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CHARACTERIZATION OF FOOD PRODUCTS BY CHEMICAL, PHYSICAL AND SENSORY PROPERTIES: A COMBINED APPROACH

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OBJECTIVES

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Sensory evaluation mainly focuses on measuring the responses of people to sensory properties of foods and on relating these properties to consumer acceptance (Stone and Sidel., 2005).

In recent years, there has been a growing interest in this field and much greater use of the sensory analysis that has been driven by a variety of factors and many applications to product development and product quality control.

Sensory and consumer testing allows insights into human behaviour and perception and involves the study of the intrinsic and extrinsic factors that influence liking, choice, purchase or consumption across a product category of foods and emotional benefits (Kemp at al., 2009).

In any event, also the development and the application of fast and non-destructive instrumental analyses to measure food characteristics such as appearance, aroma, taste, and texture could represent a useful tool for quality assurance and valorization of food products. Moreover, the study of the link between instrumental and sensory measures is fundamental: it allows clarifying the relationship between these two approaches and highlights how it is possible to combine both approches for providing much information, to improve the data interpretation, to verify the products compliance to specific criteria and, possibly, to predict results (Tzia et al., 2015).

These considerations lead to the two main objectives proposed in this doctoral dissertation.

The first objective is the study of how certain analytical characteristics (chemical, physical and sensory) can discriminate products belonging to different standard or product categories as a function of its compositional and/or technological variables.

The second objective is the relationship identification between the sensory profile and some chemical-physical parameters and variables used for characterization, valorization and quality testing of a food product.

This study also aimed to understand the main drivers of the consumer's overall liking.

A series of partial aims providing a better understanding of consumers' sensory acceptance have been proposed in the different sperimentations carried out in this PhD project.

In particular, the trials have been carried out to:

- ✓ verify whether some product information may have an influence on the satisfaction expressed by the consumer;
- ✓ identify possible drivers of liking;
- ✓ understand how the sensory characteristics are perceived by the consumer.

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SUMMARY

SUMMARY

This dissertation shows the results achieved during the realization of the doctoral research which title is "Characterization of food products by chemical, physical and sensory properties: a combined approach". It has been divided in two main sections: the Introduction as the first section, and the Experimental part, Results and discussion as the second section.

Introduction shows an overview of the sensory methodology focusing on the three categories in wich sensory tests are classified by type (discriminative, descriptive, and affective tests). Some common and new methodologies for sensory characterization are included, along with usage and limitations about the related approaches. There are also reported some instrumental tools commonly used to measure some quality attributes. In particular, the available types of instrumental measurements suitable for the investigation of instrumental–sensory relationships are summarized.

Experimental Part, Results and Discussion is divided in six chapters related to the studies conducted on different analytical characteristics (chemical, physical) linked to the sensory profile, to the quality of different food products, and to the consumers responses.

Chapter 1 describes a study on sunflower and hazelnut cold-pressed oils aimed to define the sensory profile of both types of vegetable oils and to evaluate their volatile profiles by Solid Phase Micro Extraction (SPME) coupled with Gas Chromatography Mass Spectrometry (GC-MS).

Chapter 2 includes a study investigating the effectiveness of the Flash Gas Chromatography Electronic Nose (FGC E-nose) as a tool to rapidly discriminate among commercial virgin olive oils (VOOs) characterized by a different geographical origin as shown in the label. The results obtained from this innovative analytical approach were compared with those coming from Solid Phase Micro-Extraction (SPME) coupled with Gas Chromatography Mass Spectrometry (GC-MS).

Chapter 3 makes reference to a work selecting sensory and instrumental information for designating *Taralli*, that is an Italian snack food typical of the Southern Italian region (Apulia) currently become very popular worldwide. For sensory characterization purposes, conventional profiling has been applied on samples by different producers. All samples have been also subjected to physical analysis of appearance and textural proprieties. Moreover, significant changes in sensory characteristics during storage time have been valued by a discrimination test (triangle test).

Chapter 4 shows a preliminary investigation in which different commercial categories of Italian cooked hams have been characterized using an integrated approach based on both sensory and fast instrumental measurements. Therefore, a set of Italian products belonging to different categories (cooked ham, "selected" cooked ham, and "high quality" cooked ham) have been evaluated by classical descriptive analysis and by the application of analytical instruments such as electronic eye and texture analyzer.

Chapter 5 reports quality evaluation and consumers' acceptance of a set of different extra virgin olive oils (EVOOs) purchased on the Italian market. The main objectives have been the detection of elements that can lead to the products' acceptability and to the quality recognition by evaluating the influence of information about organic or conventional production on consumers behavior.

Chapter 6 summarizes the results of a work conducted on "flavored olive oils" obtained by adding different kind of spices and aromatic herbs. The study aimed to check the possible influence of aromatization process on the product quality to characterize the volatile fraction of different samples and to test consumer acceptance.

INTRODUCTION

INTRODUCTION

1. Sensory test methods

Sensory evaluation is defined by the Institute of Food Technology as "a scientific method used to evoke, measure, analyze and interpret those responses to products as perceived through the senses of sight, smell, touch, taste and hearing" (Anonymous 1975).

This constantly evolving discipline is now recognized as a scientific field in its own right. In fact, sensory attributes are considered as a determinant key of product features including quality, functional and emotional benefits (Kemp et al., 2009).

Sensory evaluation can be divided into two categories of testing: objective and subjective (Sidel and Stone, 2005). The main difference is related to the different judges involved; in objective testing, the sensory attributes are evaluated by a panel of selected and trained assessors whereas, in subjective ones, the response (acceptability and/or preference) of potential consumers of products is measured.

The power of sensory evaluation is realized when these two elements are combined to reveal insights into the way in which sensory properties drive consumer acceptance and emotional benefits (Kemp et al., 2009).

The sensory profile of a product may provide much information about its safety, nutritional value and give details about the efficiency of the manufacturing operations and the quality of raw materials. Therefore, linking sensory properties to physical, chemical measurements is also fundamental because allows to check the quality of food products and the maintaining of the designed standard and desirable quality level (Tzia et al., 2015).

The wide use of sensory techniques is directly related to its positive contribution and variety of applications to: product development (new product, pilot plant, cost reduction, ingredient/process change, ingredient/purchase specifications); product quality (sensory

specifications, production benchmarking, manufacture quality, shelf-life and stability, distribution product); marketing (monitor competitors, advertising/claim support, category review, product optimization) (Sidel and Stone, 2005).

1.1 Discrimination test

Discrimination tests are some of the most common sensory methods employed in sensory science. These procedures were applied for determining whether a perceptible sensory difference or similarity exists between samples of two products. These test may be divided in two category in function of the availability of all information by subjects (overall difference test) or not; in the latter case, assessors are directed to focus on one specific attribute or property (attribute-specific test).

The list of all different discrimination test is showed in **Table 1**.

Discrimination tests are rapid techniques applied in sensory study with different aims (Kemp et al., 2009):

- ✓ Examine products as pre-screening for subsequent sensory test (descriptive or affective);
- ✓ Select, train and monitor assessors;
- ✓ Determinate sensitivity thresholds;
- ✓ Quality control;
- ✓ Investigate the effect of ingredient/process changes

These sensory procedures can be performed by both naïve and experienced assessors, however, to avoid making mistakes in statistical processing of data (missing a real difference or finding a false difference), subjects should be screened for their sensory skill or, in other words, for their ability to detect difference between products (Sidel and Stone, 2005).

Precision regarding a particular population of assessors increases as the size of the panel enlarges, and also with their training and with exposure to the product. Discrimination test are generally carried out in testing booths under conditions that prevent communication among assessors and the occurrence of bias according to

International Organization for Standardization (ISO) for Discrimination test (ISO 8588:1987; ISO 8587:1988; ISO 4120:2004; ISO 10399:2004; ISO 5495:2005).

Moreover, to avoid that assessors identify samples from the way in which they are presented, samples should be prepared in an identical manner (i.e. same apparatus, same vessels, same quantities of product) and different means of masking some characteristics (e.g. colored lights for masking appearance) are commonly used but should be thoroughly checked to ensure that it is effective.

The procedure carried out during discriminatory tests has two different modes of response from assessors: "forced choice" or "no differences". The 'forced choice' mode dictates that a decision must be made and a sample selected in response to the question whereas 'no difference' option allows the assessors to report that the samples do not differ with regard to the question asked. In the choice of the right mode to be apply it is necessary to take into account the different subjects interviewed (trained or naive assessors) and, possibly, a different statistical approach to process results.

Statistical significance testing is used to analyze the data and determine whether or not samples are deemed to be different or similar. Published tables are available in the literature for determining the number of correct judgments required for statistical significance (Stone and Sidel, 2004).

Discrimination Test	
Overall difference test	Attribute-specific Test
Triangle*	Paired comparison
Duo-Trio*	n-Alternative Forced-Choice
Difference from control test	Ranking test
Same-different test	
A-non-A	

Table 1. Discrimination test classification according to Kemp et al., 2009. *test also applied to determine the degree of similarity between products.

1.2 Descriptive test

Descriptive analysis (DA) represent one of the most applied techniques in sensory science (Lawless, and Heymann, 2010).

The method allows a precise sensory description of a product by the evaluation and quantification of sensory differences between products.

This technique is used extensively, particularly in the food, beverage, and personal care industries (Heymann et al., 2014). Their success is probably due to the possibility to link quantitative descriptive data to different kind of data provided by both sensory analysis and instrumental measurements. This is particularly interesting for identifying those product attributes that are most important to consumer preference and, on the other hand, for understanding the chemical and physical components of a product that affect sensory characteristics (Kemp et al., 2009).

Descriptive analysis is a sensory methodology that provide a quantitative description of product taking into account all the sensations that are perceived (visual, auditory, gustatory, olfactory and kinesthetic) when the product is evaluated.

This procedure is usually applied in controlled conditions, under the direction of a panel leader whose role may change as a function of the descriptive methodology used.

The realization of the methodology involves several steps (Kemp et al., 2009):

- ✓ Selection and training of assessors
- ✓ Generating attributes and references
- ✓ Agreement on attributes
- ✓ Determining of assessment protocol
- ✓ Rating intensity
- ✓ Scale design

The assessment protocol should control from bias and it is important to allow a suitable time to complete evaluations including the breaks between samples to prevent judges fatigue.

Moreover, to improve the quality of results, a randomized distribution of sample, the use of a control sample and the practice to carry out replicate assessments, have to be used.

The performance of the panel should be monitored in terms of discrimination power, agreement between panelists and reproducibility during training to achieve the most accurate, reliable and consistent results as possible. In case of unsatisfactory results, poorly performance individual assessor or attributes that are not discriminating between samples, can be removed from the data.

Statistics is an essential part of the sensory evaluation process, providing the necessary information to reach conclusions about a study.

There are different category of statistics applied by sensory researchers (descriptive, inferential, correlation/regression and multivariate methods) clearly summarized by Sidel and Stone (2005).

Descriptive statistics provide an overall view of individual score but it can mask or distort information; these methods are grouped into measures for central tendency (mean, mode and median) and dispersion (range, variance and standard deviation).

Inferential statistics are generally applied to evaluate the probability (or risk) of concluding that a perceptible difference exists between samples when one does not. The proper choice of statistic test to be used minimized the possibility of making a wrong decision. This category includes non-parametric and parametric tests. The difference is mainly that in first case, data from groups or products are without constraints about the shape of distribution (Binomial test, Chi-square (x^2), Cochran Q, Friedman, Kruskal-Wallies and others). On the other hand, the application of parametric test, is recommended for data that show a normal distribution (t-test, analysis of variance and post-hoc test) (Granato et al. 2014).

When the objective of the research is the study of relationship between different set of data, correlation or regression measures are used to better understand the link between sensory data and other variables or use them to predict sensory attributes and consumer drivers.

Several test in addition to those already described are also applied to simultaneously examine multiple variables (Multivariate analysis of variance, Discriminant analysis, Principle component analysis, Factor analysis and Cluster analysis).

Some of the most common types of classical descriptive methodology are described in **Table 2.**

Classical descriptive methods				
Technique	*Principle*	*Advantages*	*Disadvantages*	*Reference*
Consensus Profiling	Assessors work as a group to agree on attribute and intensity ratings	Quick and low cost	No statistical analysis	Cairncross, S.E. and Sjöstrom, 1950
Texture profiling®	A panel of 6-10 trained assessors selected according to their ability to discriminate textural differences using a category scale	Reliable results, easy data interpretation and communication	Extensive Training	Brandt *et al.* 1963; Szczesniak 1963; Szczesniak *et al.* 1963
Flavour profiling®	A panel of 4-6 trained assessors who work individually and then discuss in an open session using a category scale	Reliable results, easy data interpretation and communication	Extensive Training	Cairncross and Sjöstrom 1950
Spectrum™ methods	A panel of 12-15 trained assessors who work individually using a 5-point numbered absolute scale	A full qualitative and quantitative description can be produced	High degree of panel training and maintenance to achieve is required	Meilgaard at al. 2007
Quantitative Descriptive Analysis QDA®,	A panel of 8-15 trained assessors who work individually using a line scale and in replicate (2-6)	Reliable technique that produce reproducible results also among different panel	High degree of panel training and maintenance to achieve is required	Stone et al. 1974

Table 2. Classical descriptive methods (Kemp et al., 2009; Sidel and Stone, 2005).

Descriptive analysis provides reliable data but, as previously mentioned, requires an extensive training of the panelists to align and standardize the sensory concepts of the panel. Moreover the development of a comprehensively and accurately vocabulary able to describes the product and the maintenance of the panel in terms of resources are time-consuming, expensive and represent the main drawbacks of this methodology.

Recently, in response to these limitations, there is a need to develop faster and more cost-effective methods of descriptive analysis. Several methods have been developed as alternatives to conventional profiling as showed in recent reviews (Valentin et al.,2012; Varela and Ares, 2012); some of these are summarized in **Table 3**.

These methods do not require training; can be performed by trained or semitrained assessors, or even naive consumers; are cheaper and better meet the needs of companies due to the reduction of time. The application of these rapid methodologies for sensory characterization also with consumers gives the opportunity to know the elements that affect their perception of the quality, drive their choices and to and better understand their language.

	Rapid descriptive methods				
	Technique	*Principle*	*Advantages*	*Disadvantages*	*Reference*
DESCRIPTIVE METHOD	Free choice profiling FCP	Untrained assessors with an individual attribute lists and who rate them for intensity.	Fast, no training	Results can be difficult to interpret	Williams and Langron 1984)
	Flash profiling FP	Untrained assessors who chooses and then uses own words to comparatively evaluate products	Fast, no training	Not suitable when the products to be evaluated are too numerous and for quality control	Dairou and Sieffermann 2002
	Check all that apply CATA	List of attributes (words or phrases) from which choose those consider appropriate to describe a product.	Fast; data are not onerous to analyze;	Does not give direct information regarding attribute intensities	Adams et al., 2007; Lancaster and Foley, 2007
DYNAMIC METHOD	Time intensity TI	Continuously evaluation of the intensity of a sensory attribute over a period of time	Give information on dynamics of the sensory profile of a stimulus over consumption	Evaluate one attribute at a time; extensive training	Sjöström 1954; Larson-Powers and Pangborn 1978
	Temporal dominance of sensations TDS	Study of the sequence of dominant sensations of a product during a certain time period	Evaluate several attributes simultaneously; give information about the sequence in which attributes are perceived	Not suitable for study the kinetics of specific attributes	Pineau et al. 2009
SIMILARITY METHOD	Free Sorting Task FST	Evaluation of global differences	Fast and easy to understand and perform.	Difficulty to interpret results, poor precision	Lawless et al., 1995
	Projective Mapping PM and Napping	Evaluation of global differences	Fast and more discriminant than FST	Difficult to understand and perform and to interpret results.	Risvik et al. 1994; Pagès 2005

Table 3. Rapid descriptive methods (Valentin et al.,2012; Varela and Ares, 2012).

1.3 Affective test

The main purpose of consumer tests is to assess the subjective response (acceptability and/or preference) utilizing untrained people who are representative of the ultimate users of the product.

This methodology allows to get a lot of information on consumer perception, liking, habits and opinions concerning products under study (Cardello and Schutz, 2006).

The application of affective tests involves not only the sensory researchers but also food industry and companies which, from product concept to market launch, can apply them to monitor the products characteristics and their performance. Consumer testing are also important to check the maintenance of a quality standard, to improve and optimize the production or to support the advertising and communication actions (Kemp et al., 2009).

When a consumer test has to be performed, it is important to define some key aspect. Firstly, the number and the type of assessors who should be in large numbers as possible and selected according to the object of the study as well as different variables such as geographical regions, demographics, psychographics, lifestyle, product usage (Meilgaard et al., 1999; Kemp et al., 2009). Another aspect that could affect the test results is the test location. The choice of which location to use is partly a function of cost and partly a function of the research objective of the study

There are three type of test location: laboratory, hall and home use; their features are showed in **Table 4** reproduced as the original present in Kemp et al., 2009.

The design of questionnaire is another critical point; its preparation should be considered to:

- Do not ask for more than what is required;
- Minimize the number of questions and products to avoid fatigue;
- Provide appropriate instruction to the assessor on how to perform the assessment;
- Define the right order of questions to ensure that responses do not influence later questions;
- Define the number of different type of scaled used (e.g. hedonic, just about right (JAR) etc.).

TEST LOCATION		
	Advantages	Disadvantages
Laboratory test	Relatively high response rate; controlled conditions; Immediate (computerised) feedback; low cost; several products can be assessed per consumer.	Not representative of the natural context; Important attributes can be missed; Number of questions that can be asked is limited; Respondents not always representative of population.
Hall test	High number of respondents; Respondents from general population; Several products can be assessed per consumer; More control over how product is tested.	Unrepresentative surroundings; Less control than in a laboratory test; Important attributes can be missed; Number of questions that can be asked is limited.
Home use test	Relatively high number of respondents; Product tested under real conditions; Ability to test product under repeated use conditions; Ability to gain realistic information concerning intention to purchase.	More nil returns and missing responses; No control over product use; Time-consuming; Slow feedback; Small number of products; Generally more expensive.

Table 4. Advantages and disadvantages of test locations (Kemp et al., 2009).

The affective tests used for the collection of consumer sensory data can be divided in qualitative and quantitative methods (Meilgaard et al., 1999; Kemp et al., 2009).

The qualitative affective tests measure the subjective responses of a group of consumers to the sensory properties of the products, giving them the opportunity to set up an open discuss regarding their opinions, feelings and attitudes. This kind of test is usually used to discover and understand if there are consumers needs that are still not satisfied and thus can help to identify market trends in consumer behavior or in their use of a product. Various methods are used including one-to-one in-depth interviews, group interviews and, most commonly, focus groups.

The quantitative tests provide the responses of a group of consumers to a series of questions regarding either preference or acceptance of products. They are applied to determine the factors affecting overall preference or liking or to measure consumer responses to specific sensory attribute of a product. According to the different task of the

test (choice or rating), quantitative affective test can be classified in preference and acceptance test, respectively (Meilgaard et al., 1999).

Affective methods are easy to apply and allow to obtain a lot of information regarding consumer behavior. However, these methods have some limitations mainly related to understanding the choices made by consumers. Moreover, it should be taken into account that it involves subjects which use a reduced vocabulary to describe the products and they are not trained in the use of the scale and so, often, make some mistakes when filling out questionnaires. To overcome such drawbacks, different statistical techniques can be applied to process affective test data with the aim to linking consumer, sensory and product data (physical and chemical variables) (Kemp et al., 2009).

The preference mapping, for example, represent a multivariate techniques applied to relate the hedonic and descriptive data and allows visualize results using perceptual maps easily interpretable.

Different type of preference exist and can be divided in internal and external preference map. In addition, conjoint analysis and other modeling techniques (partial least squares PLS regression) can be used to built models on consumer choice, to predict consumer behavior, sensory properties or liking.

2. Combining instrumental and sensory methods

Despite the growing interest in the developing and application of sensory methods, until recently many commercial companies and food providers to verify if products belonged to specific standards in terms of sensory quality have almost exclusively used instrumental methods.

The interest in the application of instrumental methodologies in different industries is in part based on the possibility to reduce the variability and the subjectivity which some linking to the sensory data provided by human judgments (Sidel and Stone, 2005). In addition, food and beverage industry can meet a lot of difficulties in the implementation of sensory techniques mainly due to difficulties in setting up, management and maintaining of sensory panel in terms of time and economic investments (Kilcast, 2013).

Lawless & Heymann (1998) have suggested to restrict the use of instrumental measurements for repetitive, tiring or dangerous evaluations. In any case, the application of sensory analysis is necessary because it represent the only discipline able to understand and describe the whole range of characteristics resulted from the stimulation of all our senses by phisicochemical properties of the food (Kilcast, 2013).

Sensory evaluation should be included in the quality control program of the food industry in order to satisfy specific requirements of consumers. Moreover, sensory properties are of great interest also in the research and development of new products (Tzia et al., 2015).

The sensory characteristics of food are generally grouped into three modalities: appearance, flavour and texture. These modalities are, however, not independent of one another as confirmed by their definition proposed by International Standards Organization (ISO, 2008) that included also the possible interrelationships between appearance vs smell, flavor vs tactile and visual sensations, texture vs vision and taste (Kilcast, 2013).

The best approach for establishing the relationships between sensory and instrumental analysis requires firstly defining which sensory properties have to be measured by instrumental methods and therefore are to be imitated. Afterwards, it is necessary a careful selection of right test procedures (for both sensory and instrumental analysis) to

be used and of appropriate statistic tools for correlating data. The last step, should be the validation of the found sensory–instrumental relationships as a predictive tool (Kilcast, 2013).

Before starting with statistical analysis it is very important to carry out a visual inspection of numerical values to check for any anomalies that would lead to a loss of significance of the results achieved. In general, when we compare instrumental and sensory analysis for finding a relationship, the correlation analysis is the statistical model most commonly used to relate the intensity of a sensory attribute to a measured instrumental parameter, to set of instrumental parameters and to use instrumental data to directly model consumer liking (Kilcast, 2013; Macfie, 2007).

On the other hand, regression analysis (liner regression or multiple linear regression) is used to fit a linear mathematical function between two variables or between a variable and a set of variable, respectively.

Instrumental data can be processed also by multivariate approaches; Reineccius (2006) identifies an approaches in which the first step is to explore data set by statistical methods such as principal component analysis (PCA), factor analysis (FA), cluster analysis and multidimensional scaling (MDS) for searching relationships, trends or clusters of samples. Only subsequently, specific methods including principal component regression (PCR), canonical correlation analysis (CCA) and partial least squares regression (PLS) are applied.

Finally, there are alternative approaches to statistical ones such as artificial neural networks (ANN), fuzzy logic analysis and belief rule-based (BRB) models to combine instrumental and sensory data that have been recently developed and used to support quality analysis on food as reported by Kilcast (2013).

A summary of the main types of instrumental measurements linked to the key sensory modalities is presented in the following paragraphs.

2.1 Appearance measurements

Appearance is a key factor in determining the sensory quality of food products and its perception by the consumer. In fact it represents the first feature that potential buyers evaluate during the purchase; if negative impression is perceived at point of sale, is possible that the purchase will not be successful (Kilcast, 2013; Tzia et al., 2015).

Appearance includes all visual characteristics of food quality such as color, size, shape, particle size, distribution of pieces, glitter, sheen and gloss, and wholeness (Cardello, 1998; Keast, 2010). Among these, colour is considered as the most important of all the visible characteristics (Hutchings, 2013).

Colour is a physical characteristic related to the light which is measurable in terms of intensity and wavelength (Kramer et al., 1970) and is mainly affected by the presence of water-soluble pigments (anthocyanins, flavonoids) or fat-soluble (chlorophylls and carotenoids) (Tzia et al., 2015).

In addition, many reactions that may occur during the product production, processing and storage (e.g. oxidation, enzymatic browning, caramelization, Maillard reactions) are responsible for colour changes. Variation in expected colour can be also related to the presence of defects that indicate a deterioration of the product due to problems occurrence during processing or storage (Tzia et al., 2015).

A recent review on colour measurement of food products, discusses the most common instrumental method and procedure applied in colour analysis: colorimeters, spectrophotometers and computer vision system (Pathare et al., 2013).

Colorimeters measure colour using a light source for illuminating sample. The reflected light from the object goes through three filters (red, green and blue) to simulate the observer functions and in particular the three types of cones in the retina of the human eye. Colorimeters are easy to use and also their data are easily interpretable but allow to obtain results relative of only a standard observer and a standard illuminant and therefore are function of the apparatus used. Moreover, calibrated standards of colours in the range of those of analyzed products, are needed as reference material for most accurate results.

Spectrophotometers measure the spectral distribution of the light absorbed/transmitted or reflected by a sample as a function of a specific wavelength. The advantage of this

procedure over colorimeter is that both transmittance and reflectance are inherent and relative properties of the objects which do not depend on either the illumination or the observer, but, at the same time data obtained do not provide any correlation with the sensory perception of colour by eyes. Moreover this method is simple and non-destructive but difficult to carry out for routine quality control (Pathare et al., 2013).

Computer vision system (CVS) represent an accurate, fast and objective alternative for colour measurements. CVS allows the evaluation of the whole surface of the samples and to also analyze separate areas with different colour characteristics (Brosnan et al., 2004).

The CSV mainly includes two parts: the hardware, consisting of the lighting system, the image acquisition system and the computer; the software, for the processing and image analysis. This is the core of the computer vision technique; the image processing also involves a series of operations, carried out in order to increase the quality of images collected, by removing the geometrical distortions and noise of the acquisition system, improving the focus and the standardizing of the illumination (Brosnan et al., 2004; Pathare et al., 2013).

Image analysis has found a variety of different applications in the food industry (fruits and vegetables, meat and fish, bakery products and grain) and therefore provides one interesting alternative for an automated, non-destructive and cost-effective technique for the colour evaluation (Brosnan et al., 2004).

2.2 Flavour measurements

Flavor is a complex sensation which occurs during the eating of a food and result from different chemical and physical stimuli (aroma, taste and trigeminal response) (Reineccius and Peterson, 2013).

Aroma compounds are volatile molecules perceived by nasal receptors directly through active or passive sniffing (orthonasal route), or released from the mouth during eating and then passed into the nasal cavity (retronasal route) (Meilgaard et al., 1999; Keast, 2010). The amount of volatiles that release from a product is affected by the temperature, the nature of the compounds (volatility), and the surface properties of food (i.e., softness/hardness, porosity, and wateriness/dryness) (Meilgaard et al., 1999)

The odour response is complex, with around 2500 odorous chemicals found in food (Taylor and Roberts, 2004); however, only a limited number of these can be perceived and contribute to the characteristic aroma of a food depending on its concentration, odor quality and sensory threshold value (Kilcast, 2013).

Moreover, odor system could be useful in detecting off-flavours linked to spoiled food or to the presence of contaminations (Stewart and Amerine, 1982). Odor of food products are strongly influenced by their processing and storage; certain processes are performed just to obtain a characteristic flavor (eg, ripening, maturation, heat treatment) or to eliminate unpleasant odors (deodorization of vegetable oils). It also to be taken into account possible antagonism / synergism effects in flavour perception resulted from the mixing two or more odors (Tzia et al., 2015).

Taste, detected by receptors located on the tongue and other oral surfaces, includes gustatory perceptions (salty, sweet, sour, bitter and umami) caused by soluble substances in the mouth and, as well as smell, is determinant for food acceptance (Reineccius and Peterson, 2013).

Sour taste is associated with hydrogen ions supplied by organic acids (vinegar, fruits, and vegetable) and acid salts and its intensity depending more on the hydrogen-ion concentration than on the total acidity. Different acids are able to produce different sour sensation and the main variables involved are the nature of the acid group, pH, titratable acidity, buffering effects, and the presence of other compounds (i.e., sugars) (Tzia et al., 2015).

Salty taste is due to ions of low molecular weight salts, most commonly of sodium chloride. The taste of salts depends on the nature of both the cation and the anion (Stewart and Amerine, 1982).

