "Pesto Genovese", several samples of ground basil leafs, pine nuts, walnuts, garlic, Parmigiano, Pecorino and Padano PDO cheese, and of extra-virgin olive oils were also analyzed. Moreover, to obtain "reference" *pesto* samples several samples were prepared in laboratory, using basil leafs, pine nuts, cheese and extra-virgin olive oil in different ratios.

3. Results and discussion

Considering the high susceptibility of the analyzed samples to chemical and enzymatic oxidation, HSSE extraction was realized at 25 °C, thus avoiding heat catalytic action. Though in sorptive extraction techniques full equilibration is not essential for an accurate determination, three extraction times (60, 120 and 180 min) were considered. The equilibrium partition between PDMS stir bars and sample headspace was already reached after 60 min, but a 120 min extraction time allowed to obtain a better reproducibility for most investigated analytes, without leading to sample oxidation neither in *pesto* nor in its ingredients.

In order to avoid oxidation and degradation reactions and to approach the human body's temperature, a 40 °C TDS2 desorption temperature was set. Though this temperature was lower than reported before (Bicchi et al., 2002; Cavalli et al., 2003), it must be emphasized that in those studies higher temperature had been used also for HSSE sampling, thus requiring stronger thermodesorption conditions.

As far as CIS4 injector is concerned, in this study it was equipped with an empty glass liner. In fact, a Tenax-filled liner would trap the analytes with less than five carbons (Baltussen, Sandra, David, & Cramers, 1999) more efficiently, but would also differently affect the adsorption/desorption behaviour of each analyte and its identification.

The analysis of the volatile fractions of *pesto* and its ingredients showed that each ingredient gives a significant and clear contribution to the whole *pesto* aroma, although the amount of volatile compounds originated from basil should generally prevail. In fact, the analyses of the volatile fractions of pure ingredients allowed singling out some peculiar components for each ingredient. Moreover, the total MS integrated peak areas of basil aroma were 3–10 folds those of ground pine nuts, walnuts, cheese and of extra-virgin olive oils, whereas ground garlic, which had higher values of total MS integrated peaks, is normally used in trace amounts in high quality "Pesto Genovese". At the beginning of this study, the major contribution of basil to the whole *pesto* aroma, together with the restriction on using only PDO basil "Basilico Genovese" had lead to assume homogeneity in analogous samples, i.e., SPG and IPG groups, but the first analytical results clearly showed some variability in the headspace composition of both IPG and SPG samples. An immediate consequence of this variability was that IPG and SPG samples cannot be distinguished into two different groups, since the differences of composition between them were not statistically significant. It is likely that plant growing conditions, i.e., light exposure, minimum and maximum temperatures, and plant height, had affected the biosynthesis of the volatile compounds and thus, their final content in basil. Nevertheless, the variability of the headspace composition certainly was also an effect of using different ingredients in variable proportion.

As far as the different volatile compounds are concerned, terpenes, sesquiterpenes and terpenoids were generally more than 60% of the total volatile fraction of the analysed "Pesto Genovese" samples (Table 2), thus confirming the prevalence of basil aroma that is very rich of these compounds. The amounts of terpenes generally ranged between 12% and 40% of the whole volatile fraction, with the exception of one SPG sample reaching 49%, but they were affected both by their variability in basil and by their content in pine nuts. In fact, the high contents of limonene, α-pinene and β-pinene detected in pine nuts involved their significant contribution to the final amounts of these terpenes in *pesto*, while other terpenes, such as α- and β-ocimene, α- and γ-terpinene, and terpenoids were not detected in significant amounts either in pine nuts or in other ingredients. The analysis of ground basil leafs and pine nuts samples showed that their absolute and

Table 2
Relative amounts (Percentage of the whole MS integrated volatile fraction) of the most significant volatile compounds of "Pesto Genovese" samples