The main source of sweetness in foods are sugars but also organic compounds such as alcohols, certain amino acids, aldehydes, and glycerol contribute to this sensation.

Another primary taste is bitter whose perception is mainly due to the presence of organic compounds such as alkaloids (quinine), xanthins (caffeine), glucosides of phenolic compounds (oleuropein), amino acids or inorganic compounds (magnesium chloride) (Tzia et al., 2015).

The fifth taste is called umami and is sensed in different receptors than those of the primary tastes. It is most commonly associated with the taste of monosodium glutamate but can also be elicited by certain L-amino acids and nucleotides (Cardello, 1998).

A third component of flavor is represented by chemical feeling factors, which stimulate the trigeminal nerve ends in the mucosa of the eyes, nose, and mouth (perceptions of astringent, burnt, heat, cold, pungency, metallic etc.). Astringency has generally been attributed to the presence of tannins; coolness is characteristic of menthol (mint flavor), while hotness, that is also referred to pungency, characteristic of spices (piperine in black pepper, capsicum in red pepper) or caused by the presence of alcohol. Metallic taste can be generated by salts of metals, such as iron or copper, and is observed in canned foods as well as an aftertaste (Tzia et al., 2015).

The flavor compounds can be evaluated primarily using chromatographic approaches (both gas chromatography and high-performance liquid chromatography) either alone or in combination with mass spectroscopic techniques.

Headspace analysis is one of the options for instrumental determination of volatile compounds in foods and beverages; there are several solutions to isolate and concentrate the volatile compounds from the matrix, such as steam distillation/extraction (SDE), solvent or supercritical CO_2 extraction and the solid phase microextraction (SPME) that has become the most used technique for analysis in food (Reineccius and Peterson, 2013).

Finally, there are some alternative techniques that are increasingly being employed for study volatile compounds in food analysis which are extensively described by Wardencki et al. (2013).

The first method is the gas chromatography-olfactometry (GC-O), enabling the differentiation of a multitude of volatiles in odour-active and non-odour-active, related to their existing concentrations in the matrix under investigation and that associates the resolution power of capillary GC with the selectivity and sensitivity of the human nose (Plutowska and Wardencki, 2008, 2012).

In addition, two types of equipments based on electronic sensors are also increasingly applied: electronic nose (e-nose) and electronic tongue (e-tongue) (Deisingh et al., 2004; Ciosek et al., 2004, 2006; Apetrei et al., 2010).

In particular, e-nose performs an entirely aromatic analysis (volatile compounds) in the gaseous phase, without separating the aroma into individual aromatic components, whereas e-tongue can be used for recognition (identification, classification, discrimination), of flavour components of medium and low volatility in the liquid phase (Leake, 2006).

Both types of equipments are quick-acting, easy to operate and allow to generate a unique fingerprint characteristic for the analyzed matrix (Wardencki et al., 2013).

2.3 Texture measurements

Texture properties represent another important aspect of food quality that group all mechanical, geometrical and surface attributes of a product perceptible by means of mechanical, tactile and, sometimes, visual and auditory receptors (ISO 11036:1994).

When a food or a drink is consumed, a combination of texture qualities is perceived sequentially in this order (Lawless and Heymann, 1998; Heath and Prinz, 1999):

- visual texture, relating to surface characteristics evaluated by slicing or pouring;
- auditory texture, resulted from stimulation of acoustic receptors by the different sounds associated with consumption (during handing and chewing) of specific foods;
- tactile texture by touch (direct) or by a tool as knife, fork, spoon (indirect);
- oral tactile texture on the tongue including kinesthetic, mouthfeel, and phase change, responsible for all nongustatory oral perceptions linked to tactile, pain and temperature sensations.

Only one or the combination of these senses may be used to perceive the texture of the various food products (Tzia et al., 2015).

Many types of instrumental measurements have been developed with the aim to evaluate texture perception in all its components (Bourne, 2002; McKenna, 2003; Kilcast, 2004; Rosenthal, 1999), but the most of these allow to measure only individual categories of texture characteristics (mechanical, geometrical or characteristics related to moisture and fat content) and do not give information about the whole texture profile of a product. Despite the existence of such limitations in the application of instrumental parameters, they are widely used in quality control also due to the difficult realization of the texture sensory profile (Bourne, 2002; Kilcast, 2013).

Texture measurements generally employed can be classified in fundamental, empirical and/or imitative according to their measurement principle (Bourne, 2002). Fundamental tests measure specific and well-defined mechanical properties which can be independent of the measurement method; empirical methods measure those mechanical properties that are not well defined and related to practical experience linked to some aspects of textural quality and they are easily linked with the sensory perceptions. In addition,

imitative tests use specific instruments that mechanically reproduce the real product conditions of product consumption.

Considering the force/deformation methods applied to solid and semisolid food, there are two different approaches of measurements: destructive or nondestructive (Lu, 2013).

Destructive force/deformation methods are usually applied for providing information about the average quality for a batch of food items and tend to correlate better with sensory textural properties than are nondestructive methods. Their main drawback is the sample destruction during the process of measurement. On the other hand, nondestructive ones are usually applied to fresh raw or unprocessed food products which are highly variable in their textural characteristics and so less suitable to be assessed by destructive methods. Examples of non-destructive methods for texture measurement include sound input techniques, near-infrared techniques (NIR), nuclear magnetic resonance (NMR) and magnetic resonance imaging (MRI) (Kilcast, 2013).

Destructive measurement can be empirical (puncture, compression, shear, twisting/torsion, tension, bending) or fundamental (young modulus, poisson ratio, and shear modulus, yield strength, failure strength, creep test, relaxation test, dynamic test) (Lu, 2013).

An alternative to the application of conventional destructive techniques able to provide data related to a single parameter is the texture profile analysis (TPA) technique (Friedman et al., 1963; Szczesniak et al., 1963). This is an universal method for the measurement of the texture, based on the application of two dynamometer cycles (compression and decompression). The test provides numerous texture parameters (hardness, cohesiveness, viscosity, springiness, adhesiveness fracturability, chewiness, gumminess) reproducing the conditions applied during a mastication (imitative test) and therefore easily correlated with sensory analysis (Szczesniak et al., 2002; Lu, 2013).

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EXPERIMENTAL PART, RESULTS AND DISCUSSION

Chapter 1

Quality evaluation of sunflower and hazelnut cold-pressed oils by a sensory approach*

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ABSTRACT

The quality of sunflower and hazelnut cold-pressed oils (8 and 10 samples, respectively), all purchased on the Italian market, was evaluated by quantitative descriptive sensory analysis (QDA®). For this aim, the sensory profile sheets for the two typologies of vegetable oils were defined and a specific training of the panelists was carried out in order to obtain a satisfactory performance level of the panel monitored through the use of PanelCheck open-source software. The volatile profiles by solid phase microextraction (SPME) coupled with gas chromatographic and mass spectrometry analysis (GC-MS) were also studied to investigate the possible correlations between sensory attributes and the main volatile compounds and/or classes present in the aroma fraction of sunflower and hazelnut cold-pressed oils.

Keywords: cold-pressed vegetable oils, hazelnut oil, sensory analysis, sunflower oil, volatile compounds.

1.1 INTRODUCTION

In the last few years several vegetable oils produced by mechanical extraction without the use of any solvent, known as cold-pressed or virgin oils, have emerged and are now available on the market [1]. While virgin olive oil (VOO) is clearly defined by European Union regulations, a certain confusion about defining and characterization of other cold-pressed or virgin vegetable oils exists. In fact, definitions as *virgin*, *cold-pressed*, *not-refined* can be found on the labels. The Codex Alimentarius Standard for Named Vegetable Oils [2] clearly distinguishes between "virgin oils" and "cold-pressed oils" defining the former as "*obtained without altering the nature of the oil, by mechanical procedures, e.g. expelling or pressing and the application of heat only*" and the latter as "*obtained, without altering the oil, by mechanical procedures only, e.g. expelling or pressing, without the application of heat*". Subsequent purification of the oil by settling, filtering, centrifugation and/or washing with water vapor is possible for both types of oils. The application of heat in the course of the whole process is not allowed for cold-pressed oil, therefore cold-pressed oil is also virgin oil, but virgin oil is not necessarily cold-pressed oil.

Sunflower (*Helianthus annuus L.*) is one of the four major annual oilseed crops produced in the world [3]. Oil extracted from sunflower seeds is the second most produced virgin oil in Europe (after virgin olive oil) and is known for its high content in δ-tocopherol, a lipophilic vitamin (vitamin E) and antioxidant [4, 5]. Sunflower seeds oil is an excellent source of essential fatty acids, in particular linoleic acid, required by the human body and able to decrease the cardiovascular disease risk [6]. Three typologies of sunflower oil, characterized by different percentages of oleic acid (low, medium and high) are available on the market. The typology with a high content of oleic acid is gaining more and more importance due to the nutritional recommendation to replace a significant quantity of the more oxidizable ω-6 fatty acids (such as linoleic acid) with mono-unsaturated fatty acids (such as oleic acid) in order to reduce the risk of atherosclerosis [7]. Moreover, the high-value of this kind of sunflower seeds oil consists of its higher oxidative stability than oils lower in oleic acid, which is a desirable property for heating treatments and storage [8].

Cold-pressed sunflower oils, obtained without application of heat, retain some aromatic components that are characteristic of the original seeds; many volatile

compounds, that remain in the sunflower oil after cold pressing the seeds, play a key role to give its peculiar aroma to the product [4, 9]. The detailed profiling of the volatile substances is a source of information to classify and qualify samples on the basis of the sensory profile (aroma and taste), the technological impact or, more generally, the quality attributes [10-13]. Different authors [9] studied the volatile components that may contribute to the formation of the flavour of the sunflower oil extracted by cold pressing; they found that the principal compounds were terpenes, together with small amounts of hexanal, terpenic alcohols and/or other aldehydes. More recently, Krist [14] and Bendini [15] confirmed that β-pinene can be found as the predominant component in the headspace of all the examined cold-pressed sunflower oils.

Italy is the world's second largest producer of hazelnuts (*Corylus avellana* L.) after Turkey. Italian hazelnut cultivars are highly valued by the food industry for the quality and sensory characteristics of their nuts [16]. Hazelnuts, like the other nuts, are high-energetic food, rich in fats and proteins; they are also valuable sources of fiber, phytonutrients, and antioxidants, such as vitamin E [17]. The particular fatty acid composition of hazelnuts, rich in monounsaturated fatty acids, primarily oleic acid [18,19], can play a recognized beneficial effect on human health [20, 21]; on the other hand, hazelnuts are easily susceptible to rancidity. In fact, during storage the lipid fraction can be subjected to hydrolysis and oxidation, resulting in undesirable odors and flavors and in the reduction of the nutritional value of the kernels. A minor part of the production of the nut is consumed as such, whereas the major part undergoes a roasting process and is finally used, for example, in confectionery or as the main ingredient in cocoa/hazelnut spreads. Since raw hazelnuts have a rather bland aroma, it can be assumed that the odorants responsible for the characteristic hazelnut smell are generated by the roasting procedure from odorless precursors present in the raw nut [22]. The main purpose of roasting is to improve the desirable flavor, color, and to give a crispy and crunchy texture [23-25]. Volatile components of natural and roasted hazelnuts have been investigated by several researchers [22, 26-27]. Among volatile aromatic compounds detected in roasted hazelnuts, 5-methyl-(*E*)-2-hepten-4-one (filbertone), has been reported as a primary odorant (nutty-roasty and hazelnut-like) of roasted hazelnuts [22, 28-29]. Hazelnuts are thick-shelled tree nuts whose oils can be either consumed crude, preferably from roasted seeds, or refined. The chemical composition of hazelnut oils depends on

geographical origin, hazelnut variety and the extraction process [30, 31]. The lipid profile of hazelnut oil seems to be very similar to olive oil, which explains the great problem that the current methodologies have in detecting the presence of a fraudulent addition of hazelnut oil to olive oil at low percentages [1, 32]. The overall quality of cold-pressed/virgin oils, from a chemical and sensory point of view, is related to the oil extraction technology applied. Such quality levels can be very heterogeneous in the market, thus making the consumers uncertain and confused about the real quality of the cold-pressed/virgin oils. Those oils, obtained without the application of heat,,retain some volatile components that are characteristic of the original primary vegetable material and that play a key role to give its peculiar aroma to the product. Many edible oils labeled as cold-pressed/virgin are indeed "washed" by hot water steam, through a mild deodorized process, which eliminates almost completely these volatile markers. For this reason, a conjoint analysis between sensory characteristics and volatile profiles, could be considered a successful approach to obtain evidence for a misdeclaration of the production method [1].

Trained sensory panels are important tools for assessing the quality of food and non-food products [33]. Quantitative descriptive analysis (QDA®) is a well-known descriptive method which allows you to quantify the characteristics of the product, thus enabling a statistical treatment of data. QDA® is appropriate when detailed information on the sensory profile, identification and quantification of the attributes are required. This kind of sensory method allows you to compare similar products, testing correlations with instrumental and chemical measures, and can be used to define the standards for quality control. Qualified assessors who have undergone a specific training are essential to provide reliable and consistent results [34]. However, some problems related to the training, stability, and maintenance of the quality of such panels exist. Different methods have been developed that may help to achieve better panel performance [35-39]. These techniques can detect the lack of precision (repeatability), disagreement (reproducibility), and the ability or inability to discriminate among the samples. This type of information is very useful for improving data quality in future sessions through increased and more targeted training on problematic issues [33]. A very helpful tool is the software PanelCheck, an open-source program for quick and efficient analysis of sensory data, providing results as easy-to-understand graphs and tables.

In this work, the setting up of the profile sheet and the methodology to train the assessors were explained and the application of a QDA® methodology both on a set of 8 sunflower oils and on a set of 10 hazelnut oils was discussed. Moreover, the training of the panel was monitored and evaluated through the use of PanelCheck free software. The volatile profiles, evaluated by solid phase microextraction-gas chromatographic and mass spectrometry analysis (SPME-GC-MS), were also studied to highlight the presence of target compounds responsible for peculiar sensory notes or markers of negative sensory attributes. Moreover, a study of fatty acid composition was carried out in order to characterize the samples.

1.2 MATERIAL AND METHODS

1.2.1 Samples of cold-pressed sunflower oil

Eight samples of sunflower oils (coded from S1 to S8) were purchased on the Italian market: five oils (S2, S3, S4, S6 and S8) were bought at the supermarket, the other three (S1, S5 and S7) directly on the web sites of the companies. All the samples were obtained by mechanical processing, but three of these sunflower oils (S6, S7 and S8) were declared as washed with water vapor. All samples were produced from organic sunflower seeds. Samples were stored at room temperature (15-20°C) and protected from the light before analysis. In Table I label information of samples is summarized.

Table I - Main information present on the label of the sunflower oil samples.

Samples code	Label information	Packaging	Best before
S1	Sunflower oil obtained from first cold pressing	Dark glass bottle (750 ml)	03/06/2014
S2	Crude sunflower oil obtained by mechanical pressing	Dark glass bottle (750 ml)	15/09/2013
S3	Cold-pressed sunflower oil	Dark glass bottle (750 ml)	15/03/2014
S4	Crude sunflower oil obtained from only physical processes: pressing without solvents and filtered	Dark glass bottle (500 ml)	14/12/2014
S5	Crude sunflower oil obtained from first cold pressing. The processing of raw seeds, not preheated, occurs only by mechanical cold pressing	Dark glass bottle (750 ml)	01/03/2015
S6	Sunflower oil obtained from first cold pressing. Use of only physical processes: pressing without solvents, filtered and purified by water vapour	Dark glass bottle (750 ml)	08/07/2014
S7	Cold-pressed sunflower oil. Use of only physical processes: cold pressing, filtration and washing with water vapour	Dark glass bottle (750 ml)	30/10/2014
S8	Sunflower oil obtained without overheating the seed and the press before the pressure and without adding heat during the extraction procedure. The deodorization is carried out by water vapour at a controlled temperature	Dark glass bottle (750 ml)	05/08/2014

1.2.2 Samples of cold-pressed hazelnut oil

Different typologies of hazelnut oils were purchased directly on the web sites of the companies: all samples (coded from H1 to H10) were cold-pressed hazelnut oils. As reported in the label, four oils (H2, H5, H7 and H8) were obtained only from toasted hazelnuts, three oils (H1, H4 and H6) were obtained only from raw hazelnuts, one sample (H3) was obtained from raw and toasted hazelnuts, instead for two samples (H9 and H10) this information is not reported on the label. Samples were stored at room temperature (15-20°C) and protected from the light before analysis. In Table II label information of samples is summarized.

Table II - Main information present on the label of the hazelnuts oil samples

Samples code	Label information	Packaging	Best before
H1	Hazelnut oil obtained from mechanical extraction of raw hazelnuts of Tonda Gentile Trilobata variety (origin Piemonte)	Dark glass bottle (100 ml)	2014
H2	Hazelnut oil obtained from mechanical extraction of toasted hazelnuts of Tonda Gentile Trilobata variety (origin Piemonte)	Dark glass bottle (100 ml)	2014
H3	Hazelnut oil obtained from mechanical extraction of raw and toasted hazelnuts of Tonda Gentile Trilobata variety (origin Piemonte)	Dark glass bottle (100 ml)	2014
H4	Hazelnut oil obtained by cold pressing of raw hazelnuts (origin Piemonte)	Dark glass bottle (100 ml)	01/08/2014
H5	Hazelnut oil obtained by cold pressing of toasted hazelnuts (origin Piemonte)	Dark glass bottle (100 ml)	01/08/2014
H6	Hazelnut oil obtained by cold pressing of raw hazelnuts Piemonte IGP (variety Tonda Gentile Trilobata)	Clear glass bottle (100 ml)	30/11/2014
H7	Hazelnut oil obtained by cold pressing of toasted hazelnuts Piemonte IGP (variety Tonda Gentile Trilobata)	Clear glass bottle (100 ml)	31/05/2015
H8	Hazelnut oil obtained by cold pressing of toasted hazelnuts of Tonda Gentile delle Langhe variety (origin Piemonte)	Clear glass bottle (100 ml)	01/10/2015
H9	Hazelnut oil obtained by cold pressing	Dark glass bottle (250 ml)	01/02/2016
H10	Hazelnut oil obtained by only physical processes: pressing without solvents and filtered	Dark glass bottle (250 ml)	08/06/2014

1.2.3 Sensory evaluation of sunflower and hazelnut cold-pressed oils

The procedures for selection, training and monitoring of the assessors (A), the choice of appropriate descriptors and measure scales and the evaluation of results were developed according to ISO 13299:2003.

All the sunflower and hazelnut cold-pressed oils were evaluated, with 3 replicates, by a panel composed of eight trained panelists (five female and three male aged 20-50 years old). All the panelists were recruited on the basis of their previous experience in descriptive sensory analysis, in particular they were fully trained assessors for virgin olive oil (staff and PhD students at the Campus of Food Science, University of Bologna,

Cesena, Italy). The panel worked in a panel room and each assessor carried out the sensory analysis in a single booth. In both cases (sunflower and hazelnut cold-pressed oils), the panelists were specifically trained and samples were evaluated using a quantitative descriptive method (QDA®).

During the training phase, each panelist received oil samples and found perceivable product attributes by identification of appearance, aroma, taste and flavour attributes to be used in describing the oil samples. The panel decided whether descriptors (previously selected by a step of free discussion among panelists) were redundant (so should be removed from the list of attributes) or if there were terms that should be added. The final list of attributes was defined and the panel appropriately detailed each one (as reported in Table III and Table IV). Panelists also identified possible reference standards for the proper rating of the selected attributes. The references were all presented to each panelist and specific training sessions were carried out to develop their right recognition and to decide relative anchor points on intensity scales (Table III and Table IV). After the calibration sessions, all differently coded samples were randomly presented to the panelists for evaluation. The panelists rated the samples with the intensities of attributes on an unstructured 100 mm scale with well-defined anchor points from 0 (not perceivable) to 100 (perceivable at the level of saturation). A small white plastic cup, usually used for take-away coffee, was employed: around 10 g of oil was poured into the plastic cup. Samples were analyzed at room temperature and before the olfactory and gustatory phases the assessors were asked to slightly warm the oil by holding the plastic cup in the hands, covering and rolling it. For smell and taste evaluations, it was requested to record first the intensities of attributes perceived by orthonasal routes and then those by retronasal routes. It was advised to spit out the oil after the analysis and between one analysis and another it was required to reset the mouth using sparkling or natural water and crackers. The PanelCheck software was used to control the performance of the panel. When the panel leader found anomalous results, the analysis was repeated.

Table III - Sensory attributes and reference standards, acronyms and anchor points used in the sensory evaluation (QDA®) of cold-pressed sunflower oil samples.

	Descriptors	Acronyms	Definitions	References	Anchor points
Appearance	*Yellow colour*	YC	Intensity of yellow colour	A selected deodorized sunflower oil	Weak (20%)
				A selected cold-pressed sunflower oil	Average (50%)
				A selected cold-pressed sunflower oil	Strong (80%)
Odour (orthonasal sensations)	*Raw sunflower seed*	SS-O	Odour reminiscent raw sunflower seed	Raw sunflower seed in a cup	Strong (100%)
	Toasted sunflower seed	TSS-O	Odour reminiscent toasted sunflower seed	Toasted sunflower seed in a cup	Strong (100%)
	Herbs/flower	HF-O	Odour reminiscent of rosemary and/or chamomile	Rosemary and chamomile in two cup	Strong (100%)
	Rancid/fried oil	RF-O	Odour characteristic of strongly oxidized oil or fat	COI standard for rancid defect of olive oil	Strong (90%)
	Grain/hay	GH-O	Odour reminiscent of grain and/or hay	Grain and hay in a cup	Strong (100%)
Taste (retronasal sensations)	*Raw sunflower seed*	SS-R	Taste reminescent raw sunflower seed	Raw sunflower seed in a cup	Strong (100%)
	Toasted sunflower seed	TSS-R	Taste reminescent toasted sunflower seed	Toasted sunflower seed in a cup	Strong (100%)
	Roasted/burnt sunflower seed	BSS-R	Taste reminescent roasted/burnt sunflower seed	Burnt sunflower seed in a cup	Strong (100%)
	Rancid/fried oil	RF-R	Taste characteristic of strongly oxidized oil or fat	COI standard for rancid defect of olive oil	Strong (90%)
	Grain/hay	GH-R	Taste reminiscent of grain and/or hay	Grain and hay in a cup	Strong (100%)

Table IV - Sensory attributes and reference standards, acronyms and anchor points used in the sensory evaluation (QDA®) of cold-pressed hazelnut oil samples.

	Descriptors	Acronyms	Definitions	References	Anchor points
Appearance	*Yellow colour*	YC	Intensity of yellow colour	A selected deodorized sunflower oil	Weak (20%)
				A selected cold-pressed sunflower oil	Average (50%)
				A selected cold-pressed sunflower oil	Strong (80%)
Odour (orthonasal sensations)	*Raw hazelnut*	RH-O	Odour reminiscent raw hazelnut	Raw hazelnuts in a cup	Strong (100%)
	Toasted hazelnut	TH-O	Odour reminiscent toasted hazelnut	Toasted hazelnuts in a cup	Strong (100%)
	Sunflower seed/grains	SSG-O	Odour reminiscent sunflower seed and grains	Sunflower seed and grains in a cup	Strong (100%)
	Rancid/fried oil	RF-O	Characteristic odour of strongly oxidized oil or fat	COI standard for rancid defect of olive oil	Strong (90%)
	Burnt hazelnut	BH-O	Odour reminiscent burnt hazelnut	Burnt hazelnut in a cup	Strong (100%)
Taste (retronasal sensations)	*Raw hazelnut*	RH-R	Taste reminiscent raw hazelnut	Raw hazelnuts in a cup	Strong (100%)
	Toasted hazelnut	TH-R	Taste reminiscent toasted hazelnut	Toasted hazelnuts in a cup	Strong (100%)
	Sunflower seed/grains	SSG-R	Taste reminiscent sunflower seed and grains	Sunflower seed and grains in a cup	Strong (100%)
	Rancid/fried oil	RF-R	Characteristic taste of strongly oxidized oil or fat	COI standard for rancid defect of olive oil	Strong (90%)
	Burnt hazelnut	BH-R	Taste reminiscent burnt hazelnut	Burnt hazelnut in a cup	Strong (100%)

1.2.4 Analysis of headspace of sunflower and hazelnut cold-pressed oils

An aliquot of 0.1 g of 1-penten-3-one (internal standard dissolved in refined sunflower oil) to a concentration of 1 mg kg^{-1} was weighed into a 10 mL vial and the oil sample was added up to 1.0 g; the vial was closed with a silicone septum and conditioned at 40°C for 2 minutes without magnetic stirring. After 2 minutes of sample conditioning, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 µm, 2 cm long from Supelco Ltd., Bellefonte, PA) was exposed to the sample headspace for 30 min and immediately desorbed for 5 min at 240°C in the gaschromatograph with a split ratio of 1:10. Volatile compounds were tentatively identified and quantified by quadrupolar mass-selective spectrometry (in the 30–250 amu mass range) coupled with GC, using a GCMS-QP2010 gaschromatograph (Shimadzu Co., Kyoto, Giappone) coupled with an Autosampler AOC-5000 Plus (Shimadzu Co., Kyoto, Giappone). Analytes were separated on a ZB-WAX column 30 m × 0.25 mm ID, 1.00 µm film thickness (Phenomenex, Torrance, CA, USA). Column temperature was held at 40°C for 10 min and increased to 200°C at 3°C min^{-1}. After 3 minutes, the temperature increased to 240°C at 10°C min^{-1} and remained stable for 5 minutes. Helium was used as a carrier gas with a flow of 1 ml min^{-1}. Peaks identification was based on the comparison of their mass spectrum data with the spectra present in the National Institute of Standards and Technology (NIST) library (2008 version). Relative amounts of volatile compounds were expressed respect to internal standard as milligrams per kilogram of oil. Previously, in fact, a calibration curve was carried out by weighing 0.1 g of 1-penten-3-one at different concentrations (0.1, 0.5, 1.0, 5.0 and 10.0 mg kg^{-1}) in a vial and adding deodorized sunflower oil up to 1.0 g.

1.2.5 Fatty acid composition of samples

The fatty acid composition of oil samples was determined as fatty acid methyl esters (FAME) by capillary GC analysis after alkaline treatment. This was obtained by mixing 0.05 g of oil dissolved in 2 mL of *n*-hexane and 1 mL of 2 N potassium hydroxide in methanol. After centrifugation, 1 mL of the upper phase was drawn and diluted with 4 mL of *n*-hexane in a flask. One microlitre was injected into a split (split ratio 1:20) GC port set at 240°C; a fused silica capillary column (30 m length, 0.25 mm i.d.), coated with CPSil-88 (0.25 mm film thickness, Varian, Palo Alto, CA), was utilized. A flow rate of

1.25 mL min^{-1} of helium as a carrier gas was used. The FID was at 240°C. The initial oven temperature was kept at 120°C for 1 min and raised to 240°C at a rate of 4.0°C min^{-1} and maintained for 4 min. For each chemical determination, three replicates were prepared and analyzed per sample.

1.2.6 Statistical treatment of data

The open-source software PanelCheck 1.4.0 version was used to evaluated the assessors and panel performance (Figure 1 and Figure 2). The software XLSTAT 7.5.2 version (Addinsoft) was used to elaborate sensory and volatile mean data by principal components analysis (PCA). Before PCA analysis, the data were standardized, normalized and centered (Figure 3 and Figure 4).

1.3 RESULTS AND DISCUSSIONS

1.3.1 Fatty acid composition of cold-pressed sunflower oils

In the market there are different categories of sunflower oils classified according to the content of oleic acid: high oleic acid (75-91%), mid oleic acid (43-74%) and low oleic sunflower seeds oil (14-42%). All the samples (Table V), except one, fell into the last category of sunflower oils because their content of oleic acid ranged between 31% and 39%, only the sample S7, that showed a content of oleic acid equal to 52.2%, was classified into the category of mid-oleic acid.

Table V - Volatiles and fatty acids of cold-pressed sunflower oils (mean ± standard deviation values) grouped in the main chemical classes. TOT VOs, total volatiles tentatively identified and quantified (more than the classes indicated in the table); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; *Trans* isomers, sum of the *trans* isomers of oleic and linoleic acids.

		S1	S2	S3	S4	S5	S6	S7	S8
Volatile compounds (mg 1-penten-3-one kg^{-1} oil)	*Terpenes*	2.37±0.11	1.72±0.08	1.30±0.08	0.64±0.04	1.97±0.09	<LOQ	<LOQ	0.02±0.00
	Acids	0.37±0.03	0.85±0.04	0.44±0.04	1.29±0.07	0.31±0.01	0.08±0.01	0.09±0.02	0.06±0.01
	Aldehydes	0.30±0.02	0.98±0.08	0.43±0.04	0.75±0.05	0.40±0.03	0.21±0.01	0.18±0.02	0.22±0.01
	Alcohols	0.22±0.01	0.19±0.02	0.24±0.02	0.32±0.01	0.16±0.01	0.04±0.00	0.05±0.01	0.06±0.01
	Ketons	0.04±0.00	0.05±0.00	0.04±0.00	0.07±0.01	0.02±0.01	<LOQ	<LOQ	<LOQ
	Tot VOs	4.06±0.24	4.46±0.26	2.99±0.21	3.39±0.18	3.26±0.13	0.33±0.02	0.31±0.04	0.38±0.02
Fatty Acids (%)	*Oleic*	38.68±0.07	32.47±0.35	33.77±0.29	34.66±0.06	31.66±0.07	33.58±0.02	52.15±0.12	33.10±0.09
	Linoleic	49.24±0.11	55.76±0.30	54.34±0.43	53.44±0.13	55.17±0.06	54.71±0.06	37.25±0.14	54.04±0.14
	Trans Isomers	0.08±0.08	0.09±0.04	0.07±0.04	0.08±0.03	0.10±0.01	0.07±0.02	0.06±0.02	0.13±0.01
	SFA	10.84±0.01	10.69±0.07	10.71±0.02	10.38±0.05	11.93±0.03	10.61±0.09	9.25±0.64	11.11±0.09
	MUFA	39.75±0.05	33.41±0.04	34.82±0.13	35.87±0.15	32.72±0.11	34.55±0.09	53.32±0.34	34.61±0.50
	PUFA	49.41±0.06	55.90±0.10	54.47±0.13	53.75±0.11	55.35±0.14	54.84±0.10	37.43±0.30	54.28±0.41

1.3.2 Fatty acid composition of hazelnut oils

Concerning the fatty acid composition, monounsaturated fatty acids (MUFA) made up the largest portion (from 78.6% to 84.6%) followed by polyunsaturated fatty acids (PUFA) (from 6.2% to 13.4%) (Table VI). Among the MUFA, oleic acid was the predominant in all the hazelnut oils, ranging from 78.2% in sample H10 to 84.1% in sample H1. Samples H9 and H10 showed a fatty acid composition different from the other samples, with a concentration of linoleic (ranged between 11.9-13.3% respect to an average of 8.0%, see Table VI) and linolenic acid (1.2% respect to an average of 0.2%, data not shown) higher than the other samples and, as reported above, a lower percentage of oleic acid.

1.3.3 Evaluation of panel performance and QDA® on samples

The training of the panel has been monitored and evaluated through the use of PanelCheck software: it's a program for rapid, free, and efficient analysis of sensory profiling data, both in the case of one (our case) or multiple panels. The software provides an intuitive and easy-to-use graphical user interface that handles all statistical computations in the background and visualizes results in different types of plots. During the training, several sessions directed by the panel leader were necessary before arriving at the appropriate reproducibility and repeatability of individual tasters.

About sunflower oils, for example, the panel during the training phase had difficulties with regard to the odor attribute of toasted sunflower seeds and hay/grain; while with regard to the analysis carried out on hazelnut oils, the attribute that was reminiscent of burned hazelnut as well as the yellow color resulted in it being the most difficult to evaluate. To cope with disagreements about the yellow colour it was decided to create a colour scale with the oils (as explained below) and, in order to decrease disalignments among the assessors and between replicates, it was decided to taste again and again until they disappeared.

Table VI - Volatiles and fatty acids of cold-pressed hazelnuts oils (mean ± standard deviation values) grouped in the main chemical classes. TOT VOs, total volatiles tentatively identified and quantified (more than the classes indicated in the table); Filbertone, 5-methyl-(*E*)-2-hepten-4-one; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
Volatile compounds (mg 1-penten-3-one kg^{-1} oil)	*Terpenes*	<LOQ	<LOQ	<LOQ	0.01±0.00	<LOQ	<LOQ	<LOQ	<LOQ	0.02±0.00	0.11±0.01
	Pyrazines	<LOQ	1.71±0.02	0.83±0.03	<LOQ	0.65±0.04	<LOQ	0.77±0.03	0.78±0.03	<LOQ	<LOQ
	Acids	0.49±0.04	1.30±0.03	0.93±0.02	0.58±0.07	0.71±0.04	0.32±0.06	0.49±0.01	1.21±0.03	0.13±0.02	0.61±0.09
	Aldehydes	0.20±0.01	0.35±0.03	0.21±0.00	0.24±0.01	0.25±0.03	0.26±0.01	0.35±0.02	0.57±0.02	0.61±0.04	0.26±0.02
	Arom Hydroc	0.04±0.00	0.03±0.01	0.08±0.00	0.14±0.02	0.11±0.01	0.18±0.01	0.06±0.01	0.07±0.00	0.09±0.01	0.06±0.00
	Ketons	0.28±0.02	1.09±0.03	0.63±0.02	0.30±0.03	1.50±0.03	0.38±0.03	1.59±0.02	0.58±0.01	0.06±0.00	0.40±0.03
	Esters	0.01±0.00	0.22±0.02	0.18±0.01	<LOQ	0.24±0.02	0.03±0.01	0.24±0.02	0.22±0.01	<LOQ	0.04±0.01
	Furans	<LOQ	1.19±0.03	0.44±0.00	0.03±0.00	0.31±0.01	0.05±0.00	0.48±0.00	0.40±0.00	0.08±0.00	0.07±0.00
	Filbertone	<LOQ	0.04±0.00	0.04±0.01	<LOQ	0.06±0.00	<LOQ	0.06±0.00	0.06±0.00	<LOQ	<LOQ
	Tot VOs	1.55±0.06	6.39±0.03	3.69±0.04	2.10±0.11	4.38±0.10	1.73±0.07	4.53±0.06	4.79±0.09	1.45±0.05	2.16±0.18
Fatty Acids (%)	*Oleic*	84.10±0.15	81.75±1.85	83.60±0.02	82.66±0.09	81.35±0.07	82.96±0.83	82.71±1.80	83.53±0.21	78.55±0.07	78.22±0.04
	Linoleic	6.32±0.16	7.50±0.15	6.11±0.04	7.02±0.03	8.53±0.07	6.53±0.63	5.92±0.31	7.35±0.11	13.27±0.07	11.89±0.04
	SFA	9.02±0.05	9.70±1.17	9.62±0.02	9.71±0.07	9.49±0.08	9.81±0.23	9.90±0.29	8.55±0.15	7.58±0.01	8.27±0.11
	MUFA	84.58±0.15	82.33±1.83	84.17±0.04	83.19±0.09	81.87±0.06	83.53±0.80	83.24±1.79	83.99±0.25	79.03±0.10	78.64±0.06
	PUFA	6.40±0.18	7.98±0.68	6.21±0.04	7.10±0.03	8.64±0.08	6.66±0.69	6.87±1.50	7.45±0.11	13.39±0.09	13.09±0.05

The case of cold-pressed sunflower oils

The first step was to select and practice an appropriate vocabulary, in order to explain and share the perceived sensations. For this purpose, the panelists worked together, trying attributes appropriate for cold-pressed sunflower oils. The brainstorming activity led to a development of a provisional sheet for the evaluation of the product. In addition, it was arranged for the preparation and definition of reference standards. Through the training of the panel, Table III has come to define the attributes: about the appearance, the only selected attribute was the yellow color, since all the samples were filtered and limpid. As standard references of this descriptor, three samples of sunflower oils with an intensity of yellow equal to 20%, 50% and 80% of the maximum intensity (anchor points) were chosen. Concerning the olfactory sensations (O), the panel distinguished five different descriptors, quantifying them with the appropriate intensity line and with reference to the appropriate standard: hints of raw and toasted sunflower seeds (SS and TSS), hints that remind you of herbs/flower (HF), the smell of grain/hay (GH) and finally, as the main defect, the smell of rancid/fried (RF) were quantified. During the tasting phase, the odor of roasted/burnt seeds sensation (BSS) was also selected. With regards to the taste evaluation (R), attributes were the same of the smelling but, in addition roasted/burnt sunflower seeds (BSS) were also measured. From the elaboration of sensory data with the PanelCheck software, important information about the discriminatory ability and reproducibility of the panel were obtained: to assess these qualities, the three-way ANOVA for each sensory descriptor estimating the effects of the product, the judge and the replicates was used (Figure 1). With such an approach it is possible to identify the descriptors that have no significant effects for the product. In this case, the panel is unable to distinguish between the products for that particular descriptor. If a descriptor does not show significant effects on the court, it can be said that this particular descriptor is well-understood by the panelists and used in a similar way by all the judges. It is important to verify that the replication factor and their interactions are not meaningful to say that the panel has a good reproducibility.

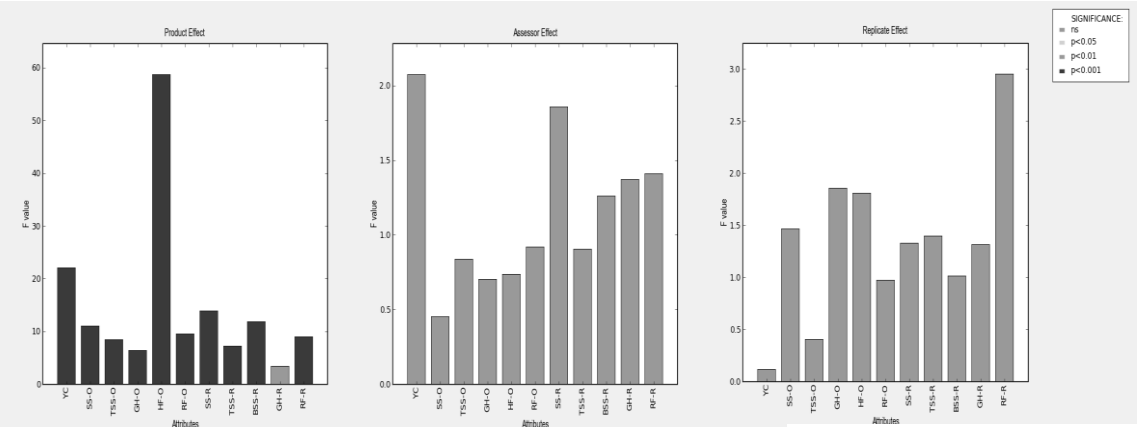

Figure 1 - Evaluation of panel performance by PanelCheck software. 3-way ANOVA of sunflower oil samples about assessor, re-plicate and product. For the meaning of attribute codes see Table III.

The case of cold-pressed hazelnut oils

At present, there are no scientific studies that have carried out the sensory analysis of cold-pressed hazelnut oils by a quantitative descriptive analysis (QDA®). Moreover, only very few studies in the literature have assessed the sensory characteristics of raw and toasted hazelnuts [22, 27, 29, 40]. As a consequence of the training sessions, only the intensity of the yellow color (YC) was selected as an appearance attribute since all the samples collected were limpid. With regard to the odor and the taste, the panel distinguished five different attributes perceived both by orthonasal routes (O) during the smelling phase and by retronasal routes (R) during the tasting phase. Assessors considered as pleasant attributes, the intensity of raw hazelnuts (RH-O, RH-R), the intensity of toasted hazelnuts (TH-O, TH-R) and the intensity of sunflower seeds/grains (SSG-O, SSG-R). Instead, the panelists considered as unpleasant attributes the intensity of rancid/fried oil (RF-O, RF-R) and the intensity of burnt (BH-O, BH-R). Furthermore, the panelists identified two other odor descriptors, that reminds you of coffee and cocoa, and were evaluated only as “present” or “absent”. Special references (Table IV) of known flavors were selected to have standards for the training and calibration of the panel and to make an unambiguous assignment of the sensations and possible attributes. The data obtained from sensory analysis were processed with the PanelCheck software: between the many graphs established by the program the Tucker-1 was selected (Figure 2). The test result Tucker-1 is a two-dimensional graph, which provides a visual representation of the

level of agreement among the judges and an estimate of the information that each provides to describe the products and differentiate them. The graph shows two ellipses: inside ellipse represents 50% of explained variance between the samples, the outer ellipse represents 100% of the explained variance. The more a judge is positioned near the outer ellipse, the greater is the information that the model derives from its assessments to describe the samples. Moreover, the more the judges are positioned near one another, in a limited space, the greater is the agreement within the panel in the use of that specific descriptor. This graph has the feature to provide information on the reproducibility of the panel and provides information on the systematic variation for each combination judge-attribute.

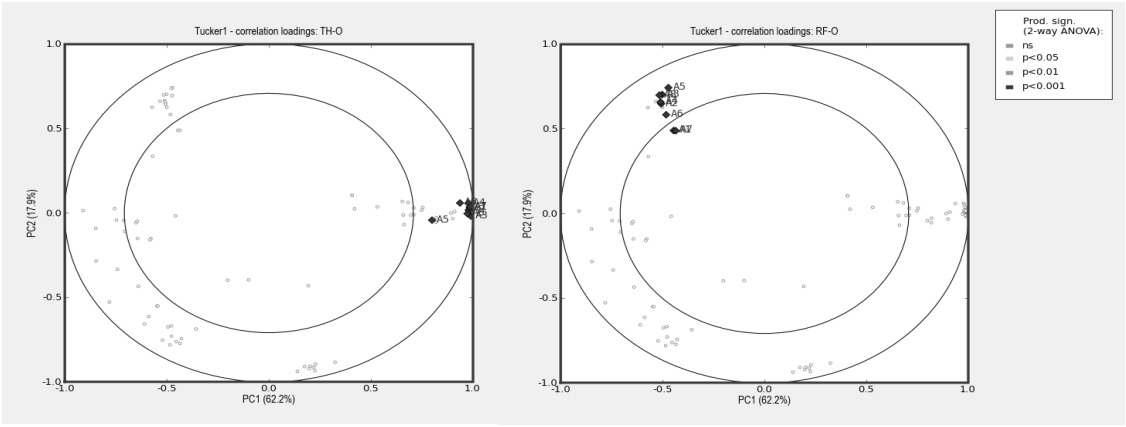

Figure 2 - Evaluation of panel performance by PanelCheck software. Consensus among the assessors (A) of hazelnuts oil samples. For the meaning of attributes codes see Table IV.

1.3.4 Conjoint evaluation between sensory characteristics and volatile profile of samples

In general, PCA elaboration shows comparison of multidimensionally expressed sensory/chemical quality of several samples (products), projected on a two-dimensional space (surface), described by two orthogonal factors used as dimensions (principal components – PC): PC1 and PC2. Percentages indicate what % of evaluated product variability is related to each PC. The sensory attributes and chemical parameters are shown as vectors: the length of each vector expresses the degree of variability of the attribute/parameter (among evaluated products). The mutual direction of attribute/parameter vectors means their positive correlation if close to each other while

negative correlation are expected if the vectors go in opposite direction. No significant correlations exist when they are perpendicular [15].

The Figure 3 and Figure 4 show the distribution of the cold-pressed sunflower and hazelnut cold-pressed oils, respectively, in the factors plane, built using both sensory and chemical variables (in this case volatile compounds).

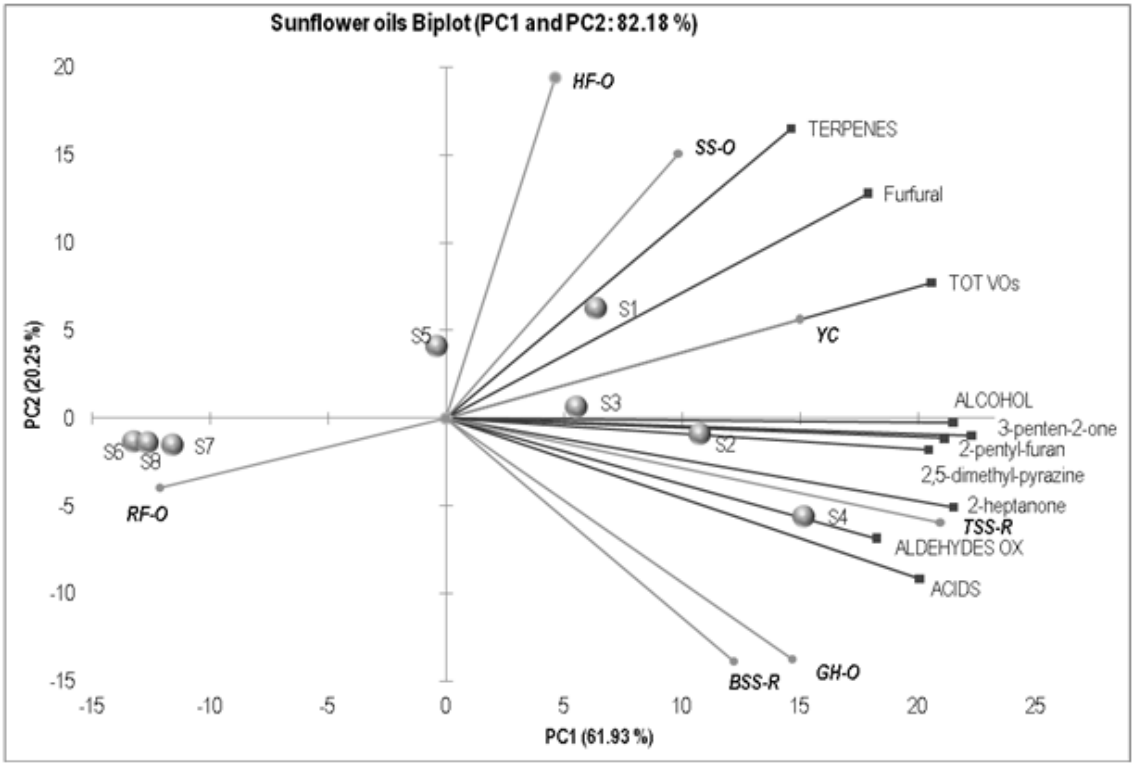

Figure 3 PCA about sensory and volatile data of sunflower oil samples. For the meaning of various codes see Table I and Table III.

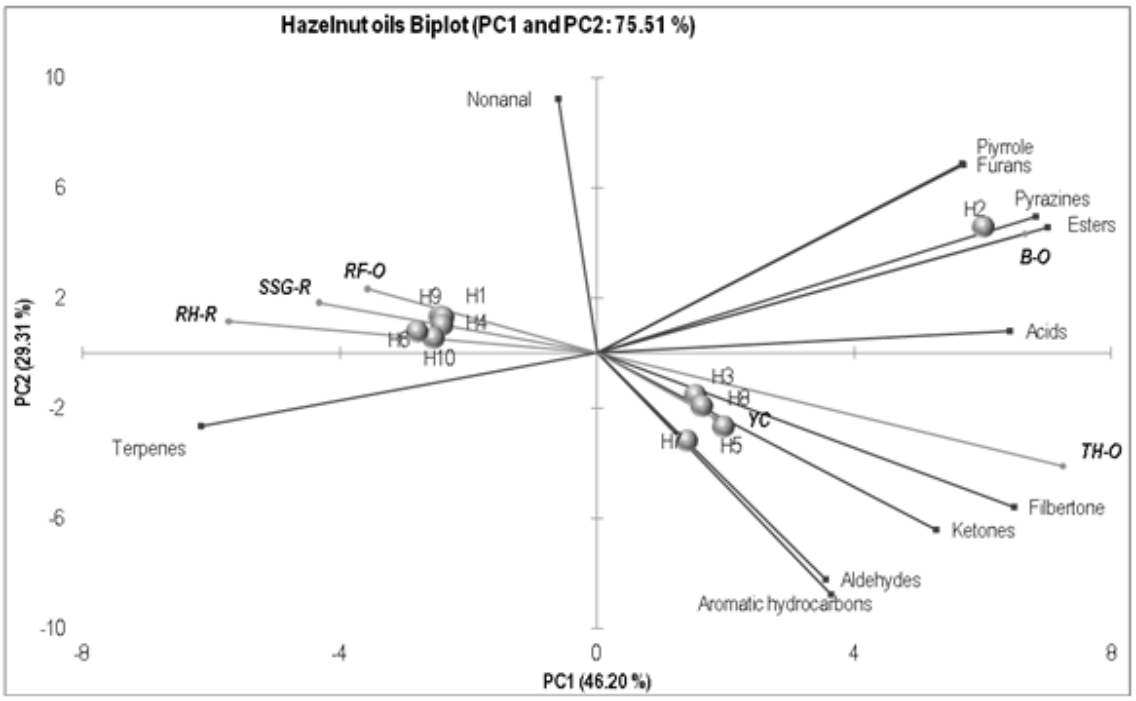

Figure 4 - PCA about sensory and volatile data of hazelnuts oil samples. For the meaning of various codes see Table II and Table IV.

The case of cold-pressed sunflower oils

In addition to sensory analysis the analysis of volatile compounds was carried out. This analysis allowed you to tentatively identify and quantify 41 compounds that were grouped in chemical classes (terpenes, acids, aldehydes, alcohols and ketones); the total content of volatiles was also calculated (Table V). Subsequently, a selection of significant compounds and classes through the analysis of the cosine squared of the variables was carried out. The sensory results and the selected volatile compounds were analyzed by PCA, in order to perform a characterization of the samples according to these variables and to check eventual correlations among them.

The first two components were responsible for 82.18 % of variance (61.93% for PC1 and 20.25% for PC2).

As shown in Figure 3, it is possible to highlight that PC1 was associated, in the positive direction, with all components of the analysis of volatile compounds (class of terpenes, furfural, 3-penten-2-one, 2-pentyl-furan, 2,5-dimethyl-pyrazine, class of alcohols, 2-heptanone, class of aldehydes and class of acids) and the attributes of the sensory analysis

such as: herb/flower O (HF-O), raw sunflower seeds O (SS-O), yellow colour (YC), toasted sunflower seeds G (TSS-R), grain/hay O (GH-O) and roasted/burnt sunflower seeds G (BSS-R); the only attribute on the negative direction of PC1 is the rancid/fried O (RF-O). Concerning the association of values in PC2, in the positive direction we found: herb/flower O (HF-O), raw sunflower seeds O (SS-O) and yellow colour (YC) regarding the sensory analysis, together with the class of terpenes and furfural. All the rest of the variables had negative values with regard to PC2. The approximate position of the product close to certain sensory attribute/chemical parameter vector(s) allows you to conclude that the product was particularly characterized by this/these attribute/chemical parameter(s); therefore, two samples (S1 and S3) present in the first quadrant (positive values for PC1 and positive value for PC2) were characterized, above all, by the presence of raw sunflower seeds attributes (SS-O), as well as by a higher content of terpenes and furfural, but, also, by the absence of notes resembling toasted sunflower seeds (TSS-R), grain/hay (GH-O), roasted/burnt sunflower seeds (BSS-R) and rancid/fried flavor (RF-O). The sample S2 is located between the first and second quadrant (positive values for PC1 and negative value for PC2), this tells us that resulted characterized by a high presence of 3-penten-2-one, 2-pentyl-furan, 2,5-dimethyl-pyrazine and the class of alcohols, while the flavor and the odor of rancid/fried oil (RF-O) were absent. The sample S5 is located between the first and fourth one, so it was characterized by a high present of hints of herbs/flower (HF-O). The sample S4 is located in the second quadrant, so the odor and the flavor of toasted sunflower seeds (TSS-R), grain/hay (GH-O), the flavor of roasted/burnt sunflower seeds (BSS-R) and the presence of 2-heptanone and of the class of aldehydes and acids were the most peculiar traits. The other three samples (S6, S7 and S8) are located in the third quadrant (negative values for PC1 and negative values for PC2), so the rancid/fried odor and flavor (RF-O) was their main characteristic. All the three samples are similar because they were washed with water vapor, this means that the oil has lost its peculiar olfactory attributes resulting more neutral from the sensorial point of view.

The case of cold-pressed hazelnut oils

Concerning the volatile compounds of hazelnut oils, about 80 compounds were tentatively identified and many of these were grouped in different chemical classes: furans, pyrazines, esters, ketones, aldehydes, aromatic hydrocarbons, alcohols and terpenes (Table VI). Sensory results and the content of volatile compounds were analyzed by PCA to perform a characterization of the samples according to these variables and to check eventual correlations among them. The first two components were responsible for 75.51% of variance (46.20% for PC1 and 29.31% for PC2). As shown in Figure 4, it is possible to highlight that PC1 was associated, in the positive direction, to attributes of yellow color (YC), toasted (TH-O) and burnt (BH-O) hazelnuts both by orthonasal perceptions and in the negative direction to attributes of rancid/fried oil by orthonasal perceptions (RF-O), raw hazelnuts (RH-R) and sunflower seeds/grains (SSG-R) both by retronasal perceptions. In particular, PC1 was associated with the positive direction to the class of acids, ketones, esters, pyrazines, aldehydes, furans, aromatic hydrocarbons. Moreover, the "filbertone" (5-Methyl-(*E*)-2-hepten-4-one) was considered separately from the class of ketones as it has been recognized as one of the main compounds characterizing toasted hazelnuts. In fact, a lot of studies showed that the content of "filbertone", responsible for the typical hazelnut smelling, increases substantially after the roasting, but its precursors are yet unknown [22, 28, 29]. Concerning the location of products on the PC1/PC2 surface, if close to each other it means that those products are similar (taking into account the combination of all the evaluated attributes), if they are far away from each other it means that they differ strongly. The approximate position of the product near certain attribute/chemical parameter vector(s) allows you to conclude that the product has this attribute/chemical parameter(s) particularly expressed. Therefore, five samples (H1, H4, H6, H9 and H10) present in the fourth quadrant (negative values of PC1 and positive of PC2) were characterized, above all, the presence of raw hazelnuts (RH-R) and sunflower seeds/grains (SSG-R) attributes, as well as by a higher content of terpenes, which are typical compounds of sunflower seeds [15]. However, for H6 and H9 samples the presence of a light note of rancid/fried (RF-O) was also detected. The presence of H3, H5, H7 and H8 samples in the second quadrant was due to the high intensity of toasted hazelnuts notes. About volatile compounds, a high content of ketones and in particular of "filbertone" (5-Methyl-(*E*)-2-hepten-4-one) resulted peculiar of these

samples. In addition to filbertone other ketones may play important roles in hazelnut aroma. For example, also the compound (*E*)-3-penten-2-one was reported to be responsible for a fruity odor in roasted hazelnut [29]. Furthermore, the sample H7 showed the higher content of aldehydes and aromatic hydrocarbons (Table VI). The total content of aldehydes increases during roasting because of the Strecker degradation [27]. Among these, 2- and 3-methylbutanal were reported to be responsible for fruity, malty, nutty, and chocolate-like odors in roasted hazelnuts [29]. This can explain why the attributes of coffee and cocoa have been evaluated with greater frequency in these samples. Only one sample, H2, was present in the first quadrant due to the burnt note and the high content of pyrazines, furans and esters. In fact, pyrroles, pyrazines and furans are formed through the Maillard reaction during the roasting process. They possess mostly burnt aroma notes and are found among the volatiles of most heated foods [29].