Compounds	IPG[a]				SPG[a]			
	Min	Max	m[b]	SD[c]	Min	Max	m[b]	SD[c]
Total terpenes and terpenoids	42.1	91.7	73.3	15.6	32.8	90.6	65.2	17.2
∑*Terpenes*	12.0	25.4	20.4	5.0	12.7	48.8	25.6	11.4
α-Pinene	2.0	3.4	2.8	0.5	1.9	6.4	3.3	1.4
β-Pinene	2.1	4.6	3.1	0.9	2.3	7.5	3.7	1.6
Limonene	0.9	13.3	4.7	4.1	1.8	16.5	7.5	5.2
∑*Sesquiterpenes*	0.2	1.5	0.6	0.5	0.0	0.9	0.4	0.3
α-Bergamotene	0.1	0.5	0.3	0.2	0.0	0.9	0.4	0.2
∑*Terpenoids*	30.0	64.7	52.3	12.3	20.1	58.8	39.2	12.0
1,8-Cineole	14.2	40.9	26.1	7.9	16.5	51.9	29.1	10.0
Linalool	14.2	41.0	26.1	7.9	1.7	24.0	8.9	9.4
Camphor	0.1	0.9	0.4	0.3	0.0	2.0	0.8	0.6
Total lipoxygenase cascade products	0.1	11.6	3.9	3.6	0.6	29.4	10.3	8.9
Hexanal	0.0	1.3	0.4	0.5	0.0	4.9	1.2	1.4
(*E*)-2-hexenal	0.0	6.3	1.3	2.0	0.0	19.0	6.1	5.6
Total polysulfides from garlic	0.0	18.9	2.3	6.2	0.0	2.3	0.7	0.8
Diallyl-disulfide	0.0	8.0	0.9	2.6	0.0	1.6	0.6	0.6
Total aldehydes, ketones, alcohols and fatty acid esters from cheese	2.0	8.0	4.5	2.1	1.4	49.9	14.8	13.8
2-Pentanone	0.0	0.2	0.1	0.1	0.0	9.7	1.6	3.0
2-Heptanone	0.0	0.6	0.2	0.2	0.0	11.8	1.4	3.4
Ethyl butanoate	0.0	11.5	1.7	3.7	0.0	7.5	1.4	2.3
Methyl exanoate	0.0	2.0	0.7	0.7	0.0	0.7	0.3	0.3
Ethyl exanoate	0.0	12.1	1.9	3.8	0.0	11.9	2.6	3.5
Ethyl octanoate	0.0	1.7	0.2	0.6	0.0	1.4	0.2	0.4
Total alkenes from virgin olive oil	0.0	2.1	0.9	0.8	0.0	3.0	0.6	1.7

[a] IPG, industrial manufactured "Pesto Genovese"; SPG, shop manufactured "Pesto Genovese".
[b] m, mean.
[c] SD, standard deviation.

relative contents of monoterpenes were quite different. In fact, in pine nuts the amounts of limonene, which formed up to 80% of their whole volatile fraction, were largely higher (expressed as MS integrated peaks with respect to the internal standard) than in basil, and limonene/β-pinene ratio was up to 140 (see Fig. 1). Moreover, α-pinene/β-pinene ratio was always close to 10. In basil leafs limonene/β-pinene ratio was $\leqslant 1$ and α-pinene amounts were approximately half the β-pinene amounts, with a α-pinene/β-pinene ratio always close to 0.5. Walnuts, which could be used instead of pine nuts, had only negligible amounts of terpenes. Thus, the amounts of limonene, together with α-pinene/β-pinene and limonene/β-pinene ratios, appeared very promising for detecting the real presence of pine nuts in *pesto* and for discriminating among pine nuts, walnuts and other ingredients, such as *Anacardium* fruits, that are a cheaper alternative to the valued pine nuts and might be used to produce low quality products.

Several sesquiterpene hydrocarbons were detected in *pesto* samples (Table 2): although they should origin only from basil leafs, their amounts were always definitely lower than those of terpenes, and, except for one IPG sample, their sum was $\leqslant 1\%$ of the total volatile fraction. In this fraction, the content of α-bergamotene seemed to be typical of "Pesto Genovese", since the amounts of this compound were quite constant and always more than 50%, often 100%, of total sesquiterpenes. Moreover, some preliminary analyses of samples of *pesto* obtained with non-PDO basil showed that in these samples α-bergamotene was often absent.

The amounts of terpenoids appeared quite variable (Table 2), with high 1,8-cineole and linalool contents and low borneol, bornyl acetate, camphor, carvacrol, estragol, α- and δ-terpineol, and thymol contents. Though in *pesto* they should mainly originate from basil leafs, traces of some terpenoids were found also in one pine nuts sample. Nevertheless, the great detected variability of terpenoids content in the analyzed "Pesto Genovese" samples clearly reflected the high variability found also in the basil leafs of "Basilico Genovese" cultivar.