1.4 CONCLUSIONS

Considering the results discussed above, some conclusions can be drawn. The attributes of sunflower seeds and herbs/flowers together with the terpenic content could be considered the vectors which are able to qualify the good sensory quality of cold pressed/virgin sunflower oils. The toasted sunflower seeds can also be perceived as a positive note if not combined with the roasted/burnt seeds sensation. In fact, excessive heating during the oil extraction process can lead to the formation of several Maillard reaction products that, as it is well known, can be responsible for odor notes resembling roasted/burnt and also for the darkening of the yellow color. The treatment of cold-pressed oil with water vapor seems to significantly reduce the total volatile compounds and, in particular, the molecules responsible for peculiar and positive notes; at the same time, these more neutral oils were affected by the sensory defect of rancid/fried linked to the lipid oxidation. Toasted and raw hazelnuts attributes were not related to each other; mainly on the basis of these two attributes, a clear separation of the cold-pressed hazelnut samples into two different groups was carried out. The different classes of volatile compounds were also useful for this purpose. In fact, the content of “filbertone” (and the sum of ketones) were positively related with the attribute of toasted hazelnuts, while terpenes were linked to the attributes of raw hazelnuts and sunflower seeds/grains.

Finally, the burnt note was found to have a greater intensity in one sample, resulting in a correlation with a high content of pyrazines, pyrroles, furans and esters compounds that originated from the roasting processes.

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Conflict of interest

The authors have no conflict of interest to declare.

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Chapter 2

Rapid direct analysis to discriminate geographic origin of extra virgin olive oils by flash gas chromatography electronic nose and chemometrics

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ABSTRACT

At present, the geographical origin of extra virgin olive oils can be ensured by documented traceability, although chemical analysis may add information that is useful for possible confirmation. This preliminary study investigated the effectiveness of flash gas chromatography electronic nose and multivariate data analysis to perform rapid screening of commercial extra virgin olive oils characterized by a different geographical origin declared in the label. A comparison with solid phase micro extraction coupled to gas chromatography mass spectrometry was also performed. The new method is suitable to verify the geographic origin of extra virgin olive oils based on principal components analysis and discriminant analysis applied to the volatile profile of the headspace as a fingerprint. The selected variables were suitable in discriminating between "100% Italian" and "non-100% Italian" oils. Partial least squares discriminant analysis also allowed prediction of the degree of membership of unknown samples to the classes examined.

Keywords: Extra virgin olive oil; Geographic origin; FGC E-nose;SPME/GC-MS; Headspace volatile compounds; Non-target analysis; PCA; PLS-DA

2.1 INTRODUCTION

In an increasingly globalized world, certification of food quality is one of the most important goals for scientists in the agri-food sector. Consumer demand of traceability and authenticity of food products is also increasing, and the international agencies dealing with food quality have recently published specific guidelines in this regard (FAO, 2003). Extra virgin olive oil (EVOO) is a typical Mediterranean food product characterized by a multi-millenary tradition that arouses great appreciation among consumers. Within the Mediterranean basin, Italy is a key producer of olive oil. The vast economic interests may give rise to illegal activities aimed to increase profit, such as a false declaration of geographic origin, thus falsifying traceability and, consequently, authenticity of the product. The European Union (EU) has recently concluded a decennial *iter* to establish regulations about olive oil with the aim of regulating production and commercialization of this important product. Regulation EU No. 1019/02 defined how to correctly pack and label oils, and the last Commission Implementing Regulation, 2013 EU No. 1335/13 made it obligatory to indicate the geographic origin on the label. In EU Regulation No. 29/2012 (European Commission Implementing Regulation, 2012), it is reported that in order to ensure that consumers are not misled and the olive oil market is not distraught, information concerning the geographic area in which olives are harvested and olive oil is obtained should be stated on the packaging or labels. For greater clarification, the document also defines that simple provisions as 'blend of olive oils of European Union origin' or 'blend of olive oils not of European Union origin' or 'blend of olive oils of European Union origin and not of European Union origin' should be stated for labeling of origin.

The mandatory necessity of certifying the geographical origin makes it highly desirable to assess origin not only by documentation of verification, but also by rapid analytical methods. In this regard, it is necessary to apply high performance instrumental analytical methods, and the large number of variables imposes the use of chemometrics, whose outputs provide useful and easy-to-visualize information extracted from data while simultaneously discarding useless information (analytical noise and redundant information).

There is an urgent need to extend the representativeness of a database established on

chromatographic, spectroscopic, and spectrometric compositional data profiles to clearly identify the most promising techniques in order to confirm the geographic origin of EVOOs and verify the conformity of label-declared geographic origin, as well as to provide one or more harmonized methods for sharing markers that are useful to check the product's conformity to specific standards (e.g., geographic origin). All the factors identified by compositional analysis of EVOOs are important. Mass spectrometry together with various spectrometric and chromatographic analytical techniques have been applied to determine the chemical composition, and many of these instrumental analytical techniques have been used in tandem with chemometrics (Gouvinhas, De Almeida, Carvalho, Machado, & Barros, 2015; Azizian et al., 2015; Mendes et al., 2015; Sinelli et al., 2010; Diraman & Dibeklioğlu, 2009). In this context, adulteration of EVOOs has been studied by liquid chromatography (HPLC), gas chromatography (GC), and linear discriminant analysis (LDA) using fatty acids (FA) and triacylglycerols (TGs) as markers (Ollivier, Artaud, Pinatel, Durbec, & Guerere, 2006; Jabeur et al., 2014). HPLC-mass spectrometry (MS) and LDA allowed determination of the phenolic profile for discrimination of geographical origin (Taamalli, Arráez Román, Zarrouk, Segura-Carretero, & Fernández-Gutiérrez, 2012). In particular, specific volatile compounds or their classes (e.g., terpenoid compounds) have been used to discriminate EVOO samples according to geographic origin (Ben Temime, Campeol, Cioni, Daoud, & Zarrouk, 2006; Cecchi & Alfei, 2013; Vichi, Pizzale, Conte, Buxaderas, & Lopez-Tamames,, 2003; Zunin, Boggia, Salvadeo, & Evangelisti, 2005). Many EVOOs have also been classified according to their geographic origin using the combination of FA and/or TG profiles with other compounds such as sterols, polyphenols, and volatiles using conventional and new analytical approaches, as recently reviewed (Gallina Toschi, Bendini, Lozano-Sanchez, Segura-Carretero, & Conte, 2013; García-González, Luna, Morales, & Aparicio, 2009). Several publications have described the use of volatile-species distribution as a fingerprint to assess traceability, authentication, and non-degradation based on head-space sampling and GC in tandem with several chemometric tools: analysis of variance (ANOVA) and correlation analysis (Cecchi & Alfei, 2013); principal components analysis (PCA) (Cimato et al., 2006); LDA (Pouliarekou et al., 2011); PCA and hierarchical clustering analysis (HCA) (Procida, Giomo, Cichelli, & Conte, 2005).

Among the chemical species in EVOO, many volatiles have been related with specific

sensory characteristics (Aparicio, Morales, & Alonso, 1996; Cerretani, Salvador, Bendini, & Fregapane, 2008). Over the last decade, "e-sensing" technologies have undergone important developments from a technical and commercial point of view, and electronic noses have been designed to mimic the human sense of olfaction in order to detect and recognize flavors and off-flavors in different food matrices (Peng, Tian, Chen, Li, & Gao, 2015). Moreover, the electronic nose results have been successfully correlated to those obtained with other techniques (sensory, GC, and GC–MS) (Mildner-Szkudlarz & Jelen, 2008; Lerma-García et al., 2010).

In a traditional multivariate approach, the variables are concentrations of several compounds: this means that the scientist chooses beforehand which chemical species are relevant; in contrast, when tools like PCA or partial least squares discriminant analysis (PLS-DA) are applied to *full chromatograms*, there is no risk to discard species with retention times not corresponding to chemical species already known to influence EVOO quality. The advantages of such an approach have recently been described (Melucci et al., 2013).

The aim of this study was to analyze the headspace profile of commercial EVOOs with different geographic origin using electronic nose with ultra fast gas chromatography (FGC E-nose), which is able to perform the separation on two short columns of different polarities working in parallel and detect analytes with a flame ionization detector (FID). The FGC E-nose was used to discriminate between products labeled as "100% Italian EVOO" and "non-100% Italian"coming from other countries in the EU, and in particular Spain and Greece. PCA, LDA, and HCA were applied as exploratory tools. Data processing was initially applied to datasets made from peak areas at retention times corresponding to significant species; in this case, a comparison between the non-target analysis performed by FGC E-nose and SPME/GC–MS achieved two purposes: (i) to demonstrate that the discriminating power of FGC E-nose was comparable with SPME/GC–MS; (ii) to assign FGC E-nose retention times to specific volatile compounds. In a second step, the full chromatograms, obtained on two different sets of samples analyzed in two different laboratories, were processed by applying PLS-DA as a chemometric tool.

2.2 MATERIAL AND METHODS

2.2.1 Samples

The two sets of samples named Set A and Set B were formed by 27 and 251 EVOOs, respectively, and were collected from COOP Italia before distribution by the supermarket chain (COOP Italia is a consortium that acts as a central retailer and is one of the most important supermarket chains in Italy; it also carries out marketing activities and performs quality control). Set A was composed of 5 PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication) Italian samples, 13 samples declared as produced and processed exclusively in Italy (100% Italian, I code), and 9 samples produced in countries which are members of the European Union (Mixtures, M code). All samples in Set A were collected during the 2012–2013 harvest period. Set B included 132 samples labeled as 100% Italian (I) and 119 samples labeled as non-100% Italian (M) EVOOs collected during the 2013–2014 harvest period. Even if the actual identity of the samples was confidential, all the olive oils were bottled (in dark or transparent glass bottles) in Italy. Moreover, samples considered as 100% Italian were assumed to be as declared, according to specific quality control checks, and based on chemometric control with single-class PCA models and Hotelling analysis for outliers elimination applied to confirm the geographic class. All samples were stored at 10 °C in darkness before analysis.

2.2.2 Sensory evaluation

A IOC panel test method was carried out on samples in Set A by a group of 8 selected trained assessors, all members of the Professional Committee DiSTAL. Sample evaluation was performed according to the official procedure (Reg. (EC) 640/2008). Moreover, the presence of green notes and other positive attributes were evaluated with reference to the list of descriptors for PDO EVOOs developed and agreed by the International Olive Oil Council, 2005 (IOOC/T.20/Doc. No. 22, 2005).

2.2.3 FGC E-Nose

The same type of FGC E-nose Heracles II (AlphaMos, Toulouse, France) was used for both sets of samples but in two different laboratories (Set A was analyzed in Toulouse, Set B in the laboratory of COOP in Bologna, Italy). The Heracles II was equipped with two columns working in parallel mode: a non-polar column (MXT5: 5% diphenyl, 95% methylpolysiloxane, 10 m length and 180 μm diameter) and a slightly polar column (MXT1701: 14% cyanopropylphenyl, 86% methylpolysiloxane, 10 m length and 180 μm diameter). A single comprehensive chromatogram was created by joining the chromatograms obtained with the two columns; such an approach may help in preventing/reducing incorrect identifications due to overlapping of chromatograms obtained with two different columns, and represents a useful tool for improved identification. An aliquot of each sample (2 g ± 1%) was placed in a 20 mL vial and sealed with a magnetic plug. The vial was placed in the Heracles' auto-sampler, which placed it in a shaker oven where it remained for 20 min at 50 °C, shaken at 500 rpm. Next, a syringe pierced the silicone septum of the magnetic plug and sampled 5 ml of the head space. Prior to the chromatographic separation, the 5-ml headspace aliquot was adsorbed on a CARBOWAX trap maintained at 40 °C for 65 s while the carrier gas (H_2) flowed through it in order to concentrate the analytes and to remove excess air and moisture. Subsequently, desorption was obtained by increasing the temperature of the trap up to 240 °C in 93 s and the sample was injected. The thermal program started at 40 °C (held for 2 s) and increased up to 270 °C at 3 °C s^{-1}; the final temperature was held for 21 s. The total separation time was 100 s. At the end of each column, a FID detector was placed and the acquired signal was digitalized every 0.01 s. For calibration, an alkane solution (from *n*-hexane to *n*-hexadecane) was used to convert retention time in Kovats indices and identify the volatile compounds using specific software (AromaChemBase). Samples were analyzed in triplicate or quadruplicate for both Set A and Set B.

2.2.4 SPME/GC–MS

The headspace composition was investigated by SPME coupled to GC separation and MS detection. This same analysis was performed in two different laboratories: samples in Set A were analyzed at the University of Bologna (Italy), whereas the laboratory of the University of Barcelona (Spain) performed analysis on Set B. The same kind of

instrument, a gas chromatograph Agilent 6890 N Network and a quadrupolar mass-selective spectrometry Agilent 5973 Network detector (Agilent Technologies, Palo Alto, CA, USA), provided with a split–splitless injection port and helium as the carrier gas (linear velocity of 17 cm s^{-1}) was used. Slight differences in analytical conditions were applied.

For analysis of Set A: SPME was carried out by weighing 1.5 g of sample, spiked with 4-methyl-2-pentanone (internal standard dissolved in refined sunflower oil) to a concentration of 10 mg kg^{-1} in a 10 mL vial fitted with a silicone septum. The vial was placed in a water bath at 40 °C and maintaining the oil sample under magnetic stirring for 2 min (conditioning) and then a DVB/CAR/PDMS fiber (50/30 μm, 2 cm long from Supelco Ltd., Bellefonte, PA) was exposed for 30 min in the headspace of the sample. After exposition, the fiber was retracted into the needle and immediately desorbed for 3 min in the injection port of a gas chromatograph (250 °C). Compounds were separated on a ZB-WAX column 30 m, 0.25 mm ID, 1.00 μm film thickness (Chemtek Analytic, Bologna, Italy). Column temperature was held at 40 °C for 10 min and increased to 200 °C at 3 °C min^{-1}. The ion source and transfer line were at 180 °C and 230 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy in the 20–250 amu mass range, 2 scans s^{-1}.

For analysis of Set B, SPME extraction was performed according to Vichi et al. (2003) and differed from the method applied for Set A only for use of a different internal standard, 4-methyl-2-pentanol (Sigma–Aldrich, St. Louis, MO). The fiber was then desorbed at 260 °C in the gas chromatograph injection port for 5 min. Separation of compounds was performed on two columns with distinct polarity: Supelcowax-10 and Equity-5 (both 30 m x × 0.25 mm I.D., 0.25 μm film thickness), both purchased from Supelco (Supelco Ltd., Bellefonte, PA, USA). The column temperature was held at 40 °C for 5 min and increased to 200 °C at 4 °C min^{-1}. The injector temperature was 260 °C, and the transfer line temperature was 280 °C. Electron impact mass spectra were recorded at 70 eV ionization energy in the 30–300 amu mass range, 2 scans s^{-1}.

Identification of volatile compounds was mainly carried out by a comparison of mass spectral data with information from the National Institute of Standards and Technology (NIST) library (2005 version) and checked with pure standards. Linear retention indexes

were also calculated and compared with those available in the literature. Relative amounts of volatile compounds were expressed as mg of internal standard per kg of oil, applying a response factor of 1. All determinations were carried out in triplicate or duplicate for Set A and Set B, respectively.

2.2.5 Software

The FGC E-nose data processing was carried out with Alphasoft V12.44 and AroChembase software. XLSTAT version 2011.1.03 software (Addinsoft, USA) was used to elaborate ANOVA and PCA on Set A. Preliminary PCA on Set B and PLS-DA were performed using The Unscrambler version 9.8 (CAMO, Norway).

2.2.6 Chemometrics

In this work, a first explorative step was carried out using peak areas that were automatically calculated by the software that controls each instrument. All data based on peak area were pre-processed by autoscaling.

Principal component analysis is a well-known chemometric procedure which rotates the original space to another one whose versors are the principal components (PCs) oriented along directions containing the maximum explained variance (EV) and mutually orthogonal. Score and loadings plots are obtained, allowing for easy visualization of samples and variables and verification of their role in the analytical problem. Hotelling analysis, applied to PCA scores, calculates the covariance ellipsoid corresponding to 95% confidence level (and visually draws it on the scores plot); therefore, samples falling outside of the ellipsoid are those in the multivariate Gaussian tails and may be considered outliers and discarded from further analyses. Linear discriminant analysis is a multivariate classification tool which rotates the original space, but unlike PCA its aim is to maximize separation between classes, minimizing at the same time distances between objects in the same class; in this way, new objects may be projected onto this new scores space and assigned to one of the classes of the training set. HCA may also be applied to identify eventual sub-classes by calculating multidimensional Euclidean distances between objects and grouping those closest to each other. In the present investigation, it was highly expected that various sub-categories may be included in the very broad category "non-100% Italian" (M, for example mixtures from Spain, Greece, Italy).

Once the preliminary exploration by PCA, HCA, and LDA was completed, the work was extended by creating models, or equations involving experimental variables. A very useful response variable is the degree of belonging of objects to the possible classes involved in the analytical problem. The main interest was in quantifying the degree of belonging to class I (y_I) and the degree of belonging to class M (y_M). Few tens of objects are available while up to thousands of variables (digitized signal) are generated by a FGC E-nose chromatogram. Thus, the only adequate modeling tool is PLS regression (in particular, PLS-DA), which exploits PCs and maximizes both EV and correlation between regressors (the variables, that is the chromatographic signals at various retention times) and the response (degree of belonging, y). The choice of using full chromatograms has important advantages: (i) no pre-selection of significant retention times is needed, thus by-passing the non-target character of FID signals; when no pre-selection is done, the risk of discarding useful information is avoided; (ii) errors related to incorrect integration in peak-area calculation are avoided. Of course, some disadvantages must also be considered when using whole chromatograms as predictors: a number of correlated variables much higher than the number of objects may lead to overfitting, which provides modeling noise instead of useful information. However, chemometric modeling tools offer reliable methods for controlling these problems to obtain good performance of PLS-regression, based on objective measures: in particular, root mean square error (RMSE) and correlation coefficients. Predictive ability (also for LDA) was evaluated by the well-known cross-validation (CV) procedure (Brereton, 2007).

2.3. RESULTS AND DISCUSSION

2.3.1 Explorative analysis of sample Sets A and B

The exploration of Set A was considered as a preliminary step in the method development as it was the first to be analyzed and consequently taken into account to better define a chemometric approach for discriminating such a large number of olive oil samples subsequently studied. This first set of 27 samples was very useful for exploring Set B in depth and in establishing the method.

2.3.2 PCA from SPME/GC–MS peak areas of Set A

According to the sensory analysis performed by IOC panel test method, the 27 samples of Set A were classified as EVOO (8 samples) and VOO (19 samples); for EVOOs, the intensity of fruity was light (4 samples) and medium (4 samples), and the presence of secondary notes (olfactory and gustatory sensations) of almond, tomato, and grass was also found. The VOOs showed several sensory defects, although "fusty-muddy" (off-flavor of oils from olives stored in large amounts for many days before processing, or of oils left in contact with the sediment for a long period of time, both leading to anaerobic fermentation) was the most common. Other sensory defects found in VOO samples were rancid and winey-vinegary.

The volatile compounds identified and quantified in the headspace of the analyzed samples by SPME/GC–MS are reported in Fig. 1, which shows an overlap between SPME/GC–MS traces relative to the profiles in volatiles molecules for M15 (mixture, non-100% Italian), I13 (100% Italian), and I23 (Italian PDO) samples. It is interesting to note that the non-100% Italian sample (M15) showed a high content of C_6 lipoxygenase (LOX) esters (hexylacetate and (*Z*)-3-hexenylacetate), which contribute to the positive sensory notes of "sweet","fruity", and "banana-like" (Kalua et al., 2007) and, on the other hand, a tendency towards a lower content in (*E*)-2-hexenal and (*E*)-2-hexenol, both positively correlated with green sensory attributes such as "freshly cut grass", "bitter almond", and "leaves" (Angerosa, 2002; Morales, Luna, & Aparicio, 2005). Moreover, a larger peak of a compound tentatively identified as dodecene could be observed (see also Fig. 2). Generally, samples I13 and I23, respectively, 100% Italian and Italian PDO, were characterized by a major richness in compounds derived from the secondary pathway of LOX (i.e., C_5 molecules and pentene dimers).

Volatile data obtained from SPME/GC–MS were elaborated by PCA to compare the profile of volatile compounds (Fig. 2). A selection of the most discriminant volatile compounds obtained by ANOVA was performed to improve separation among samples. The first two components explained 81% of total variance (48% for the first latent variable and 33% for the second). Considering the locations of products on the PCA scores plot, it is possible to point out that the non-100% Italian samples (M) were grouped in a cluster located in the quadrant of negative values of PC1 and positive values of PC2, whereas Italian samples (100% Italian and Italian PDO/PGI, I) were concentrated mainly between the two quadrants corresponding to negative values of PC2. The different direction/location of vectors (PCA loadings) shows which molecules were involved in the aroma variations among samples, according to the previous explanation. This statistical elaboration allowed to discriminate the samples according to their different geographic origin (non-100% Italian vs. Italian), but not in terms of sensory quality: in fact, each cluster contains both VOOs and EVOOs. The application of FGC E-nose on the set of samples allowed hypothetical identification of 25 different compounds based on Kovats retention indices and the AroChembase software equipped with a library built on the scientific literature to display the associated sensory features.

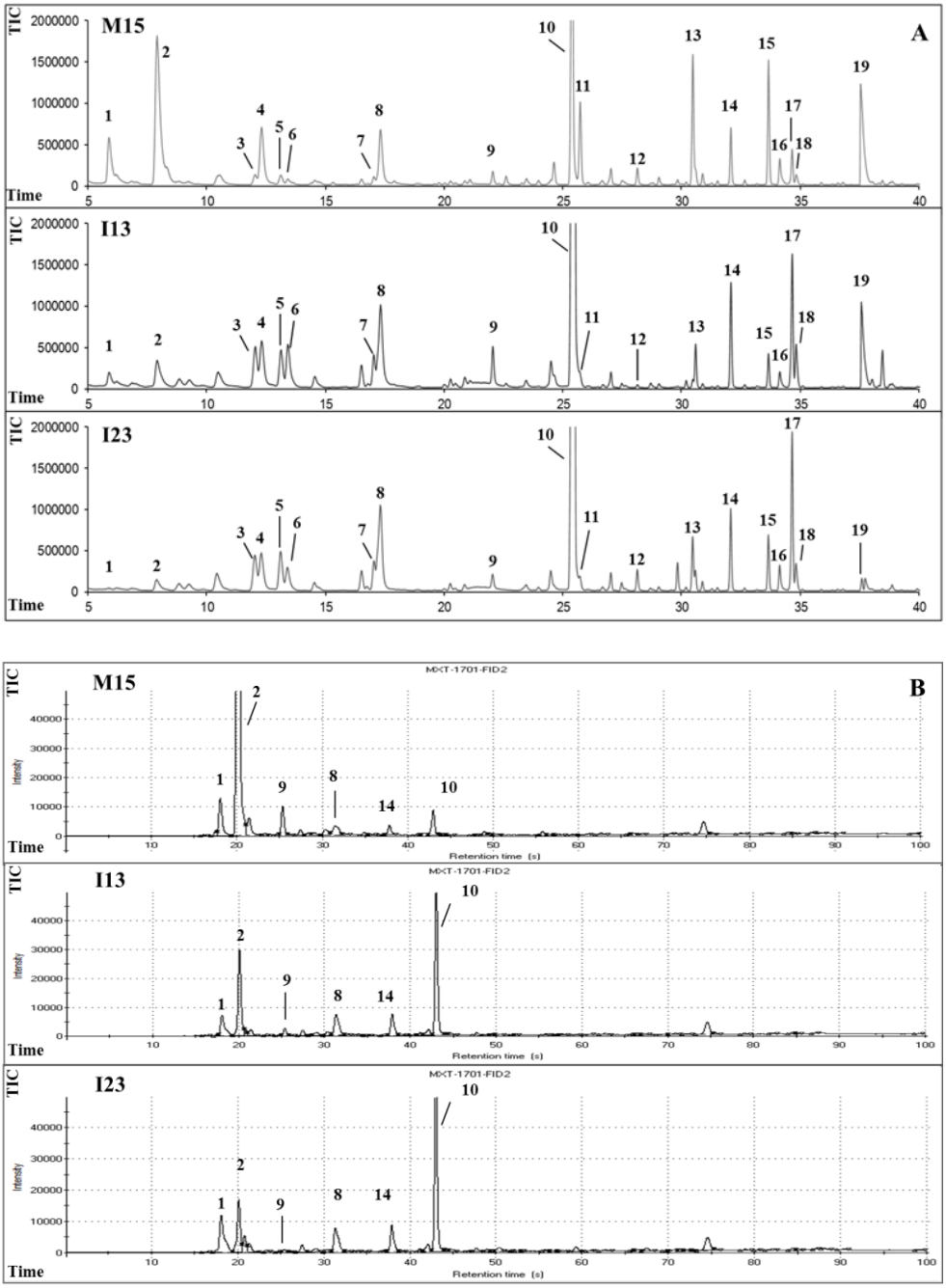

Fig. 1 (A) Overlapping of volatile GC traces obtained by SPME/GC–MS analysis (Set A). Samples: M15 (non-100% Italian), I13 and I23 (100% Italian). Peaks are reported in order of elution: 1: ethyl acetate; 2: ethanol; 3: 3 ethyl-1,5-octadiene (I); 4: IS; 5: 3 ethyl-1,5-octadiene (II); 6: 1-penten-3-one; 7: 4,8-dimethyl-1,7-nonadiene; 8: hexanal; 9: 1-penten-3-ol; 10: (E)-2-hexenal; 11: 1-dodecene; 12: hexylacetate; 13: (Z)-3-hexenylacetate; 14: hexanol; 15: (Z)-3-hexenol; 16: nonanal; 17: (E)-2-hexenol; 18: (E,E)-2,4-hexadienal; 19: acetic acid. (B) Overlapping of sensors (volatiles) as detected by FGC ENose (Set A). Samples and peak numbers according to the (A).

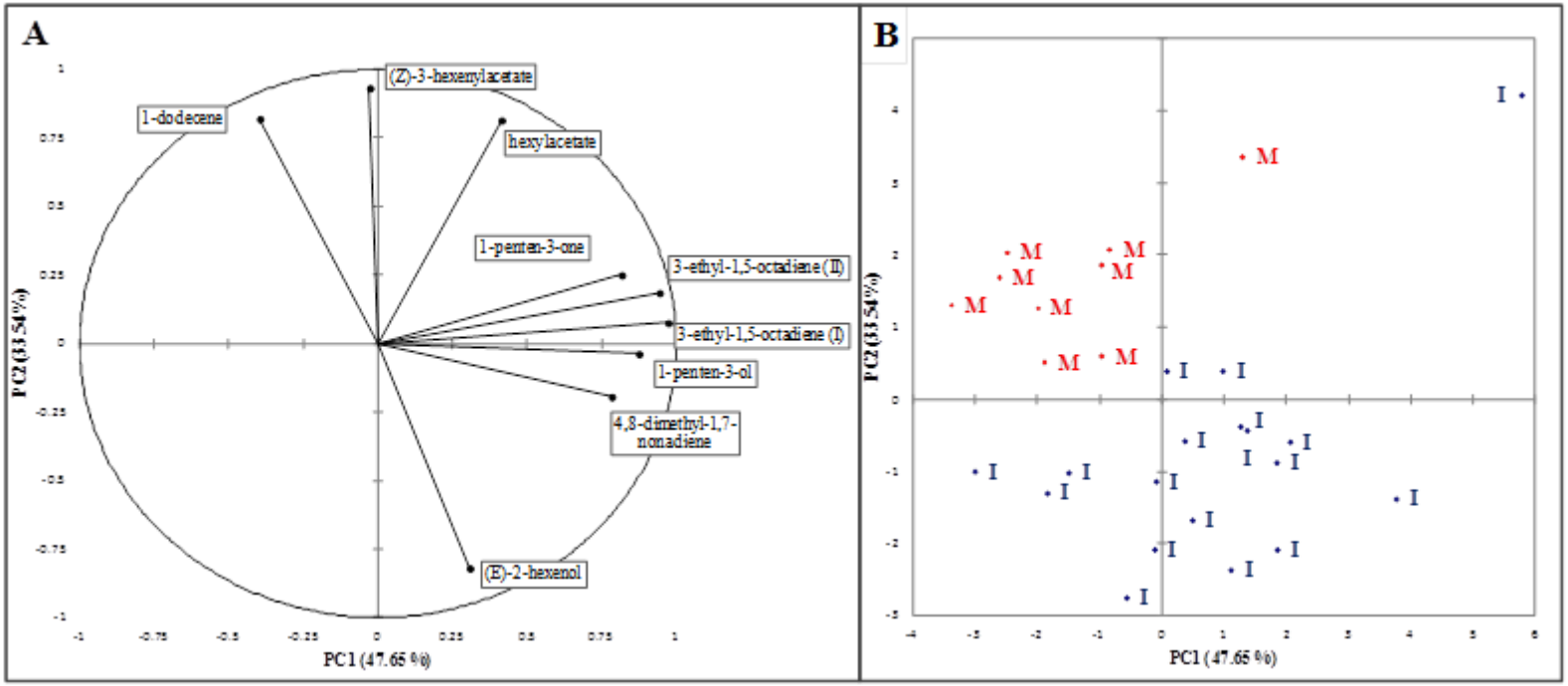

Fig. 2 (A) PCA loadings obtained using the selected variables on SPME/GC–MS data (Set A). (B) PCA score plot obtained using the selected variables on SPME/GC–MS data (Set A).

2.3.3 PLS-DA from FGC E-nose full chromatograms of Set A

Fig. 2 clearly demonstrated that the discriminating power of the volatile profile with respect to geographic origin can be identified: this preliminary result encouraged further chemometric exploration. Once the discrimination potential of PCA based on Set A, the same set was used to explore the potentials of the other key chemometric tool chosen, namely PLS-DA. In order to make this check independent of the analytical procedure (modality of introduction of volatiles in the GC column) and of the nature of chemometric variables (peak areas or full chromatograms), thus reinforcing eventual confirmation of the intrinsic discriminating power of the volatile profile, the PLS-DA was applied to full chromatograms obtained by FGC E-nose analysis of Set A. To reduce the calculation complexity, one retention time every 10 was selected: hence, the number of variables was reduced from 20,000 to 2000. For the sake of succinctness, the PLS-DA model is not reported herein, but its good performance may be summarized as follows: (i) the scores-plot is analogous to the one shown in Fig. 2 (I samples on negative PC2 values and M samples with negative PC1 and positive PC2); (ii) high total EV (96.9% in the first 2 PCs) was obtained; the plot of predicted vs. experimental responses showed low RMSE (0.071) and $RMSE_{CV}$(0.15) with high correlation (R^2 = 0.980; R^2_{CV} = 0.908).

Following the demonstration that the volatiles profile is intrinsically related to geographic origin (independently of whether the volatiles are identified in the GC column by E-nose or SPME, and independently of choosing variable peak areas or full chromatograms), in depth analysis of the large training set (Set B) was initiated.

2.3.4 PCA models based on FGC E-nose peak areas of Set B

Considering Set B, the training set to create chemometric models and the unknown set to apply models must be extracted from all 251 EVOO samples that were analyzed in quadruplicate by FGC E-nose. Each replicate corresponds to a row of the data set (object), and thus 251 samples gave 1004 objects. In this first step of multivariate analysis of Set B, the variables are the peak areas. Choosing the training set is a delicate step, because the fidelity of the characteristics declared about the samples is crucial to the model's performance. In order to obtain a very reliable and consistent training set, the following rationale was used. A PCA model was created from the 100% Italian samples, and Hotelling analysis was performed. Only objects far inside the Hotelling ellipse were

chosen; 224 objects were thus selected. The same was done with the M samples, and 269 objects were selected. Therefore, the training set was formed of 493 objects. To verify the suitableness of samples, a LDA scores plot (not reported) was created, and separation between classes was excellent (94.2% correct assignments in cross validation). This is not an obvious result: based on FGC E-nose areas, all Italian samples formed a homogeneous PCA cluster, and all M samples constituted another homogeneous PCA cluster, but LDA showed that these two clusters are separated, thus demonstrating the discriminating ability of FGC E-nose variables and hence of the volatiles profile. This preliminary exploration allowed identification of the variables that were related to high discriminating power. In order to explore eventual subgroups in M category (very wide in this case), a HCA was performed. In fact, 3 clusters were observed in the M category, termed M1, M2, and M3 (dendrogram not shown).

The PCA analysis of these 493 selected objects obtained the results reported in Fig. 3. The resulting PCA model showed good performance since 81.3% EV was obtained in calibration mode with only 6 PCs of 20 original variables. It can be seen that the centroid of the I-cluster is far distant from the centroid of the M-cluster. This is another important proof of the suitability of the volatiles profile (here represented with FGC E-nose variables) to discriminate the geographic origin with respect to 100% Italian and non-100% Italian EVOOs. However, several M2 samples in the scores plot in Fig. 3 are near the I centroid; this is not surprising, since a sample classified as "non-100% Italian" may contain a fraction of Italian EVOO. The samples with the highest distance from the centroid were from four suppliers who declared that they were from EU countries, but did not contain Italian oil. In order to avoid doubts related to the geographical origin of samples in the training set, in the subsequent discussion a sub-dataset was created where the M2 samples were discarded (439 objects remained), and M1 and M3 were joined again in a unique M class.

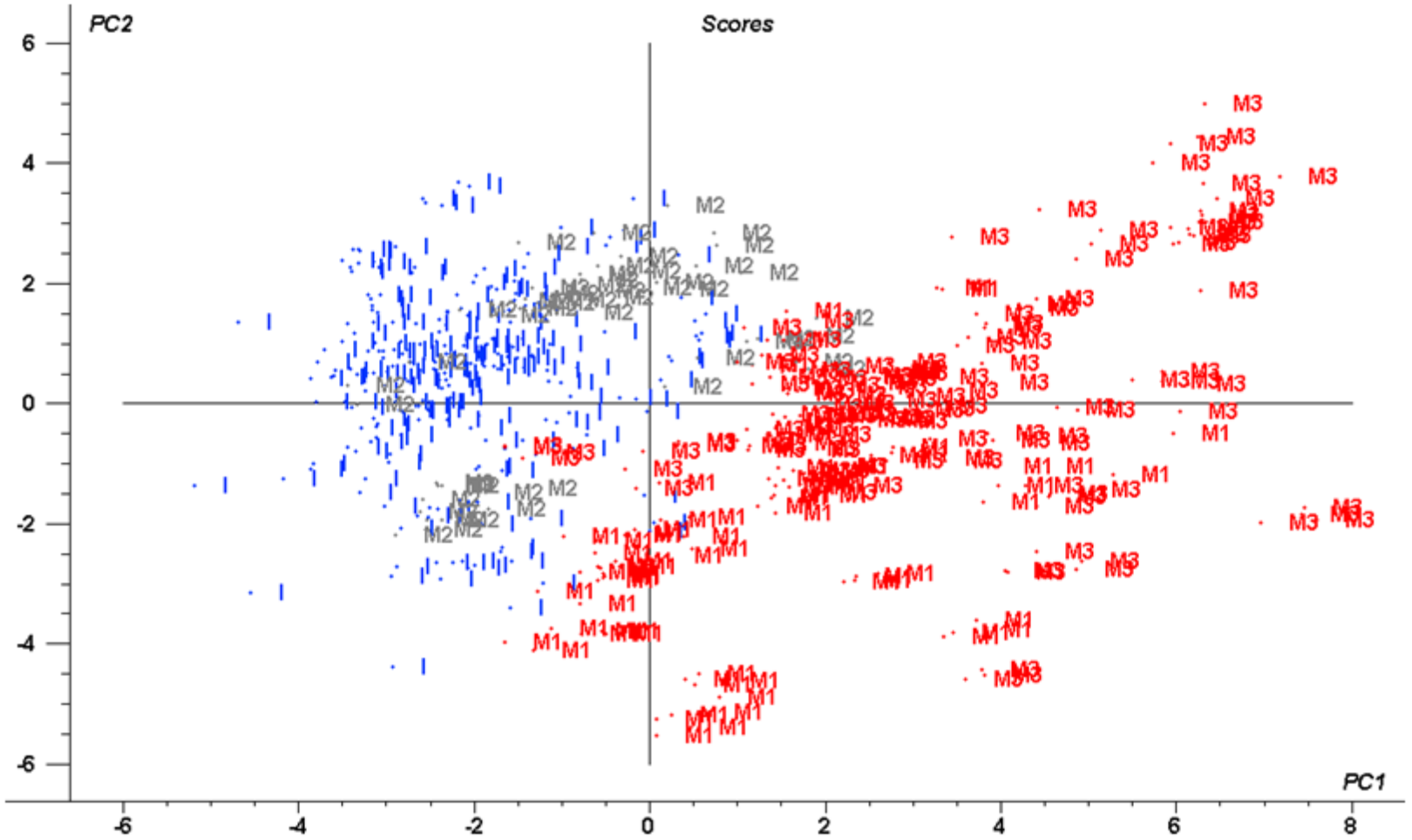

Fig. 3 Scores plot of FGC E-nose peak areas of 493-objects dataset selected by Hotelling (Set B). M1, M2, M3: clusters identified by HCA. EV = 39% along PC1, 18% along PC2. 95% EV is obtained with 11 PCs in calibration and 15 PCs in cross validation mode.

2.3.5 PLS-DA from FGC E-nose full chromatograms of Set B

To check the opportunity of using full chromatograms as prediction variables, PLS-DA was performed on the 439 object sub-dataset of Set B. This procedure is almost identical to the one used in Section 3.2, although in this case there is a much higher number of objects. The outputs relevant to the PLS-DA model are reported in Fig. 4A-C. A well-defined separation between Italian and non-100% Italian classes is obtained. Comparison between the scores plot in Fig. 4A and loadings relevant to PC1 (Fig. 4B) allows determination of which FGC E-nose retention times discriminate objects with positive PC1 scores with respect to objects with negative PC1 scores; the analogous comparison allows to study the FGC E-nose discriminating retention times along PC2. The figures of merit related to the PLS-DA response plot (calculated vs. experimental degree of belonging to Italian class) were as follows: a very low RMSE was obtained for both descriptive and predictive ability (0.203 and 0.207, respectively); very few PCs contained over 99% of variance: for each chromatogram, 2000 signals at several retention times were acquired and only 2 PCs contained an high level of information (PC1-EV: 87%, PC2-EV: 7%; total EV = 94%). Both in calibration and in validation, the slopes of response plot were very high (0.834 and 0.833, respectively) and the offsets were close to the ideal null value. Determination coefficients were also high (0.835 and 0.839). This is a very strong result, because models created in Sections 3.3 and 3.5 were obtained by two different laboratories working in a completely independent manner, and using two different sample sets from different harvest periods analyzed with different instruments and experimental conditions.

The good PLS-DA model obtained was applied to M2 samples that were used as unknowns to be predicted. In all cases, a relative standard deviation (RSD) of about 20% degree of belonging (y_I or y_M) was obtained. Predicted values for y_I or y_M that were higher than 70% were considered to correspond to "full" *I*or *M* character, respectively; values resulting lower than 30% were assumed to indicate non-belonging. The result of prediction was the following: 6 ME2 samples of 51 (11.8%) were predicted as "non-100% Italian"; 19 samples (37.3%) were predicted as "100% Italian"; the remaining 26 ME2 samples were predicted as partially "100% Italian" and partially "non-100% Italian".

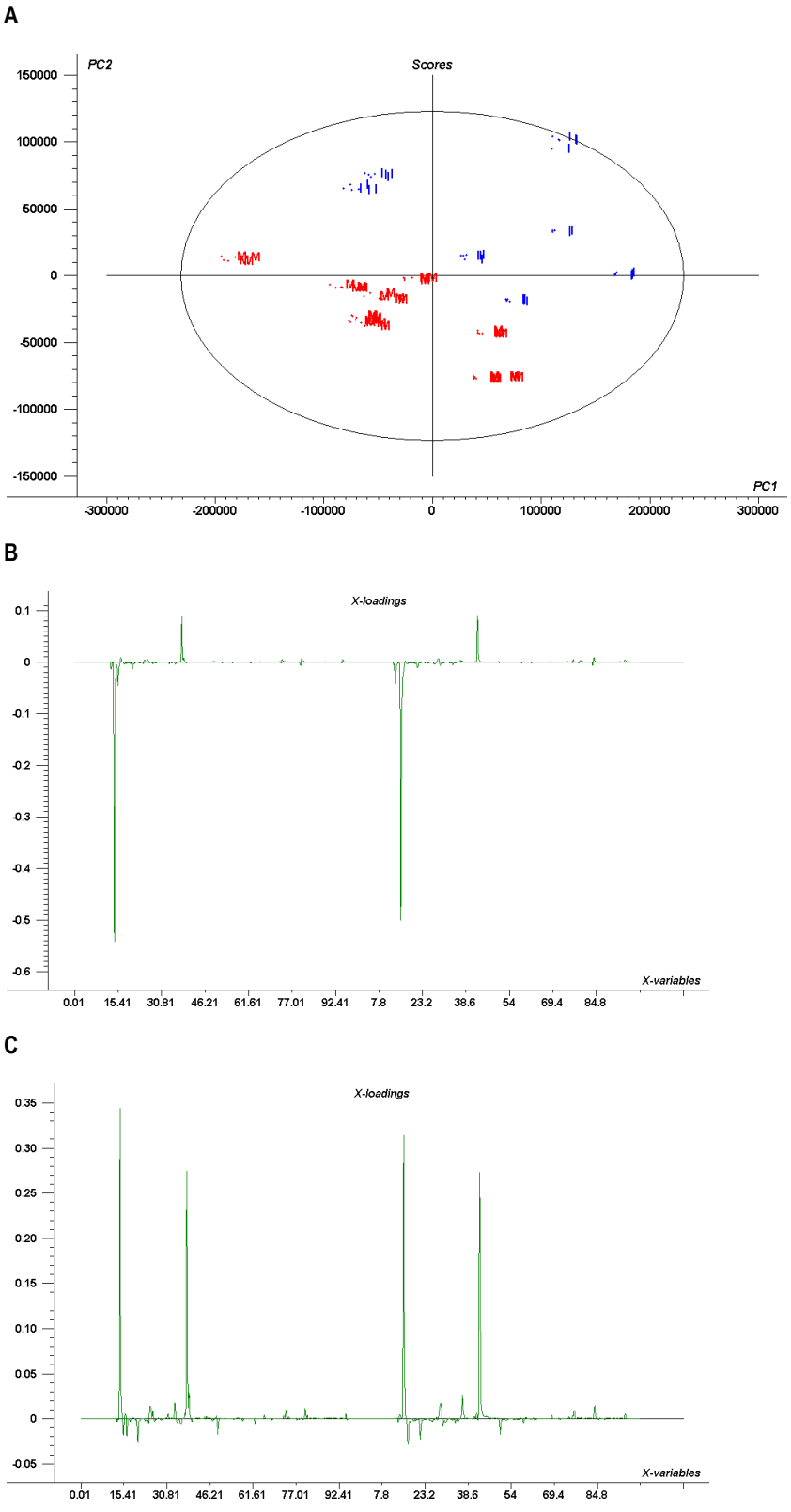

Fig. 4 PLS-DA from FGC E-nose full chromatograms of 439 objects sub-dataset (Set B). (A). Scores plot, PC1 EV: 87%, PC2 EV: 7%. (B). PC1 loadings plot. (C). PC2 loadings plot.

2.3.6 PCA models based on SPME/GC–MS peak areas of Set B

In order to compare FCG E-nose results with a well known technique such as SPME/GC–MS, a new dataset was created on the basis of the PCA shown in Section 3.4, according to the following criteria. Samples for which all the replicates gave points that were very close to the I-centroid were selected as"surely Italian samples". Samples for which all the replicates give points very close to the M-centroid were selected as "surely non-100% Italian" samples. In this way, 7 I samples and 9 M samples were extracted, and the I-M sub-dataset was obtained. The scores plot obtained from I-M dataset is reported in Fig. 5, where the Hotelling ellipse is seen.

The I-M dataset extracted from Set B was processed by SPME/GC–MS, and careful and detailed analysis of mass spectra was performed to identify molecules corresponding to significant chromatographic peaks. It must be pointed out that neither the gas chromatographic conditions nor the headspace conditions respectively employed for SPME/GC–MS and FGC E-nose were identical. Moreover, correlation analysis between SPME/GC–MS and FGC E-nose chromatograms may show eventual correspondences between species identified in SPME/GC–MS and FGC E-nose retention times. This could help in bypassing the non-target character of FGC E-nose analyses.

Since SPME/GC–MS analyses were performed in two replicates (Set B), the 7+9 samples corresponded to 14+18 objects. The species identified by SPME/GC–MS analysis were the following: 1-hexanol; 1-octanol; 1-octen-3-ol; 1-penten-3-ol; 1-penten-3-one; 2,4-decadienal; 2,4-hexadienal; 2-butenal; 2-heptanone; 2-methylbutanal; 2-methylbutanol; 2-octanol; 3,4-diethyl 1,5-hexadiene; 3,4-diethyl meso-1,5-hexadiene; 3,5-octadien-2-one; 3,7-decadiene; 3-ethyl 1,5-octadiene; 3-methylbutanal; 3-methylbutanol; 3-pentanone; acetic acid; acetone; α-copaene; α-murolene; α-pinene; benzeneethanol (2-Phenylethanol); benzenemethanol; citronellal; decanal; decane; dimethylnonadienal; (*E*,*E*)-α-farnesene; (*E*)-2-heptenal; (*E*)-2-hexenal; (*E*)-2-hexenol; (*E*)-2-pentenal; (*E*)-2-pentenol; (*E*)-β-ocimene; ethanol; ethyl acetate; formic acid; heptanal; hexanal; hexane; hexylacetate; isoamylacetate; isoamylalcohol; limonene; methanol; methylacetate; methyloctane; murolene; nonanal; octanal; octane; pentanal; propanal; (*Z*)-2-pentenol; (*Z*)-3-hexenal; (*Z*)-3-hexenol; (*Z*)-3-hexenylacetate; (*Z*)-4,8-dimethylnonatriene.

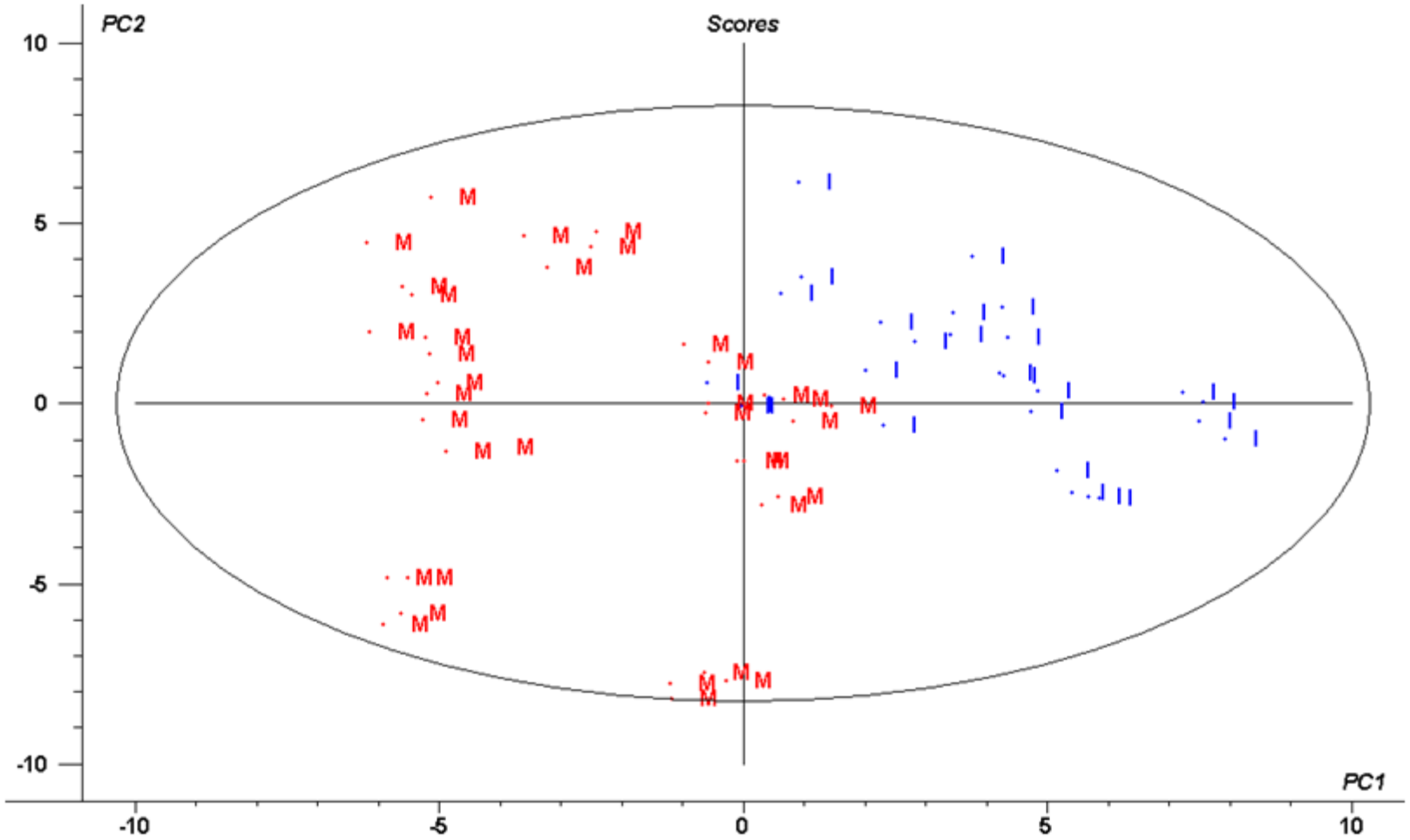

Fig. 5 Scores plot of FGC E-nose peak areas of I--M dataset (Set B). PC1-EV: 25%, PC2-EV: 16%

Each of these species was a "variable" in a dataset created by putting the I-M samples on lines and the SPME/GC–MS peak-area values (in total ion current, TIC) in the corresponding columns. There were 62 species detected, although some were detected by both the more-polar and by less-polar columns. Hence, there were 89 variables in the SPME/GC–MS dataset, which was more than the number of species detected. The PCA model obtained by the I-M SPME/GC–MS dataset is reported in Fig. 6A, where the Hotelling ellipse is seen. An excellent separation was observed between I and M clusters, thus confirming that headspace GC may discriminate the Italian quality of EVOOs. The corresponding correlation loadings plot (Fig. 6B) showed which species are especially important in discriminating samples: the molecules in the zone between the internal and the external ellipses are the most important variables; molecules with absolute values of loadings higher than 0.3 may be considered significantly relevant. It is interesting to observe that molecules relevant to a separation along PC1, namely with respect to the separation between I and M, are due to primary or secondary metabolic compounds of the LOX pathway and terpenes. This has a chemical-biological basis, since molecules derived from these enzymatic activities are known to be influenced by the cultivar and geographic origin. Comparison between Figs. 5 and 6A shows that the FGC E-nose peak areas and SPME/GC–MS peak areas yield a very similar PCA model: this confirms that headspace GC data (independently of how volatiles are brought into the GC column, i.e., FGC E-nose or SPME/GC–MS) are suitable for discriminating between 100% Italian and non-100% Italian samples, and that FGC E-nose performance in this discrimination is not significantly different with respect to SPME/GC–MS. It must be pointed out that the extraction of the training set samples from the initial samples was performed based on data pre-processing on objects obtained by FGC E-nose; the fact that these objects gave good results even with SPME/GC–MS data demonstrates that the initial choice was not a tautology: MS data are completely independent from FID data. The comparison between the scores plot and the correlation loadings plot, respectively reported in Fig. 6A and B, shows that I samples are characterized by negative PC1 scores and M samples are characterized by positive PC1 scores; this suggests thatmolecules identified by MS spectra and characterized by negative PC1 loadings and positive PC1 loadings may be related to I and M samples, respectively.

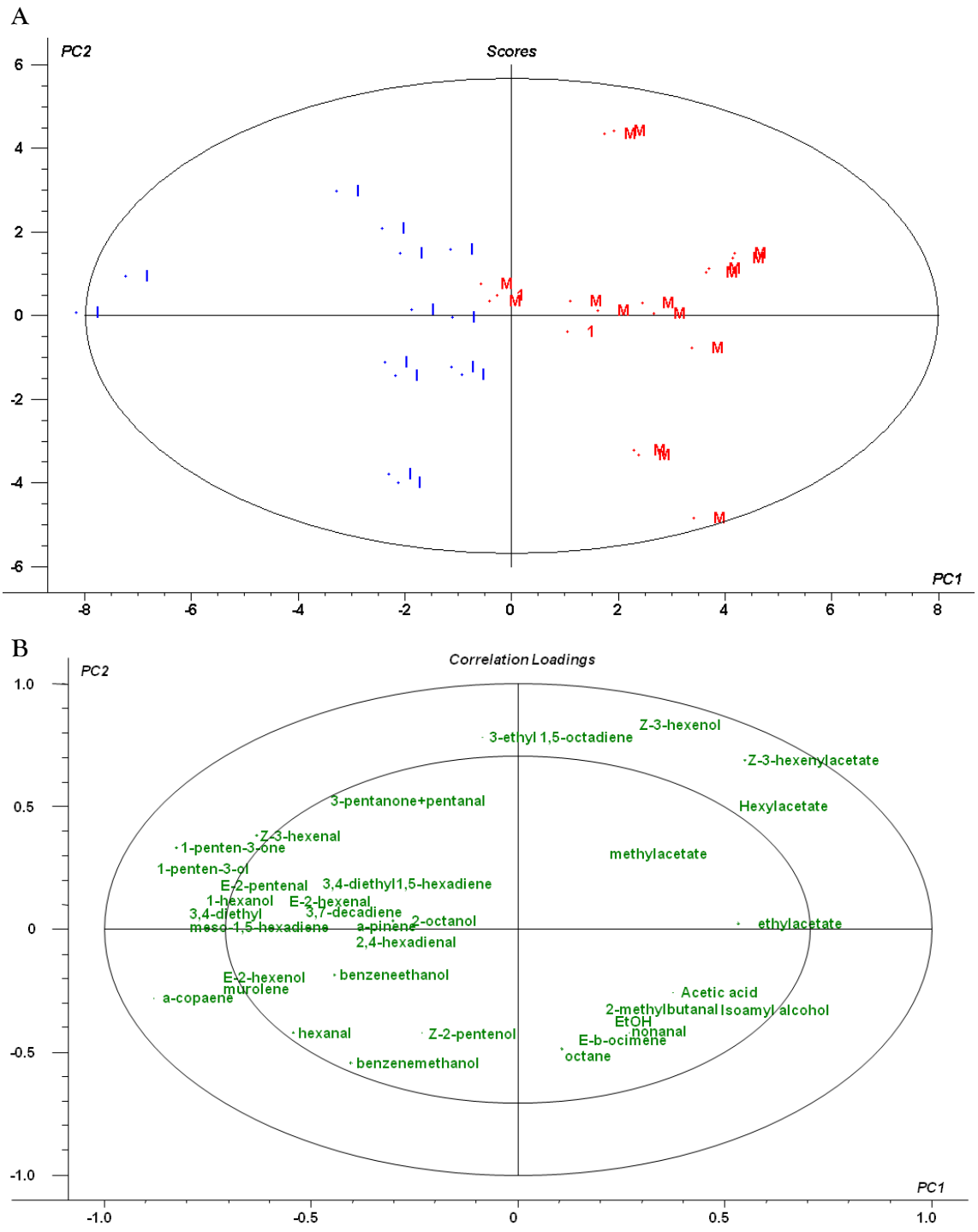

Fig. 6 (A) Scores plot of SPME/GC–MS peak areas of I–-M dataset (Set B). PC1-EV: 28%, PC2-EV: 14%. (B) Correlation loadings plot of SPME/GC–MS peak areas of I–-M dataset (Set B).