As reported above, some samples had a content of terpenes and terpenoids lower than 60% (Table 2) and higher percentage amounts of other classes of volatile compounds. For example, in one IPG sample, which had an intense and unpleasant garlic flavour, the sum of garlic polysulfide (Table 2) was close to 19% of the total volatile fraction, with diallyldisulfide (garlicine) at 8%. Since in the other SPG and IPG samples the polysulfide contents never exceeded 2% of the total volatile fraction and these samples certainly showed more pleasant and balanced taste and aroma, the amounts of total polysulfides might be useful to safeguard the peculiar organoleptic features of a high

quality "Pesto Genovese". In three SPG and one IPG samples the total content of fatty acid methyl and ethyl esters, ketones, alcohols and aldehydes ranged between 15% and 50%: since most of these compounds were not detected in other ingredients than cheese, their content showed that in these samples high cheese amounts had been used. Nevertheless, the high encountered variability of these compounds could be caused either by the different amounts of cheese or by the different kinds of PDO cheese used in the formulation. In fact, the analysis of pure PDO cheese showed qualitative and quantitative differences among them, since in Pecorino samples were detected also propyl- and isobuthyl-fatty acid esters, and, as a whole, the total integrated MS areas of the volatile fraction of PDO Pecorino cheese were 2–3 folds higher than those of Parmigiano, which, in turn, were approximately two-folds those of Padano. Moreover, it is possible that in order to cut production costs, some manufacturers made use of cheaper less ripened and not-PDO cheese, that gave a slighter contribution to the whole *pesto* aroma. In sight of an application for the PDO "Pesto Genovese", it will be advisable to choose a definite kind of cheese, or a more definite proportion of different cheese, on the basis of a strict collaboration with a panel of tasters, which defines the balanced contribution of cheese to *pesto* aroma. This would allow defining the corresponding amounts of marker compounds and, in future, checking the product compliance with the adopted regulation.

The contribution of extra-virgin olive oil to the volatile fraction of "Pesto Genovese" was difficult to single out: in fact the major volatile compounds of olive oils, such as several terpenes and C6 aldehydes, alcohols and esters formed by lipoxygenase cascade (Zunin et al., 2004; Zunin, Boggia, Salvadeo, & Evangelisti, 2005) were also detected in ground basil leafs, though their amounts were very variables. Moreover, the amounts of virgin olive oil used as ingredient in the analyzed samples might have been very different and probably lower in SPG samples, which appeared thicker than IPG samples. Nevertheless, some minor alkenes, such as dimethyl-, diethyl-, and methyl-ethyl-octadienes, which were always detected in extra-virgin olive oils and never detected in other ingredients or in refined vegetable oils, including refined olive oils, could be used as markers of the actual presence of extra-virgin olive oil in "Pesto Genovese".

4. Conclusions

The HSSE-TD-GC–MS analysis of the volatile fraction of "Pesto Genovese" allows detecting the actual presence of most of its valuable traditional ingredients and may be an important tool for unmasking their substitution with other worthless ingredients.

The detected variability of the HS composition of the analysed *pesto* samples shows that *pesto* composition is quite variable and emphasises the need for regulating the type and the amounts of the different ingredients, in order to preserve the original taste and aroma of "Pesto Genovese". Moreover, the similarity between IPG and SPG samples confirms that IPG products, largely commercialized out of Italy, can keep the peculiar sensorial features of traditional *pesto*.

In sight of an application for the PDO "Pesto Genovese", the future development of this study must involve the co-operation with panels of tasters and manufacturers. In order to optimize and standardize the peculiar and pleasant taste and aroma of "Pesto Genovese", tasters shall single out the best "Pesto Genovese" and its "recipes" among *pestos* of known composition manufactured by different producers. Then, the HSSE-TD-GC–MS analyses of the optimized "Pesto Genovese" samples will allow singling out the amounts of marker compounds of each ingredient corresponding to its optimized and balanced proportion. Finally, the HSSE analysis will be very useful for a careful control of the composition and quality of this valuable niche product.

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