2.3.7 Correlation between FGC E-nose and SPME/GC–MS data of Set B

In order to study the correlation between FID variables and MS variables, a dataset in which lines corresponded to the I-M samples discussed in Sections 3.4 and 3.6 was created; all columns relevant to FGC E-nose peak areas and SPME/GC–MS peak areas relevant to the more polar column are reported. The correlation matrix for the FID-peaks and MS-peaks was calculated, and correlation coefficients with significant or considerable values for highly discriminating FGC E-nose peaks (see Section 3.4) were observed. For instance, correlation coefficients higher than 0.8 were observed for ethanol, methylacetate, ethylacetate, 1-penten-3-one, 1-penten-3-ol, (*E*)-1-hexenal, 1-hexanol, and (*E*)-2-hexenol. This analysis shows that accurate study may lead to identification of FGC E-nose peaks, thus bypassing the shortcomings of this technique: it is a non-target analysis; when a significant signal is not linkable to a chemical characteristics, the chemometric results are less strong. It must be underlined that high correlation between retention time and a molecule does not imply that the molecule is an important variable; the present correlation analysis simply has an identification purpose. Importance of variables is determined by loadings: the important molecules are those lying in the outside elliptical ring shown in correlation loadings plot (Fig. 6B). Complete identification of FGC E-nose signal is beyond the scope of the present work, which aims to demonstrate that FGC E-nose based chemometric models are not less reliable that those obtained with SPME/GC–MS data.

2.4 CONCLUSIONS

This study demonstrates that FGC E-nose is suitable for checking geographical traceability of EVOO, even using non-target chromatographic signals of the volatile fraction as variables for multivariate analysis. As a consequence, the feasibility of comparing the geographic origin of standard EVOOs to the origin of an unknown EVOO using FGC E-nose chromatograms as a fingerprint has been assessed. A PLS-DA model, able to discriminate between oils labeled as “100% Italian” (I) and oils labeled as EU oils mixture, considered as“non-100% Italian” (M), was created. This means that when a good, reliable training set coming from a certain production year is available, it is possible to verify, through direct and rapid analysis, whether unknown samples belong to

the same statistical population as the training set. Moreover, it is possible to quantify the degree of belonging of unknown samples to the category "100% Italian". The performance of geographic discrimination of FGC E-nose was comparable with SPME/GC–MS, and the results obtained by the two techniques on the same dataset were not significantly different. Comparison between FGC E-nose and SPME/GC–MS signals allowed for eventual correlations between some FGC E-nose retention times and particular molecules identified by their MS spectra in SPME/GC–MS analysis.

Both approaches utilized to analyze volatile compounds were able to discriminate samples with different geographical origin (M vs. I), but each offers specific advantages and limitations: SPME/GC–MS provided more reliable diagnostic information on the identity of compounds thanks to the study of the specific ion fragment profile and the possibility to consult the library of mass spectra, but a lengthy time for analysis and for data processing is required. FGC E-nose was a very fast analytical tool (only 100 s of acquisition time and virtually no need for solvents), discriminating samples with a higher explained variance and allowed for comprehensive data processing with automatic identification of molecules. These results highlight the potential of FGC E-nose for rapid control of the compliance of information on geographic origin declared in the label. This analytical approach seems particularly interesting for food providers, commercial suppliers, and retailers that intend to avoid media scandals of this sector thanks to a more efficient protection and promotion of the integrity of the olive oil image. The main effort concerns the possibility to build, season by season (even by each distributor) an internal or shared and representative data base to be used to screen and control, year after year, EVOOs labeled with a specific origin.

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Chapter 3

Sensory and instrumental study of *Taralli*, a typical Italian bakery product

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ABSTRACT

Taralli is a bakery snack food, typical of the south of Italy that has currently become very popular worldwide also as savory snack on board (trains, flights) or in the vending machines and consumed in every occasion (during the morning break, as appetizer or snack, at dinner) as substitute of bread.

However, few studies have focused on its physical and sensory characteristics. The present work aims to select sensory and instrumental information able to characterize *Taralli* with similar formulation and size. For sensory characterization purposes, conventional profiling was applied on samples by different producers. All samples were also subjected to physical analysis of appearance and textural proprieties. Samples differing only for storage time were evaluated to assess changes in sensory characteristics during this period, moreover a discrimination test (triangle test) was also applied. The test results confirmed the utility of descriptive analysis for evaluation of quality characteristics. Moreover, significant differences between *Taralli* samples during the storage times were observed. This suggests that physical parameters obtained by simple and rapid instrumental tools can be an useful support in evaluating sensory characteristics.

Highlights

- Sensory descriptive analysis were effective for evaluate *Taralli* quality characteristics.
- The panel showed a good performance in terms of repeatability and understandability of attributes.
- Trained assessors were able to discriminate *Taralli* stored for different months.
- Appearance and texture physical data support sensory ones in *Taralli* characterization

Keywords: *Taralli*, bakery products, sensory characterization, image analysis, texture analysis.

3.1. INTRODUCTION

Taralli belong to a niche market of Italian food certified as Traditional Agri-food Products (Prodotti Agroalimentari Tradizionali or PAT). This typical Italian savory snack food native to the south of the peninsula (Apulia region) is *"obtained with specific methods of processing, storage and seasoning consolidated over time, standardized throughout all the italian territory concerned, according to traditional specifications, for a period of time not shorter than twenty-five years"* (Ministerial Decree, G.U. n 350, 08.09.1999).

Italian *Taralli* belong to the "bread substitute" category and are characterized by a ring-shape, a texture similar to breadsticks and are produced using specific ingredients (flour, water, vegetable oil or other kind of fat (about 20%), salt, yeast and, sometimes, with the addition of wine, fennel seeds or other herbs/spices). The production technology also provides the use of a specific equipment called *"tarallatrice"* and a boiling phase before baking, necessary for impeding the leavening of the product before baking (Pagani et al. 2007).

Despite its wide distribution on the Italian market and its daily consumption as appetizer or snack, during the morning break or at dinner, few studies have focused on this bakery product. Some authors have evaluated how different formulations, processing and storage times may affect the volatile and lipidic fraction of *taralli* (Caponio et al. 2009; Giarnetti et al. 2012; Caponio et al. 2013). In others studies, the influence of vegetable oils used in the *Taralli* formulation on sensory characteristics of the final product was considered, suggesting that textural and visual attributes are the most important parameters for product acceptability (Giarnetti et al. 2011; Caponio et al. 2011). However, these studies did not give adequate information on the physical and sensory characteristics suitable for characterization of *Taralli*.

Sensory analysis, although using standard and time-consuming sensory evaluation techniques, represent an useful tool to ensure both the quality and protection of traditional agri-food products with an identifiable geographical origin, established technology of production and defined product characteristics (Pagliarini et al. 2004; Scintu et al. 2010). Sensory evaluation of textural attributes is the primary way by which consumers judge the quality of many food products. However, often the scientist use instrumental data rather than sensory evaluation for the characterization of texture due to

the cost involved in establishing and maintaining a sensory panel and the time necessary for training and managing it (Pereira et al. 2005).

Correlations between instrumental and sensory methods of texture evaluation represent a highly controversial subject that has involved many researchers (Szczesniak 1968). Although "texture" is basically a physical property, its perception can be affected not only by chemical factors, but also by psychological and cultural aspects (Peleg 1983). Regarding the correlation between sensory (profiles) and instrumental (appearance and textural characteristics) data, some results have highlighted the possibility of this combined approach to characterize bakery product quality (Reyes-Vega et al. 1998; Gámbaro et al. 2002; Lassoued et al. 2008; Kim et al. 2009; Pagliarini et al. 2010; Laguna et al. 2012; Handa et al. 2012; Scheuer et al. 2015).

Instrumental texture tests differ from sensory oral evaluation mainly due to the absence of water and enzymes (saliva) and for the lower temperature during the execution. In fact, instrumental tests are generally performed at room temperature (e.g. 20°C, even if the new equipment allows for product temperature control during analysis), while the body temperature during sensory analysis is around 36.6°C. These are key factors that can affect correlations when testing moisture (e.g. corn flakes) or temperature (e.g. chocolate) sensitive foods (Szczesniak 1987). Moreover during a sensory analysis the characteristics perceived in the mouth include mechanical attributes (relating to the reaction to the applied force), geometrical attributes (relating to the shape, size and particle orientation inside the food) and attributes relating to perception of moisture and fat content (Scheuer et al. 2015).

In addition, different types of mechanical forces can be applied during instrumental or sensory tests. The correlation between sensory and instrumental analyses depends on several factors, as mentioned by Szczesniak (1968): improper execution of sensory tests (consumer and expert), inadequate knowledge of what instrumental tests really measure, sampling errors and heterogeneity of food products and interpretation of the meaning of correlation coefficients (Szczesniak 1987).

There are several reasons in finding correlations between sensory and instrumental measurements: 1. The need to find rapid quality control instruments and tests; 2. The desire to predict consumer response as the degree of liking and the overall acceptance of a

new product; 3.The need to understand what is being perceived in the mouth during a sensory assessment; 4. The need to develop improved/optimized instrumental methods (Szczesniak 1987; Scheuer et al. 2015).

For these reasons, in order to obtain objective results accurate and meaningful it could be interesting to perform both the instrumental analysis and the sensory evaluation; only the human senses can truly perceive, describe and quantify texture, but at the same time, instrumental methods are easier to perform, standardize, reproduce and they could reduce the complexity of sensory textural assessment (Pereira et al. 2005; Scheuer et al. 2015). Based on the above considerations, the aims of this study were: *i)* characterize *Taralli* samples using both sensory and instrumental methods; *ii)* determine the correlation between sensory attributes and physical parameters; *iii)* detect physical parameters that can support sensory analysis in a calibration panel process and in the quality prediction of the product.

3.2. MATERIALS AND METHODS

3.2.1.Samples

The study is focused on a set consisting of 7 *Taralli* samples available in the Italian market, all obtained by using similar ingredients and recipes. Three samples differed only for the storage time before analysis: 0, 6 and 12 months (St_0, St_6, St_{12}). All samples were stored in closed packaging at room temperature (20 ± 2°C) protected from light and moisture and were analyzed in multiple replicates to increase the level of repeatability. Sample characteristics are summarized in Table 1.

Table 1. Ingredients and information on *Taralli* samples.

Sample	Ingredients	Packaging type	Storage time before analysis
St_0	strong/hard wheat flour; white wine; extra virgin olive oil (18%); natural yeast; salt.	plastic pack (250 g)	0 months
St_6	strong/hard wheat flour; white wine; extra virgin olive oil (18%); natural yeast; salt.	plastic pack (250 g)	6 months
St_{12}	strong/hard wheat flour; white wine; extra virgin olive oil (18%); natural yeast; salt.	plastic pack (250 g)	12 months
S1	weak/soft wheat flour "0"; white wine; olive oil (22%); natural yeast; salt.	plastic pack (400 g)	4 months
S2	weak/soft wheat flour "00"; white wine; olive pomace oil; natural yeast.	plastic pack (500 g)	3 months
S3	strong/hard wheat flour regrind; white wine; olive oil; salt.	plastic can (1000 g)	3 months
S4	weak/soft wheat flour "0"; wine; vegetable oil/fats; olive oil (2%); brewer's yeast; salt.	plastic pack (40 g)	4 months

3.2.2 Sensory analysis

3.2.2.1 Descriptive analysis (DA)

The sensory quality of all the samples was evaluated by a panel of eight fully trained assessors (four females and four males), aged between 25 and 50 years and recruited on the basis of their previous experience in descriptive sensory analysis (staff and PhD students at the Campus of Food Science, University of Bologna, Cesena, Italy), their familiarity with the product and their availability to complete all the training and panel sessions. We used the minimum number of panelist to give a stable result also considering the number of sample evaluated (Heymann et al. 2012). The conventional profiling method was applied (Meilgaard et al. 2007).

During two 1-h sessions the DA panel generated a list of aroma, taste and texture attributes using the consensus training (Varela and Ares 2014) to identify and select a set of non-overlapping attributes that, as far as possible, permit a complete descriptive analysis of the samples under study. In order to prevent panelist fatigue, while still covering all the differences among the samples, we tried to minimize the attribute list.

Once the selection of descriptors (positive and negative), their definition and the choice of standard references have been completed, several training sessions were carried out to ensure the understanding of the lexicon by the entire panel and making sure that each panelist was comfortable with sensory descriptors and reference standards as well as practicing with the scales. The training procedure was realized also following the guidelines of the ISO 13299:2003.

The sensory training results were processed by an open source software specific for sensory analysis (PanelCheck) to test the judges' performance in terms of sensory data repeatability and discrimination ability. Panelists' evaluation sessions were performed in a closed room and each assessor evaluated samples in a separate tasting booth to reduce panelist interaction. Additional data on the sensory descriptors with their definitions and references are reported in Online Resource 1. Panelists evaluated the intensity of each attribute using a continuous scale of 100 mm anchored at their extremes (0: absence of sensation; 100: maximum of sensation intensity) in three replicates and average values were calculated.

To take into account product variability, panelists evaluated the visual attributes of 10–15 *Taralli* placed inside a plate, whereas, evaluation of other attributes (smell, taste and texture) was performed by providing judges three *Taralli* for each sample. To the panelists two or three sample in each session were given, presented in randomized blocks and labeled with random three-digit codes. Water was used to cleanse the palate during testing. A 2-min break was allowed between one sample and the next.

3.2.2.2 Discrimination test

The triangle test is one of the most widely used and applicable when the products to be evaluated are sufficiently homogeneous, with the objective to determine whether a sensory difference exists between products. Each subject invited to take part in the test receives a set of three coded samples, with the information that one is different and that the panelist should identify it. The sample choice is "forced"; therefore, subjects must provide an answer. An assessor who detects no difference between the products should be instructed to randomly select one of the samples and to indicate that his choice was only a guess in the comments section of the scoresheet. This test was carried out on three samples (St_0, St_6, St_{12}) of the set with the aim to understand if subjects were able to

identify sensory differences between these samples made by using the same ingredients (same batch), formulation and processing conditions, but differing for the storage time. The analysis was performed according to ISO 4120:2007 by untrained subjects, familiar with the triangle test procedure and with the product tested. Samples were prepared out of sight of the assessors and in an identical manner and conditions. Three sessions were carried out to obtain all possible combinations of the comparison between products (St_0 *vs.* St_{12}; St_0 *vs.* St_6; St_6 *vs.* St_{12}). For each session, the presentation order of samples was randomized. Red lights were employed to mask any samples differences in order to eliminate possible influences of product appearance (browning intensity, shape and roughness).

3.2.3 Physical analysis

3.2.3.1 Texture analysis

Textural characteristics of *Taralli* samples were determined by performing penetration test, using a texture analyzer (mod. TA - HDi500, Stable Micro System, Surrey, Godalming, UK). For the test, a 2 mm cylinder probe (P/2) penetrates the sample for 3 mm. The chosen setting to perform the test was: pre-test speed: 1.0 mm/s; test speed: 0.5 mm/s; post-test speed: 10.0 mm/s; distance: 3 mm; load cell: 5 kg; trigger force: 5 g.

Instrumental measurements were replicated at least 30 times for each sample. The maximum force (F(g)) was taken as an indication of sample hardness and linear distance (L_d) as an indication of sample crispness. The penetration test was performed on 20 *Taralli* for each sample.

3.2.3.2 Browning area evaluation by computer vision system (CVS)

Image acquisition of *Taralli* took place by positioning samples inside a black box under controlled lighting conditions (D65). A digital camera (mod. D7000; Nikon, Shinjuku, Japan) equipped with a 60 mm lens (mod. AF-S micro, Nikkor, Nikon, Shinjuku, Japan), was positioned at a fixed distance of 80 cm from the sample surface and used to acquire images (exposition time 1/2 s; F-stop f/16). Three digitalized images for each sample (nine *Taralli* in each image) were acquired and then processed with an advanced image analysis software package (Image Pro-Plus v. 6.2, Media Cybernetics, USA) using RGB scale.

Image analysis was performed by evaluating the total and differently browned areas and the following set up of a specific color model. Two pixel ranges were identified on the basis of the different chromatic characteristics considered as 'light' and 'dark' areas. The model was then applied to each digitalized image and, by evaluating all the pixels, the percentage of each chromatic area was calculated by the software.

3.2.4. Statistical analysis

The open-source software PanelCheck 1.4.0 version was used to evaluate the assessors and panel performance. The software XLSTAT 7.5.2 version (Addinsoft) was used to elaborate sensory and physical data applying a 3-way ANOVA, Principal Component Analysis and linear correlation (Granato et al. 2014).

3.3. RESULTS AND DISCUSSION

3.3.1. Descriptive analysis (DA)

The final list of 11 descriptors (Online Resource 1) included: three relative to appearance (roughness, browning intensity and browning uniformity), four describing orthonasal and retronasal routes (yeast, toasted, musty/mould and fried/rancid) and four relative to texture (hardness, crispness, dryness and greased/oiled). The attributes fried/rancid and musty/mould were considered as off-flavors. Some terms included in this list are common to other studies carried out on similar products, which confirm their importance in bakery products sensory evaluation (Bower 2000; Gámbaro et al. 2002; Vázquez et al. 2009; Elía 2011). The different ones depending on the nature of the product chosen for the study.

The 3-way ANOVA results showed that all attributes were significantly different for *Taralli* characterization, but with different levels of significance: $p < 0.001$ for roughness, browning intensity, yeast, musty/mould, fried/rancid, hardness, crispness, dryness and greased/oiled; $p < 0.01$ for toasted; $p < 0.05$ for browning uniformity. Moreover, the panel reached a satisfactory level of calibration and performance in terms of repeatability and understandability of the selected attributes because all descriptors were without significant effect for replicates.

The evaluation of samples was carried out after panel training. The intensities of the positive and negative attributes, expressed as mean value (calculated on 3 replicates), for 8 assessors and relative to the 7 samples evaluated, are reported in Table 3 and Fig.1 splitted in two groups of samples: S1, S2, S3, S4 (a) and St_0, St_6, St_{12} (b).

Samples and attributes valued by conventional profiling were projected in a two-dimensional plane consists of four quadrants to highlight the possible interactions, using the principal components analysis (PCA) (Fig. 2). The distance between attributes and samples expresses the degree of variability of the parameters between the samples analyzed; *Taralli* closely located on the plane can be considered similar in terms of intensity of perception of the attributes; on the other hand, if positioned in the opposite direction they are different. This PCA explains about 80% of the variance between samples (51% PC1 and 30% PC2) and illustrates a different distribution of the samples on the plane.

In the first quadrant (positive values of principal components) is located sample S2 which seemed very similar to sample S4, placed in the fourth quadrant: both were characterized by high intensity of the parameters roughness, greased/oiled and yeast, both perceived by orthonasal and retronasal routes. S1 and St_{12} were placed in the second quadrant, the samples were similar for the high intensity of browning and the presence of musty/mold, but were different for the fried/rancid descriptor characterized mainly in S1. In the third quadrant, St_6 was placed which showed average values for all the attributes evaluated and S3, whose position was affected by the low intensity recorded for the attributes, is in the opposite quadrant. This sample, in fact, was characterized by high hardness, crispness, toasted and by a low intensity of yeast. Finally, St_0 was very similar to St_6 but less hard, toasted and dry. Another consideration related only to the sensory profile of the three sample stored for different months (St_0, St_6, St_{12}) (Fig. 1b), is that the presence of rancid (off-flavour) appear only at the end of storage (St_{12}) confirming the essential role played by the raw materials used such as the type of oil (extra virgin olive oil or refined oil) on the possible formation of negative sensory attribute linked with the lipid oxidation (Giarnetti et al. 2012). The high oxidative stability of virgin olive oil with respect to other vegetable oils is mainly due to its fatty acid composition, in particular, to the high monounsaturated-to-polyunsaturated ratio, and to the presence of minor compounds that play a major role in preventing oxidation (Bendini et al. 2009).

Table 3. Sensory and instrumental data on *Taralli* samples. Sensory attributes were expressed as mean values (3 replicates for each sample). For penetration test, mean value (30 replicates for each sample) relative to the peak force (F) and to the linear distance (Ld). For Image analysis, mean values (27 replicates for each sample) of the percentages relative to dark and light areas identified in *Taralli* using the software Image-Pro Plus 6.2®. [a-d] Different letters indicate significant differences among *Taralli* samples (Fisher LSD, $p < 0.05$).

Sensory analysis							
	St_0	**St_6**	**St_{12}**	**S1**	**S2**	**S3**	**S4**
Roughness	37.0^{d}	50.9^{b}	62.8^{a}	38.5^{d}	67.3^{a}	26.1^{e}	44.9^{c}
Browning intensity	34.6^{d}	53.3^{bc}	61.7^{a}	59.1^{ab}	49.8^{c}	62.0^{a}	29.8^{d}
Browning uniformity	67.1^{a}	67.7^{a}	46.3^{bc}	51.0^{b}	43.4^{c}	65.5^{a}	53.0^{b}
Yeast	40.8^{a}	28.1^{b}	25.0^{bc}	18.3^{c}	39.9^{a}	9.4^{d}	36.1^{a}
Toasted	24.9^{b}	29.4^{b}	30.4^{b}	24.9^{b}	11.3^{c}	55.5^{a}	21.7^{b}
Musty/mold	2.7^{cd}	13.2^{b}	15.8^{b}	34.8^{a}	14.5^{b}	6.2^{c}	0.0^{d}
Fried/rancid	0.0^{d}	0.0^{d}	9.7^{b}	15.6^{a}	3.9^{c}	0.0^{d}	0.0^{d}
Hardness	44.9^{b}	55.8^{a}	59.4^{a}	45.0^{b}	27.5^{c}	53.6^{a}	39.1^{b}
Crispness	68.8^{a}	63.1^{ab}	60.1^{b}	44.3^{c}	39.6^{c}	64.4^{ab}	40.6^{c}
Dryness	39.9^{c}	45.5^{bc}	50.5^{b}	53.2^{b}	39.5^{c}	63.7^{a}	27.5^{d}
Greased/oiled	25.0^{b}	24.9^{b}	18.2^{c}	17.7^{c}	39.8^{a}	10.5^{d}	39.7^{a}
Penetration test							
F (g)	1576.6^{b}	1650.8^{b}	1758.5^{b}	2212.5^{a}	1158.1^{d}	1791.9^{b}	1408.7^{c}
Ld	1010.5^{b}	1061.6^{b}	1084.0^{b}	481.0^{d}	479.3^{d}	1426.2^{a}	736.0^{c}
Image analysis							
% dark area	24^{de}	59^{b}	71^{a}	47^{c}	30^{d}	55^{bc}	15^{e}
% light area	76^{ab}	41^{d}	29^{e}	53^{c}	70^{b}	45^{cd}	85^{a}

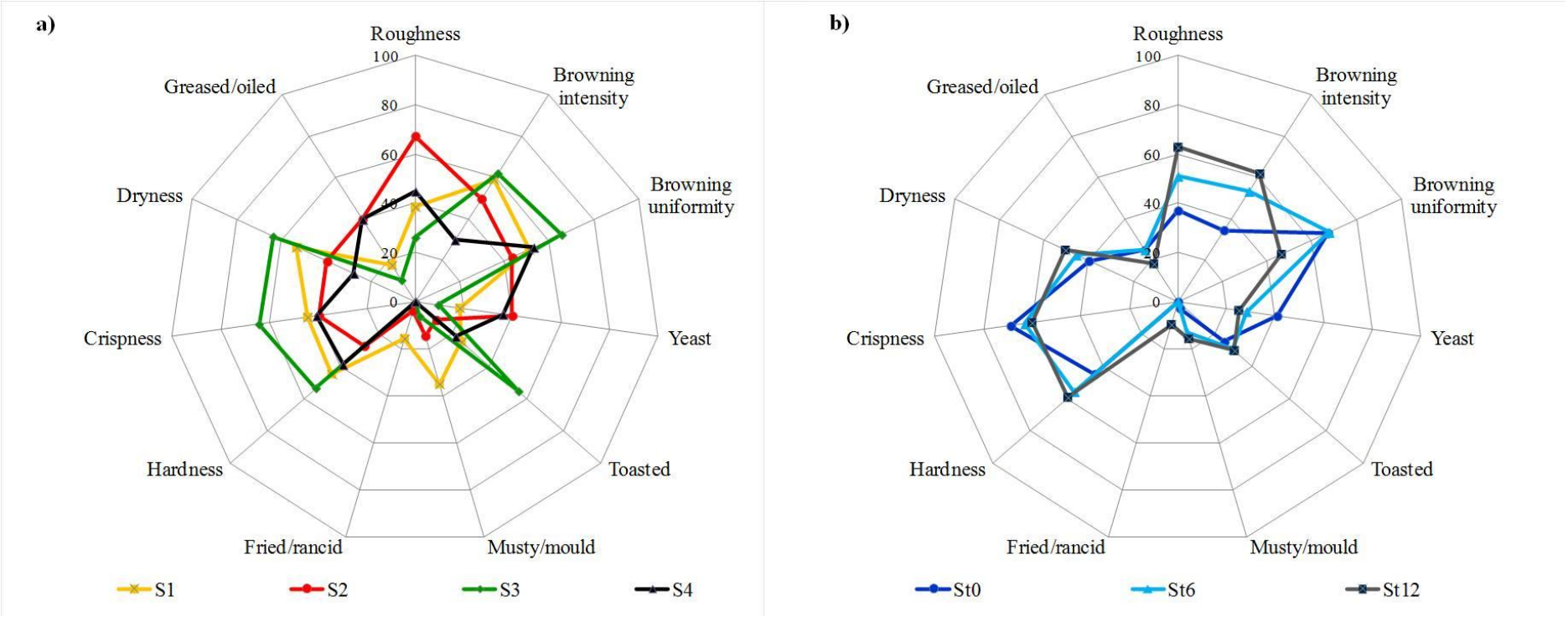

Fig. 1 Spider web graph of sensory attributes (expressed as mean values on the 100 mm scales) for *Taralli* samples. S1 (yellow line), S2 (red line), S3 (green line) and S4 (black line) (a); St_0 (blue line), St_6 (light blue line) and St_{12} (grey line) (b).

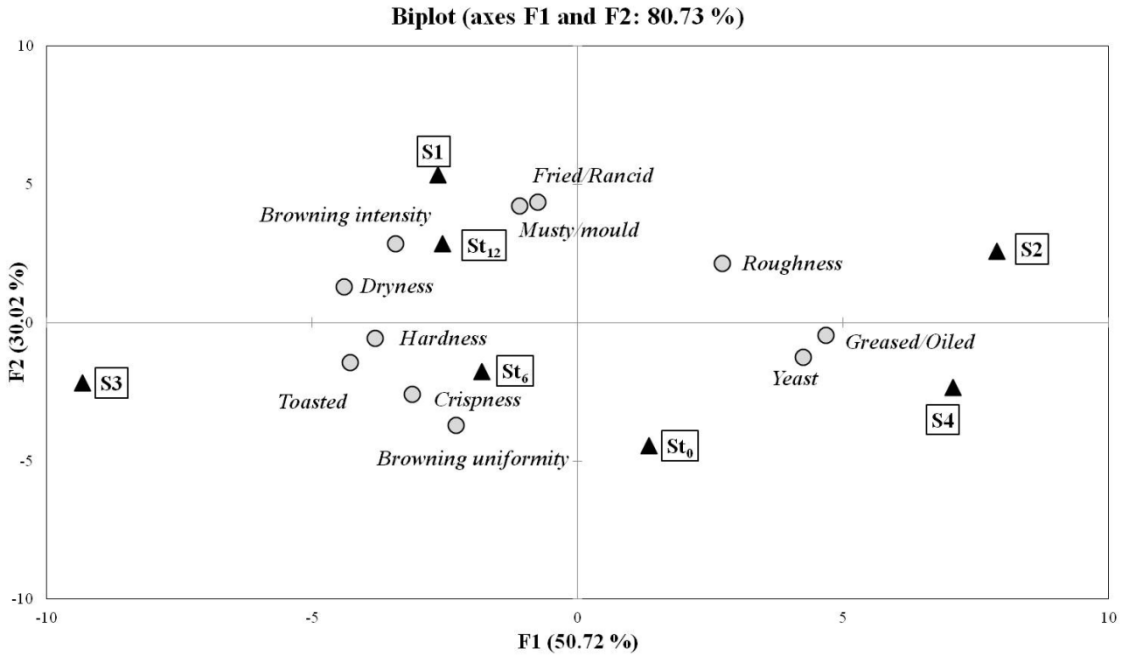

Fig. 2 PCA plot of *Taralli* samples built using sensory data

3.3.2. Discrimination test

The triangle test was carried out in three different sessions to obtain all possible combinations of comparisons between samples produced by the same company but stored for different months (St_0 *vs.* St_{12}; St_6 *vs.* St_0; St_6 *vs.* St_{12}). For triangle test, subjects with previously experience in the field of sensory analysis and with different age, gender and frequency of consumption of *Taralli* were interviewed. However, in order to increase the probability of finding a significant difference and the effectiveness of the results only assessors with familiarity with the product were selected. Data processing was performed by comparing the number of correct answers with the value reported in a double-entry statistical table of probability which indicates, in correspondence of the number of judges interviewed and of the different levels of significance, the minimum number of correct answers needed to conclude that there is a significant difference between each pair of tested products (Meilgaard et al. 1999). Results obtained in the three sessions are summarized in Table 2.

Data relative to the first session confirmed the results of DA analysis and showed statistically significant differences (α = 0.1) between St_0 and St_{12}, considering that 21 of the 48 tasters correctly identified the different sample. In this session, it was decided to interview a larger number of individuals (n=48) since it was the most important comparison considering the shelf-life of the product; if these results did not show any differences between samples at the initial and final time of production, it would be not necessary to proceed with the other comparisons.

In the second session (St_0 *vs.* St_6), only 10 of 36 subjects answered correctly and so there were no significant differences between samples. The same results were obtained even in the third session (St_{12} *vs.* St_6) in which only 9 of 36 judges gave the correct answer.

According to DA results', the sensory differences found between samples evaluated at the start (St_0) and at the end (St_{12}) of the storage time, were due to: the develpment of unpleasant odors (fried/rancid, musty/mould) related to the probable oxidation of the products and the reduction of the intensity of attributes linked to structural characteristics such as hardness and crispness.

Table 2. Sessions, number of subjects involved, number of correct answers given and significance level of the discrimination test (triangle test) conducted on *Taralli* samples with different storage time. The significance is expressed in terms of α-risk level. The X indicates that no significant perceptible difference between samples was found.

Discrimination test: *does a sensory difference exist between samples?*			
Sessions	Judges (n)	Correct answers	Significance (α)
St_0 *vs.* St_{12}	48	21	0.1
St_0 *vs.* St_6	36	10	X
St_6 *vs.* St_{12}	36	9	X

3.3.3. Texture analysis

The maximum peak force (F) corresponds to the hardness of the sample, while the linear distance (Ld) represents index of the sample's crispness. In Table 3 the average values of about 30 replicates for each sample obtained in the penetration test are reported. S1 was the hardest sample followed by S3 and the group composed of St_0, St_6, St_{12}. On the other hand, S4 and S2 were the least hard samples of the set in agreement with the results found in sensory analysis.

Regarding crispness, the same trend was observed in the DA panel: S3 was confirmed as the most crispy sample, followed by St_0, St_6, and St_{12} (mean values between 1011 and 1084), while S1 and S2 were the most friable samples showing values relative to the product between the number of peaks and the linear distance around 400 (data not shown).

Moreover, no differences in texture characteristics (hardness and crispness) between the three samples with the same composition but different production times were observed (St_0, St_6, St_{12}).

3.3.4. Browning area evaluation by computer vision system (CVS)

Images of *Taralli* were examined selecting total sample area and areas with different levels of browning, bound to NEB phenomena promoted by cooking. The images were elaborated in order to select the best combinations of threshold values (i.e. range of colour scales) to identify and segment the chromatic area visually associated to well cooked and poor cooked regions. Thus, on the basis of similar chromatic characteristics of the all samples, two colour patterns to recognize the two ROI's were built up and labeled using two virtual colours (Fig 3 a and b). On the basis of chromatic characteristics of all samples, two colour models were built up (Rocculi et al. 2005).

Specifically each *Taralli* sample was examined in order to select the colour ranges associated to well cooked portion ('dark' area, virtual colour red, Fig. 3a) and poor cooked portion ('light' area, virtual colour yellow, Fig. 3b).

This model was applied to each sample (27 replicates for each product) obtaining, the percentage of light and dark areas (Table 3). St_{12} had the highest percentage of dark areas.

S4 had the highest percentage of light areas, followed by St_0 and S2. The same colour patterns were applied and used for the analysis of all *Taralli* images.

Fig. 3 *Taralli* images and virtually coloured samples on the basis of different levels of browning area calculated with advanced image analysis software. Dark area, virtual colour red (a), light area, virtual colour yellow (b)

3.3.5. Correlations between sensory and instrumental data

Instrumental measures of textural (hardness and crispness) and appearance (browning intensity) properties were correlated with the intensity of sensory attributes used to describe the same characteristics in sensory analysis using the correlations matrix. Positive correlations, even if not particularly high, were found for both hardness and crispness measured with the two approaches (respectively, r = 0.413 and r = 0.617),

whereas the highest value of correlation (r = 0.821) was seen for the attribute browning intensity measured by sensory analysis and image analysis. The poor correlation between instrumental and sensory texture measurements could be due to several factors such as the non-homogeneity between samples and destructive nature of measurements that cannot be performed on exactly the same sample (Szczesniak 1968). Moreover, low values of correlation coefficients may be due to inherent differences in the two methods of evaluation. *Taralli* evaluated by a sensory test is subject to conditions that alter the structure of the sample. In fact, the effect of moisture and temperature in the mouth and the physical changes during chewing cause constant changes. *Taralli* samples instrumentally tested are subjected only to penetration forces (compression and cutting stress). Therefore, although samples were very similar at the beginning of the test, they were not comparable throughout the testing period (Brady and Mayer 1985).

3.4. CONCLUSIONS

The sensory methods applied in this work were effective for the assessment of different *Taralli* samples and permitted the evaluation of the quality characteristics. Eleven sensory attributes (3 descriptors related to the visual phase, 4 attributes for olfactory evaluation/aftertaste and 4 adjectives relative to texture) were useful to describe and discriminate *Taralli* samples. The sensory training and the panel's performance were monitored using the three-way analysis of variance, which showed judges had correctly understood the meaning of the used attributes and that all attributes were significant to describe the samples, especially those relating to the appearance and texture evaluation.

It was also evaluated whether it was possible to detect sensory differences among samples with the same composition, but stored for different lengths of time (St_0, St_6, St_{12}): the trained panel was able to discriminate the three samples mainly based on the evaluation of the texture attributes that change their intensity in function of the storage time. On the other hand, the triangle test provided a significant difference only between St_0 (just produced) and St_{12} (stored for 12 months). Two different physical tools able to evaluate the attributes of the browning intensity, hardness and crispness were also applied: browning intensity was detected by use of a specific computer vision system for acquisition, processing and image analysis, while the hardness and crispiness were measured by the penetration test using the texture analyzer.

A positive correlation between sensory and instrumental data were found, suggesting that simple and rapid physical tools can be useful to support sensory analysis in the evaluation of appearance of bakery products such as *Taralli*. On the other hand, between texture characteristics (hardness and crispness) evalued by the penetration test and sensory evaluation a lower correlation value were found.

This study could contribute to better defining product characteristics and its typicality; the perception of food quality during the storage time is also taken into account and could represent an useful indicator for producers. Moreover, this study suggests the use of instrumental methods able to support the sensory evaluation of physical properties of bakery products such as *Taralli*.

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Online resource 1 Sensory vocabulary used in this study: terms, definitions and reference of *Taralli* products**.**

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Sensory group	Descriptor	Definition	Reference* and its values on 0-100 scale
Appearance	Roughness	Presence of cuts, imperfections on whole surface of sample	light (0) dark (100)
	Browning intensity	Intensity of color	weak (0) strong (100)
	Browning uniformity	Uniformity of browning on whole surface of sample**	weak (0) strong (100)
Aroma and Flavor ***	Yeast	A fermented yeast-like flavor	dough made with flour, water and beer yeast (100)
	Toasted	The aroma associated with toasted notes in bakery products	weak (0) strong (100)
	Musty/mould	Intensity of musty/mouldy	weak (0) strong (100)
	Fried/rancid	Intensity of fried oil or rancid	International Olive Council defect of rancid (100)
Texture	Hardness	Intensity of the force required to first bite through the sample with the molars	weak (0) strong (100)
	Crispness	Intensity of noise during mastication	weak (0) strong (100)
	Dryness	Intensity of perception of a low content of water	weak (0) strong (100)
	Greased/oiled	Intensity of perception of soaking and exuding fat	weak (0) strong (100)

* references established by the panel after the training sessions.

** evaluated on several *Taralli* of the same sample

*** perceived by orthonasal and retronasal routes

Chapter 4

Sensory and rapid analytical methods as a combined tool for quality control of cooked ham

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ABSTRACT

In this preliminary investigation, different commercial categories of Italian cooked pork hams have been characterized using an integrated approach based on both sensory and fast instrumental measurements. For these purposes, Italian products belonging to different categories (cooked ham, "selected" cooked ham and "high quality" cooked ham) were evaluated by sensory descriptive analysis and by the application of rapid tools such as image analysis by an "electronic eye" and texture analyzer.

The panel of trained assessors identified and evaluated 10 sensory descriptors able to define the quality of the products. Statistical analysis highlighted that sensory characteristics related to appearance and texture were the most significant in discriminating samples belonged to the highest (high quality cooked hams) and the lowest (cooked hams) quality of the product whereas the selected cooked hams, showed intermediate characteristics.

Also physical-rheological parameters measured by electronic eye and texture analyzer were effective in classifying samples. In particular, the PLS model built with data obtained from the electronic eye showed a satisfactory performance in terms of prediction of the pink intensity and presence of fat attributes evaluated during the sensory visual phase.

This study can be considered a first application of this combined approach that could represent a suitable and fast method to verify if the meat product purchased by consumer match its description in terms of compliance with the claimed quality**.**

Highlights

Italian cooked pork hams were analysed by sensory and fast instrumental analysis.

Sensory analysis resulted effective to define the quality of the product.

Physical-rheological parameters are effective to support the sensory analysis.

The electronic eye allows the prediction of visual attributes.

Keywords: cooked pork ham, sensory properties, texture analysis, electronic eye.

4.1 INTRODUCTION

Cooked pork ham as meat product made from entire pieces of muscle meat, belongs to the cured cooked meat category which after the curing process of the raw muscle meat, always undergoes heat treatment to achieve the desired palatability (Heinz & Hautzinger, 2007).

Cooked pork ham is a very common product that is consumed worldwide, and is the cured meat product most consumed in Italy (ASSICA, 2014), even if it is not included among Protected Geographical Indications (PGI) or Protected Denominations of Origin (PDO) products. However, the Italian market offers a wide variety of cooked hams that are classified in three different commercial categories: cooked ham, "selected" and "high quality" cooked ham (Ministerial Decree, G.U. n 231, 04.10.2005).

According to Italian regulations, the specifications established for each class of product define the raw materials, allow ingredients and aromas, adopted processing method and some physical and sensory characteristics (visual recognition of major thigh muscles of the pork leg, water content, etc.). However, the sensory properties that characterize the product and strongly influence consumers' choice are not well defined in these specifications (Ministerial Decree, G.U. n 231, 04.10.2005).

The final quality of cooked ham depends on both the raw materials and the processing techniques. In particular, the factors that are mainly involved concern the type of meat cut, type and amount of ingredients, injected volume of brine, rate and extent of tumbling, cooking time, and temperature (Delahunty et al., 1997).

Cooked ham has a typical light pink colour as a consequence of nitrite addition. During the heating process, the colour of ham changes from red (pork meat) to pink; this physical characteristic depends primarily on the initial content of myoglobin present in the muscles used, and, consequently, is dependent upon the muscle type and age of the animal at slaughter (Toldrá, Mora, & Flores, 2010).

Visual appearance is a key factor in the consumer perception of the sensory quality of meat and meat products. In addition to the traditional color measurement (L*, a*, b* values in CIELAB colour space), various image processing techniques find widespread use as objective and non-destructive quality evaluation systems. The hyperspectral imaging

(HSI) is a promising technology that integrates conventional imaging and spectroscopy to attain both spatial and spectral information from the sample; this techniques has been successfully applied to ham quality evaluation and control allowing to collect information about different physico-chemical properties (ElMasry, Sun, & Allen, 2012; Iqbal, Sun, & Allen, 2013; Iqbal, Sun, & Allen, 2014). On the other hand, also the conventional image analysis represents an useful tool to the study of products' colour, especially considering its cost effectiveness, consistency, speed and accuracy provided by its automated application (Brosnan, & Sun, 2004). In particular, computer vision has been used for the assessment of meat products to study the relationship between the presence and/or the change of appearance characteristics such as colour, aspect, size and distribution of fat-connective tissue and specific ingredient or technological process applied (Sánchez et al., 2008; Fongaro, Alamprese, & Casiraghi, 2015).

Textural characteristics are also very important for the quality of cooked hams and depend on several factors that are related to chemical constituents (water, fat, protein, connective tissue content etc.), chemical reactions (entity of proteolysis and lipolysis prior to cooking) and processing variables such as the extent of heating (Toldrá, Mora, & Flores, 2010), cooling treatment used (Desmond et al., 2000), smoke flavourings used and storage time (Martinez et al., 2004).

Another highly appreciated characteristic in this product is represented by its flavor, which is mostly related to processing conditions, brining, and spices added. The typical flavor (smell and taste) of the product is due to quantitative variations in free amino acids and fatty acids generated by enzymatic reactions mainly through proteolysis and lipolysis, but the amount of released amino acids and fatty acids depend on the extent of resting before cooking. Further chemical modifications (i.e. Strecker and Maillard reactions) contribute to the generation of aroma volatile compounds (Toldrá, Mora, & Flores, 2010; Utrera et al., 2012). Very few studies have investigated cooked ham and its physical and chemical properties in relation with the sensory profile to characterize the product, evaluate its quality, and test consumers' knowledge and acceptance (Delahunty etal., 1997; Válková et al., 2007; Tomovic et al., 2013; Henrique, Deliza, & Rosenthal, 2015).

Others studies have focused on the classification of cooked hams manufactured with pork legs produced in different countries and with different percentages of brine injection by a

chemometric approach based on the physical and chemical parameters (Casiraghi, Alamprese, & Pompei, 2007; Moretti et al., 2009). However, the results from all these investigations are not always easily comparable because they take in account different raw materials and processing procedures (Tomovic et al., 2013).

The aim of the present study was to analyze Italian cooked pork hams belonging to the main commercial categories for quality control by applying a combined approach of sensory (descriptive analysis) and fast instrumental (image and texture) analysis.

4.2 MATERIALS AND METHODS

4.2.1 Samples

The research was carried out on commercial brands of cooked pork ham belonging to different product categories: cooked ham (CH); "selected" cooked ham (SE), and "high quality" cooked ham (HQ). The main characteristics of these three classes are reported in **Table 1.** A specific profile sheet was established: 15 samples (5 for each category) were analyzed to develop sensory vocabulary and train the panel group before sensory evaluation. The final score was the average of the scores assigned by each judge to these samples in three different sessions. Textural and appearance properties were measured on the whole set of samples. All cooked hams (pieces of about 5 kg) were stored at 4°C, vacuum packed, protected from light, and all analysis (sensory profiling and instrumental) were carried out in several replicates.

4.2.2 Sensory characterization by Descriptive Analysis (DA)

Samples were tasted by a panel of 8 assessors, balanced in terms of gender, varying in testing experience, and previously trained in the assessment of cooked ham. All of them were regular consumers of cooked ham and interested in the study. They were all trained according to ISO 8586:2012 and ISO 13299:2010. The training proceeded in 3 sessions: (i) definition of each descriptor of the sensory vocabulary and the training; in this step the panellists chose a list of 10 non-overlapping attributes that permit a descriptive analysis of the samples under study and, at the same time, represent an useful tool also for the quality control of the product; (ii) assessment of the intensity and the memorization of the scale; (iii) sensory evaluation and monitoring of performance of selected assessors in terms of repeatability, discriminatory capacity and reproducibility.

Table 1. Characteristics of different commercial categories of cooked ham (Ministerial Decree, G.U. n 231, 04.10.2005). Ingredients/additives that differ between CH, SE and HQ samples are shown in italic. [1]MDDP = moisture on deffated-deadditived product.

Category	Raw materials	Ingredients/Additives	MDDP[1]
COOKED HAM **(CH)**	Pork leg	Sodium chloride Protein (milk and soy) Starches (native or modified) Polyphosphate Sugar (dextrose, lactose, fructose, glucose syrup) Ascorbic acid Lactate Glutamate Nitrate and nitrite Wine Spices and aromas	≤81
SELECTED **(SE)**	Pork leg in which it is possible to identify at least 3 of the 4 major muscles	Sodium chloride Protein (milk and soy) Starches (native or modified) Polyphosphate Sugar (dextrose, lactose, fructose, glucose syrup) Ascorbic acid Lactate Glutamate Nitrate and nitrite Wine Spices and aromas	≤78.5
HIGH QUALITY **(HQ)**	Pork leg in which it is possible to identify at least 3 of the 4 major muscles	Sodium chloride Sugar (dextrose, lactose, fructose, glucose syrup) Ascorbic acid Lactate Glutamate Nitrate and nitrite Wine Spices and aromas	≤75.5

The conventional profiling method was applied (Meilgaard, Civille, & Carr, 2007). The final list of descriptors included 3 relative to appearance: typical appearance (recognition of major muscle), pink intensity (intensity of colour), presence of fat (total amount of fat inside the slice); 3 perceived by orthonasal and retronasal routes: overall aroma (intensity of total aroma of the product), spices and flavours (intensity of spices and other flavours), smoky (aroma associated with smoked notes in meat products); 2 gustatory: sweet (basic taste), salt (basic taste); 2 relative to the texture: cohesiveness (resistance to the product separation, to be assessed during the first 3-4 bites), juiciness (amount of juice released from the product during mastication).

Rating of the attribute's intensities was done using a linear unstructured 100 mm scale anchored at their extremes (0: absence of sensation; 100: maximum of sensation intensity) and results were expressed as the mean of three replicates. Samples were coded with three-random numbers and were presented to the assessors presented in randomized blocks. Between samples, a break with water rinses and unsalted bread sticks was suggested to reduce the carry-over effects as much as possible. To make it easier to understand and evaluate visual attributes, a group of product images were provided to each judge as references. These images were selected taking into account the previously results of the training session and were used to illustrate the maximum, the minimum or average intensity of typical appearance, pink intensity and presence of fat. Moreover, in order to standardize the testing conditions as much as possible and avoid bias, panellists evaluated visual attributes by observing the same slice of product inside a plate, whereas evaluation of other attributes (smell, taste, and texture) was performed by providing assessors with a sample minced and placed in plastic cups.

4.2.3 Image analysis

The instrumental measurement of appearance was carried out by an "electronic eye" (visual analyzer VA400 IRIS, Alpha MOS, France), a high-resolution CCD (charge-coupled device) camera combined with powerful data processing software. This instrument was equipped with an adjustable photo-camera (16 M colours) in a dedicated measurement room with standardized, controlled and reproducible lighting conditions. The camera imaging was software-monitored, embedded in the cabin for a better protection adapted to quality control environment and equipped with several lenses of

different focal length available to accurately assess very small to large products.Top and bottom lighting (2*2 fluorescent tubes) 6700°K colour temperature and IRC=98 (near D65: daylight during a cloudy day at 12 AM). It has to be turned on 15 minutes at least before acquisition for lighting stabilization. Samples were placed on a removable white tray, diffusing a uniform light inside the device's 600 × 600 × 750-mm closable light chamber and the CCD camera takes a picture.

The instrument is able to undergo automatic calibration with a certified colour checker, and image analysis (RGB scale or CIE L*a*b*) and statistical analysis were carried out using the advanced software available in the instrument (Alphasoft, version 14.0). The data processing software extracts color parameters from the picture and can then correlate these data with data from sensory panels.

4.2.4 TA-Hdi® texture analyzer

Textural characteristics of HQ, SE, and CH cooked hams were evaluated at 22°C using a TA-Hdi® texture analyzer (StableMicro Systems, UK) equipped with a 245 N loading cell. Texture profile analysis (TPA), Allo-Kramer (AK) shear force, expressible moisture (EM), and gel strength were assessed in 10 replicates for each sample.

TPA, consisting in a double compression, was run on a 1 cm-high and 2 cm-wide cylindrical-shaped sample compressed up to 40% of its initial height. The test was performed using a 5 cm-diameter aluminium probe and a time of 20 sec was elapsed between two compression cycles. Force-time deformation curves were obtained and hardness (N), springiness, cohesiveness, chewiness (N), and gumminess (N) were calculated according to Bourne (1978).

Shear force test was performed using an A-K shear cell (10 blades) and a cross-head speed of 500 mm min^{-1} according to the procedure described by Bianchi et al., 2007. From each cooked ham, a 4 × 2 × 1 cm sample was excised, weighed, and sheared perpendicularly to the direction of muscle fibers. Shear force was then calculated as N shear per g of sample.

Expressible moisture (%) was measured following the procedure proposed by Hoffman, Hamm, & Bluchel, 1982) with some modifications. A 4 × 1 × 0.3 cm sample was cut, weighed, and placed between four filter papers (Whatman No. 1). The sample was compressed through a single compression cycle with a load of 12.15 N for 5 min and the

amount of water released per gram of meat was calculated, conventionally expressed as percentage.

Lastly, gel strength (N × cm) was assessed on a 1 cm-high and 2 cm-wide cylindrical-shaped sample using a 5 mm stainless steel spherical probe according to the procedure described by Petracci et al., 2009.

4.2.5 Statistical analysis

Instrumental data (AK-shear force, gel strength, expressible moisture, hardness, springiness, cohesiveness, chewiness, and gumminess) and the intensity of each sensory attribute (typical appearance, pink intensity, presence of fat, overall aroma, spices and flavours, smoky, sweet, salt, cohesiveness and juiciness) were analyzed with a one-way-ANOVA or Kruskal-Wallis (in case of significance of the Levene test) to test the effect of market category (HQ, SE, and CH). Sensory and physical data were explored by principal component analysis (PCA). Pearson's correlations between sensory and instrumental data were performed to check possible relations. Partial Least Square (PLS) regression was also applied to predict sensory attributes by instrumental variables. A cross-validation method was employed to validate PLS models. The precision and the predictive capabilities of the models were evaluated by the coefficients of determination (R^2) and root-mean square error estimated by cross-validation (RMSECV). All statistical analysis were performed by XLSTAT 7.5.2 version software (Addinsoft).

4.3 RESULTS AND DISCUSSION

4.3.1. Sensory analysis

Before analytical evaluation of samples, the reliability of the panel's performance and training efficiency was checked to ensure reproducibility and repeatability (data not shown). Sensory profiling results (**Table 2**) showed that, in general, all visual attribute intensities followed an upward trend passing from CH, SE, and HQ samples; on the other hand, regarding texture attributes, there was a decreasing trend for juiciness and a growing trend of cohesiveness intensity going from CH, SE, and HQ. On the contrary, olfactory and taste attributes did not appear to be able to differentiate the commercial class to which a product belonged.

	Overall aroma	Spices and flavours	Smoky	Sweet	Salt	Typical appearance	Pink intensity	Presence of fat	Cohesiveness	Juiciness
CH	55^{b}	40^{ab}	10^{a}	46^{a}	49^{a}	37^{b}	33^{c}	40^{c}	39^{c}	49^{a}
SE	56^{b}	36^{b}	12^{b}	45^{a}	49^{a}	57^{a}	53^{b}	52^{b}	52^{b}	44^{b}
HQ	60^{a}	42^{a}	19^{b}	48^{a}	45^{a}	59^{a}	62^{a}	57^{a}	61^{a}	36^{c}

Table 2. Sensory data of cooked hams (n=5/group) measured by the panel of trained assessors using the DA method. CH, cooked ham; SE, "selected" cooked ham; HQ, "high quality" cooked ham. Mean values followed by different letters significantly differ between the categories (p<0.05).

These results are in agreement with previous studies present in literature which found appearance and texture sensory attributes as more significant in describing and differentiate hams than flavour descriptors (Nute et al., 1987), also when the sensory evaluation was carried out by consumers (Delahunty et al., 1997).

Fig. 1 shows the results obtained from PCA of sensory data: samples and sensory attributes with greater discriminating power were projected in a two-dimensional surface, described by orthogonal factors used as dimensions (F1 and F2) to highlight differences or similarities among analyzed samples. The first two components explained 84.87% of the total variance (66.27% for PC1 and 18.59% for PC2). In particular, almost all of HQ and SE samples were close and located between the first and the second quadrant; they were characterized, above all, by the highest intensity of pink intensity, typical appearance and cohesiveness, and, at the same time, by the lowest intensity of juiciness. In the third and fourth quadrants all CH samples and one SE sample, that showed the lowest intensity of all visual attributes and the highest value of juiciness, were positioned.

Similar results were observed also by Tomović et al., (2013) in a study performed on cooked hams processed with different carcass chilling methods (rapid and conventional) and time of deboning in which the colour panel score increased with decreasing juiciness.

Moreover, a recent study of Henrique, Deliza, & Rosenthal (2015) in which the Check-All-That-Apply (CATA) questionnaire was applied for the sensory characterization of cooked ham by consumers, showed that appearance attributes (characteristic ham aspect, intense pink colour, uniform aspect) and texture ones (juicy, tender) were positively correlated with the preference and the willingness to pay whereas a pale colour had a negative influence on liking.

In the present study the sensory traits mainly ascribed to the high quality product category are: pink intensity, typical appearance and cohesiveness.

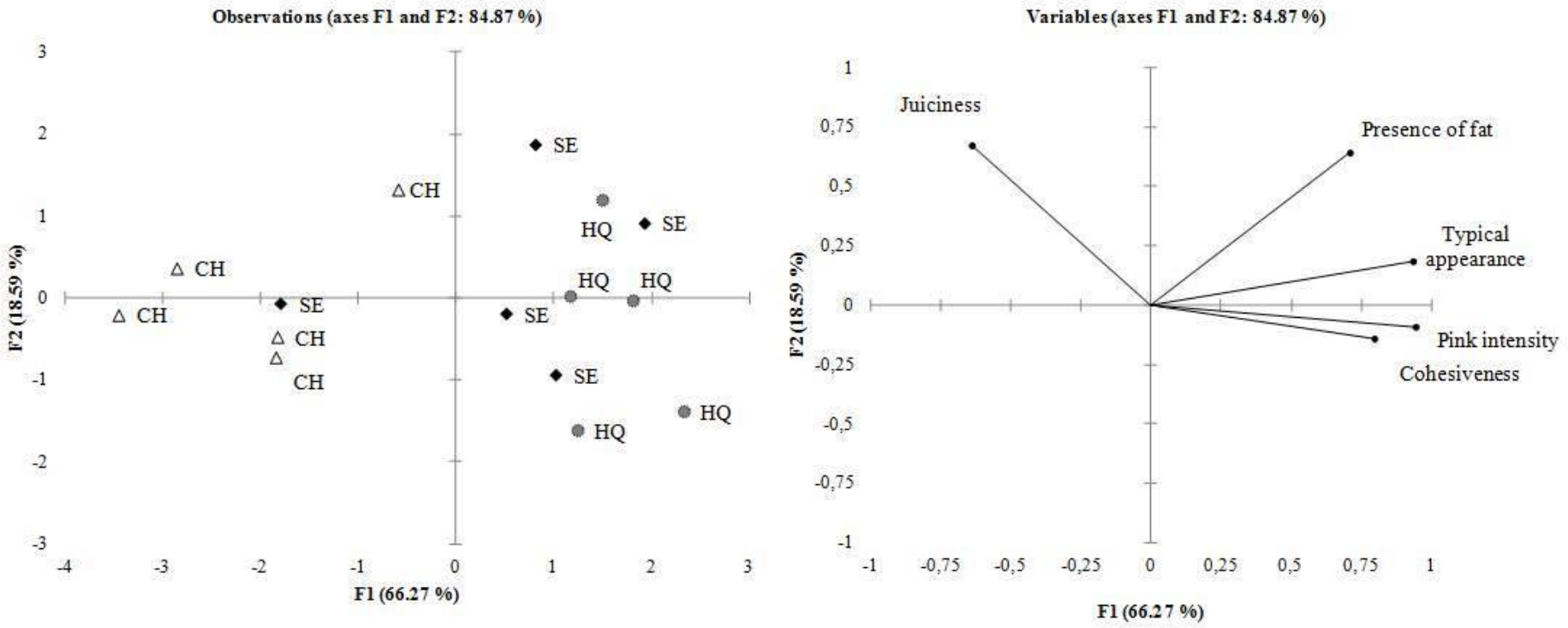

Figure 1. Principal component analysis (PCA) of sensory data evaluated by descriptive analysis (DA) (loading plot on the right side). CH, cooked ham; SE, "selected" cooked ham; HQ, "high quality" cooked ham (score plot on the left side).

4.3.2 Image analysis

To characterize the product's appearance, an "electronic eye" able to quickly assess this property using an acquired image subsequently processed by a specific software, was used. Data processing by the electronic eye allowed to obtain a colour spectra of a sample in RGB coordinates (Red, Green, Blue) that could be used to differentiate samples according to different hues and uniformity of colour. The application of the software available in the instrument (Alphasoft, version 14.0) allowed to group colour spectra in range of 16 bit for each coordinates RGB obtaining 4096 variables shown as histograms. In **Fig. 2**, some examples of colour spectra from samples belonging to each of the three commercial categories are shown. The proportion of each colour in the analyzed image, on a fixed scale of 4096 colours, is represented as a percentage. It is a color map of the object and the dashed line represents the minimum percentages of the colors displayed in the color spectra.

In particular, for CH, greater colour homogeneity described by the predominance (> frequency percentage) of a lower number of bars (colours) corresponding to different tonality of pink was seen; on the contrary, for categories "selected" (SE) and "high quality" (HQ), the trend was reversed and the number of bars increased passing from SE to HQ. These results are in contrast with Iqbal et al., (2010) who found that inhomogeneous colour surfaces characterize the lowest quality class, when the images of three qualities of pre-sliced pork with different brine injection level were compared. However, these authors indicated that the lack of homogeneity is due to the presence of discoloured sections of pork muscles while, in this study, is mainly linked to the presence and the visual recognition of major thigh muscles of the pork leg.

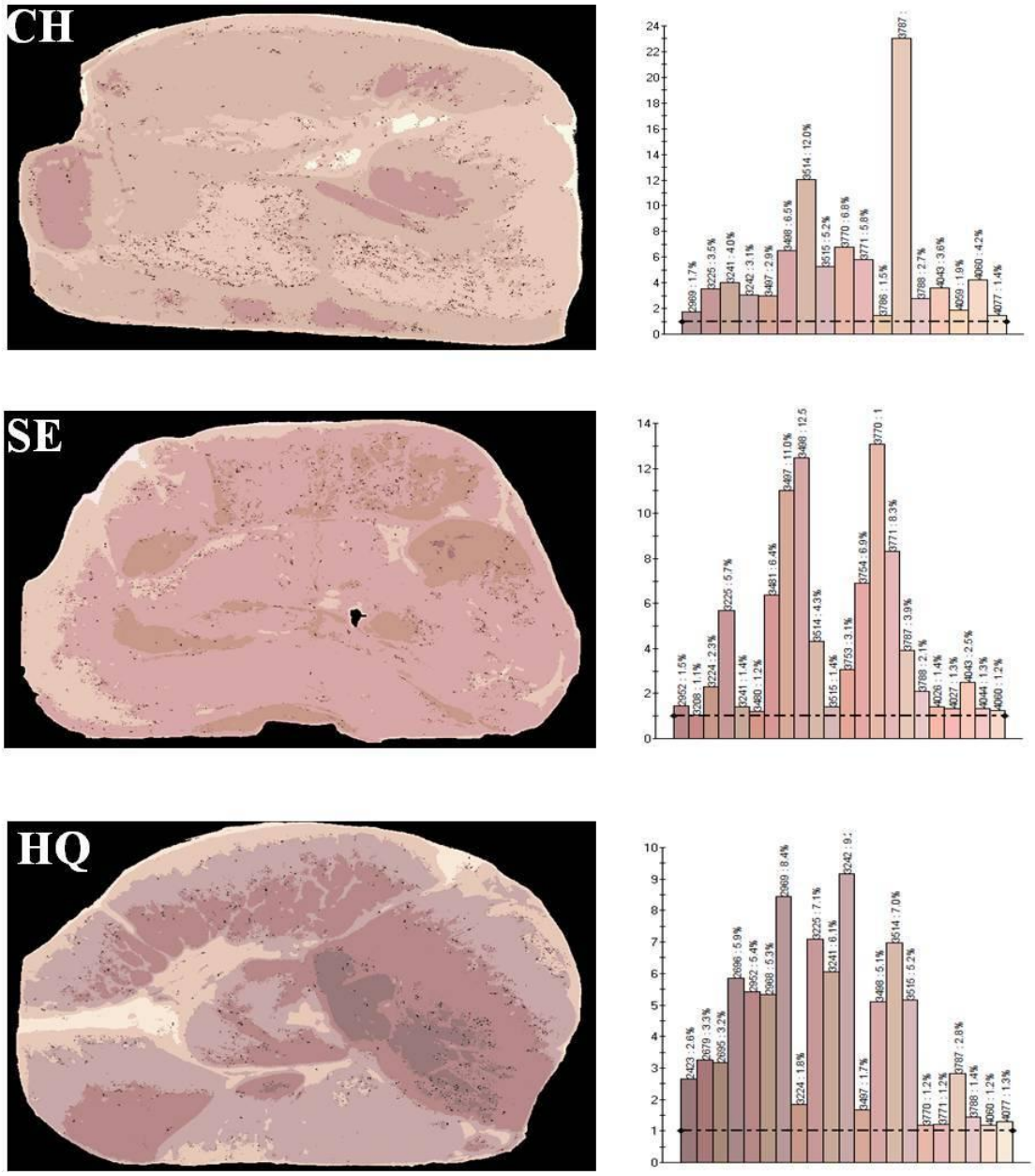

Figure 2. Examples of color spectra obtained from the data processing by the electronic eye. CH, cooked ham; SE, "selected" cooked ham; HQ, "high quality" cooked ham.

To evaluate its ability in discriminating the different categories of cooked ham, data collected by electronic eye on the five samples of each commercial class were processed by PCA (**Fig. 3**). A selection of the most discriminant variables has been performed in order to improve the separation between samples. The first two components explained 80.68% of the total variance (62.00 for F1 and 18.68% for F2). Considering the locations of products on the surface (PCA score) is possible to note that HQ and SE samples were

quite grouped in a cluster, whereas CH samples were clearly differentiated from HQ and SE but divided in two groups mainly as a function of F1. The different direction/location of vectors (PCA loadings), shows which variables (colours) were involved in the appearance variations among samples. Variable "colours-2679" which describe the strongest pink intensity affected mainly the position of HQ samples, on the contrary, variable "colours-3514" which describe the weakest pink intensity, was opposite and characterized some CH samples.

These differences were probably linked to intrinsic variable of raw material such as the different water content that affected the concentration of meat pigments and therefore the ham colour (Moretti et al., 2009).

The PCA score plot shows a good discrimination between samples: the lowest quality class (CH) was clearly differentiated from the highest one (HQ); however the intermediate category (SE) did not significantly differ from HQ and belong to the same cluster. This is in accordance with the study of Iqbal et al., (2010) who reported that it is easier to differentiate between the lowest and the highest qualities in function of their colour uniformity, homogeneity and fat content and therefore confirms the effectiveness of specific image descriptors of colour in checking the quality specifications.

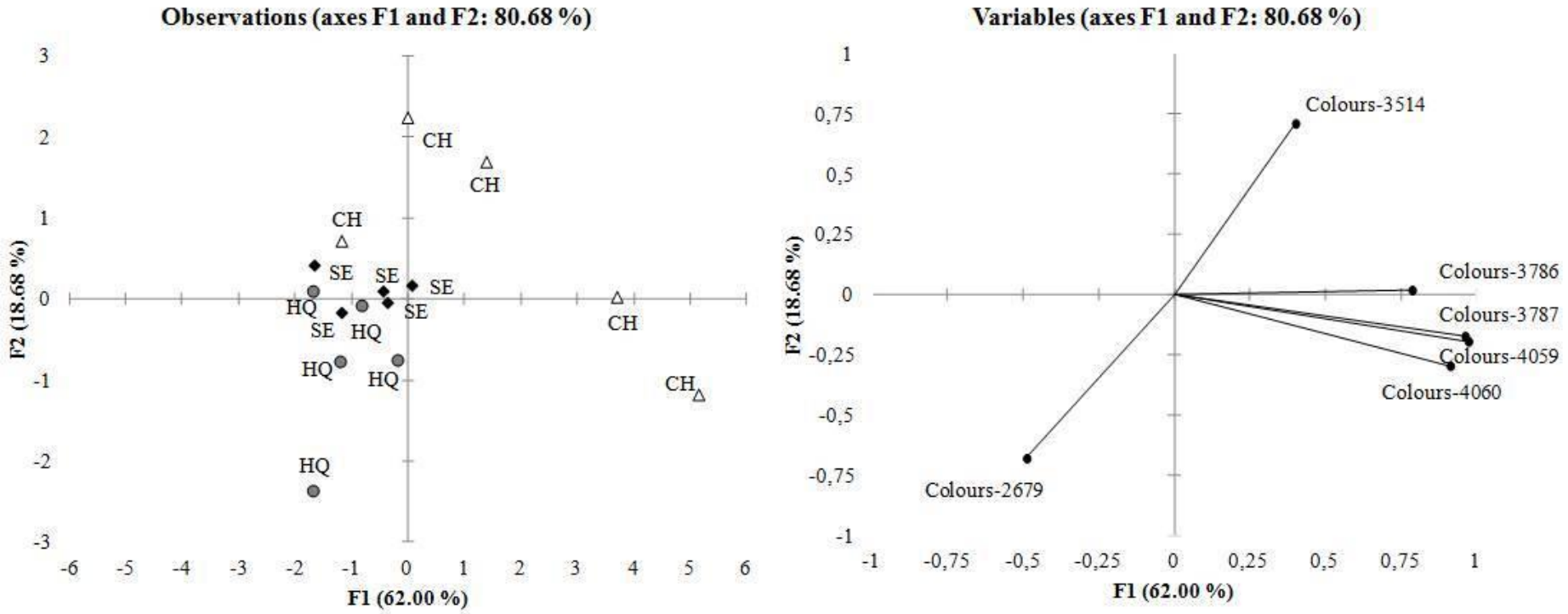

Figure 3. Principal component analysis (PCA) built using data related to visual characteristics evaluated by electronic eye (loading plot on the right side). CH, cooked ham; SE, "selected" cooked ham; HQ, "high quality" cooked ham (score plot on the left side).

4.3.3 TA-Hdi® texture analyzer

The data for gel strength, expressible moisture, sheaf force, and TPA parameters are summarized in **Table 3**. HQ ham had a lower expressible moisture compared with CH (12.9 vs. 18.6%; $p<0.05$), while SE hams exhibited intermediate values (16.5%). In addition, HQ samples had higher shear force (28.15 vs. 18.23 and 19.72 N/g; $p<0.05$) and springiness (1.62 vs. 1.29 and 1.31; $p<0.05$) than CH and SE samples, which did not differ each other. On the other hand, gel strength, cohesiveness, hardness, gumminess, and chewiness were not substantially different between groups. Overall, these results showed that instrumental traits of HQ hams are different compared with both CH and SE, which seem to be more related, especially considering textural traits. These differences were likely due to the complex dissimilarities such as raw meat characteristics, ingredients, brine injection level, and processing among products of different market categories as noted in previous studies (Casiraghi, Alamprese, & Pompei, 2007; Válková et al.,. 2007; Moretti et al., 2009; Pancrazio et al., 2015). Lower expressible moisture in HQ hams was likely due to the lower total moisture imposed by national legislation. Moreover, HQ hams had also higher shear force and springiness because whole muscles were used, and a lower brine injection level was also found by Casiraghi, Alamprese, & Pompei (2007). This agrees with the results of Válková et al., (2007) who found that shear force and springiness were negatively and positively correlated, respectively, with moisture content. Casiraghi, Alamprese, & Pompei, (2007) did not find any differences in product cohesiveness when hams with increasing brine injection level were compared.

The results of PCA analysis of instrumental texture parameters are shown in **Fig. 4**. Two principal components were extracted that accounted for 74.88 % of the variance in the 8 variables. The first PC was mainly defined by the instrumental traits of gumminess, chewiness and hardness and gel strength , while the second PC was characterised by three instrumental parameters (AK-shear force, springiness, and cohesiveness). Expressible moisture appeared to equally contribute to both PCs. A good discrimination between HQ and the other classes of products (CH and SE) was observed. Positive PC2 values were associated with HQ samples, one SE ham and one CH thus confirming that AK-shear force, springiness, cohesiveness were mainly involved in product category discrimination.

Otherwise, hardness, gumminess, chewiness, and gel strength seem to independently vary within each market category.

Parameter	Categories			sem	*p-value*
	STD	SCE	AQ		
Number of samples	5	5	5		
Gel strength (N × cm)	12.68	12.45	13.01	0.81	0.965
Expressible moisture (%)	18.6 a	16.5 ab	12.9 b	0.99	0.049
Shear force (N/g)	18.23 b	19.72 b	28.15 a	1.89	0.045
Texture Profile Analysis (TPA)					
Cohesiveness	1.68	1.62	1.88	0.05	0.113
Hardness (N)	50.47	78.97	79.07	7.94	0.252
Gumminess (N)	131.94	125.01	93.12	12.10	0.417
Springiness	1.29 b	1.31 b	1.62 a	0.06	0.033
Chewiness (N)	169.68	161.68	149.35	16.99	0.862

Table 4. Textural properties of cooked hams (n=5/group) measured by TA-Hdi® texture analyzer and reported in Newton (N). CH, cooked ham; SE, "selected" cooked ham; HQ, "high quality" cooked ham. Mean values followed by different letters significantly differ between the categories ($p<0.05$). sem = standard error of mean.

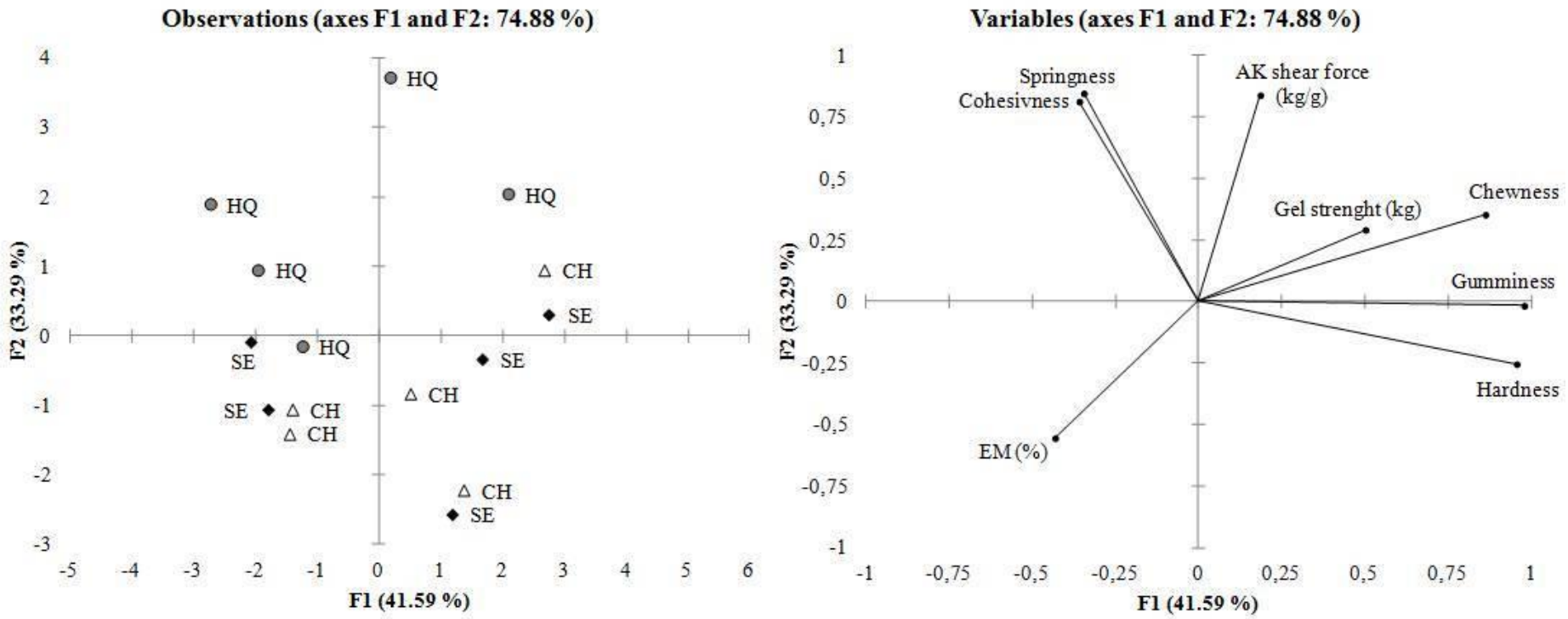

Figure 4. Principal component analysis (PCA) built using data related to textural characteristics evaluated by texture analyzer (loading plot on the right side). CH, cooked ham; SE, "selected" cooked ham; HQ, "high quality" cooked ham (score plot on the left side).

4.3.4 The relationship between sensory and instrumental data

The data obtained from both sensory and instrumental approaches were also statistically assessed to determine possible correlations; the visual attribute of pink intensity was correlated with physical parameters with Pearson's correlation coefficient ranging between 0.57 and 0.72 ($p < 0.05$).

In particular the pink intensity attribute showed a positive correlation with AK shear force (0.62) , springiness (0.57) and with the variable "Colours-2679" (0.72). A negative correlation was found, instead, with the variable "Colors-3514" (-0.66).

On the other hand, no significant correlation was discovered between the attribute presence of fat and instrumental measurements (appearance and texture), in agreement with previous studies (Válková et al., 2007).

Considering the texture sensory attributes, only juiciness showed some negative correlations with instrumental parameters of AK shear force (-0.79), cohesiveness (-0.54) and springiness (-0.63) ($p < 0.05$). Among texture instrumental parameters, positive correlations was found between: gumminess and hardness (0.95) as already observed by Válková et al. (2007), springness and cohesiveness (0.76), chewness and hardness (0.75) and also between chewiness and gumminess (0.89) ($p < 0.05$).

In addition, some correlations were obtained also among sensory attributes: pink intensity showed significant positive correlations with typical appearance (0.84) and cohesiveness (0.72) and a negative one with juiciness (-0.64) ($p < 0.05$); the latter result was in accordance with Tomović et al., (2013) who reported a similar correlation coefficient (-0.51, $p < 0.05$).

The instrumental dataset and the sensory attributes related to them was also subjected to PLS regression with the aim to estimate a prediction model for sensory characteristics. For visual and texture sensory attributes (cohesiveness, juiciness, pink intensity and presence of fat), models using data coming from electronic eye and texture analyzer were developed. All PLS results were showed in **Table 4**.

	Sensory attribute (*y*)	R^2	RMSECV
Texture analyzer	Cohesiveness	0.24	9.87
	Juiciness	0.48	37.99
Electronic eye	Pink intensity	0.95	3.24
	Presence of fat	0.88	5.84

Table 4. Coefficients of determination (R^2) and root mean square errors calculated in cross validation (RMSECV) estimated for specific sensory characteristics by PLS models built using texture and visual instrumental data.

The best results were obtained from models developed using electronic eye data that allowed an effective prediction of pink intensity (R^2= 0.95, RMSECV = 3.24) and presence of fat (R^2= 0.88, RMSECV = 5.84) as showed by **Fig. 5.** For colour prediction, the developed model was better than that obtained by Iqbal et al., (2013) in cooked, pre-sliced turkey hams though by another image system (NIR hyperspectral imaging).

4.4 CONCLUSIONS

In this investigation, the application of physical-rheological and sensory techniques were able to provide useful information for quality control of Italian cooked ham samples. Sensory analysis resulted effective in defining the profile and the quality of the product. Among sensory attributes, those relating to appearance (pink intensity, typical appearance, and presence of fat) and texture (cohesiveness and juiciness) were the most effective in describing the class of ham providing a significant discrimination especially between the lowest quality market category (CH) and the other two higher quality categories (HQ and SE).

Data obtained by electronic eye were in agreement with sensory ones; on the other hand, considering physical-rheological parameters, AK-shear force, expressible moisture, springiness, and cohesiveness were able to clearly discriminate only the premium class ("high quality") from each others.

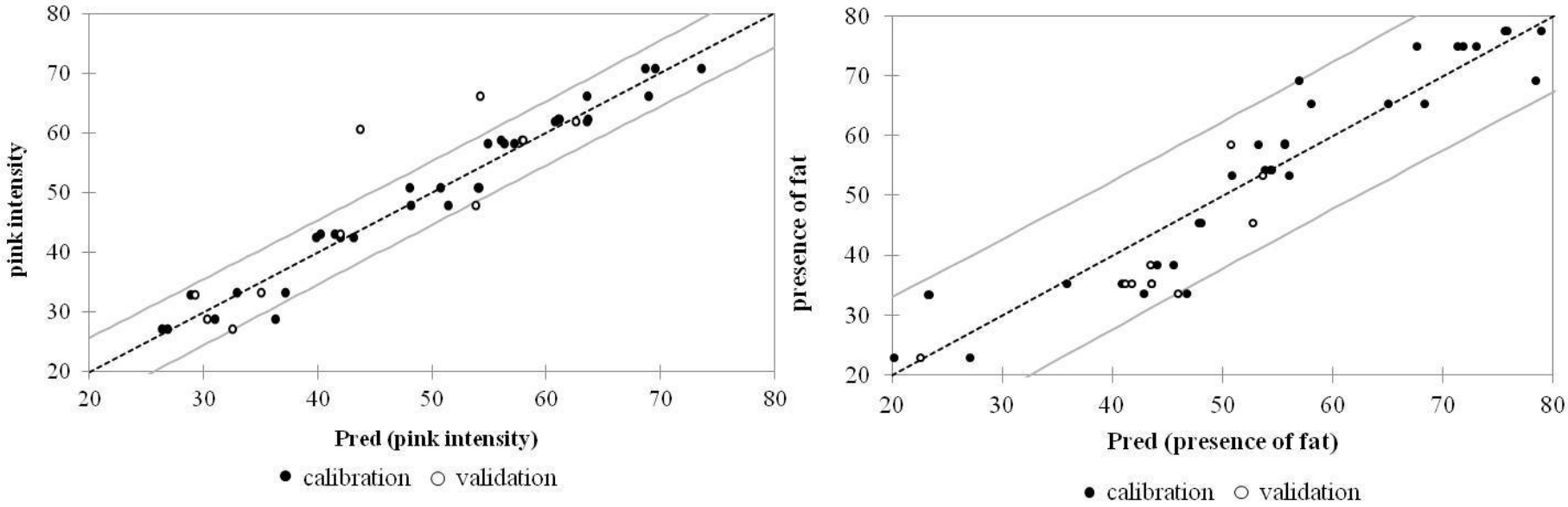

Figure 5. Predicted vs. measured plot from PLS model developed for "pink intensity" and "presence of fat" sensory attributes by means of instrumental data from electronic eye. Calibration and validation data are shown as black and white dots, respectively.

The electronic eye applied in this study allowed to develop a PLS models with a promising value of prediction of visual attribute of presence of fat and pink intensity (R^2= 0.88, RMSECV = 5.84 and R^2= 0.95, RMSECV = 3.24, respectively).

Based on these preliminary results, the use of physical-rheological parameters could be proposed to complement sensory analysis, for example in the definition of reference standards and for rapid quality control of different categories and classes of the same product. This study permitted to hypothesize a preliminary model for a fast and effective screenings to be conducted by a "one-day" experimental plan suitable for the quality control also of other categories of meat products. This analytical approach could be particularly interesting for food providers, buyers and retailers that intend to protect and promote these products to better addressing consumer needs and enhancing their competitiveness on the market. However, further efforts aimed to differentiate and certify a higher quality product and to improve consumer's knowledge and to direct them towards a more informed choice, are needed.

Acknowledgments

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Chapter 5

Do consumers recognize the positive sensorial attributes of extra virgin olive oils related with their composition? A case study on conventional and organic products.

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***Abbreviations*:** EVOO, extra virgin olive oil; LOX, lipoxygenase; JAR, just about right; VOO, virgin olive oil; LOX, lipoxygenase; P.D.O., protected designation of origin; IOC, International Olive Council; FA, free acidity; PV, peroxide value; FAME, fatty acid methyl esters; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; OA/LA, oleic acid/linoleic acid; BI, bitterness index; ESI, electrospray interface; SPME, solid phase micro-extraction; PREFMAP, preference mapping; BL, blind test; IN, informed test; CONV, conventional; ORG, organic; HU, heavy users; LU, light users; TP, total phenols; *o*-DPH, ortho-diphenols; HY, hydroxytyrosol; TY, tyrosol; VA, vanillic acid; SY, syringic acid; DOA, decarboxymethyl oleuropein aglycon; LUT, luteolin; DLA+Acpin, decarboxymethyl ligstroside aglycon + acetoxypinoresinol; API, apigenin; Oagl, oleuropein aglycon; Lagl, ligstroside aglycon.

Keywords: extra virgin olive oil, Phenolic compounds, Volatile compounds, IOC Panel test, sensory acceptance, Agricultural production method, Health, Food composition, Food analysis.

Chemical compounds studied in this article

Tyrosol (PubChem CID: 10393); Hydroxytyrosol (PubChem CID: 82755); Apigenin (PubChem CID: 5280443); Luteolin (PubChem CID: 5280445); Decarboxymethyl ligstroside aglycon or Oleocanthal (PubChem CID: 11652416); 1-penten-3-ol (PubChem CID: 12020); (*E*)-2-hexenal (PubChem CID: 5281168)

ABSTRACT

Consumers perceive the sensory characteristics of extra virgin olive oils (EVOOs), but they are not always able to relate the positive sensory attributes to the presence of healthy substances (e.g., polyphenols) and, in general, to appreciate the overall quality of the oils. In the present work, consumers' preferences and influence of information concerning the agricultural production method on consumer behavior were investigated. EVOOs samples were evaluated in terms of sensory attributes, basic chemical parameters, volatile and phenolic molecules. The results showed that the majority of the interviewed consumers appreciated "fruity" attribute, but disliked what they perceived as bitterness. Organic farming information did not affect their judgment. The chemical and sensory analyses confirmed the relationships between the presence of minor compounds and the positive sensory attributes; positive correlations were found among bitter, pungent vs. decarboxymethyl oleuropein aglycon (ranged from 23.8 to 143.8 mg kg^{-1}) and decarboxymethyl ligstroside aglycon, as well as between green notes and the volatile compound 1-penten-3-ol (C_5-LOX alcohols, 0.1-0.9 mg kg^{-1}). Nevertheless, consumers seemed indifferent to the more health-promoting EVOOs, preferring an "uneducated" sweeter taste. This result points to the need for much more consumer education concerning "genuine" and "native" taste of extra virgin olive oil and its health related properties.

5.1. INTRODUCTION

The flavor of extra virgin olive oil (EVOO), which is the combined effect of odor (perceived via orthonasal and retronasal routes), taste and chemical responses (such as pungency), makes EVOO unique and distinguishable from other vegetable oils. It is well known that the sensory quality of VOO (virgin olive oil) is mainly due to the presence of minor compounds, such as volatile and phenolic molecules (Aparicio and Guadalupe, 2002); nevertheless, the evaluation of profiles in these minor compounds are not recognized among the numerous official chemical parameters provided by European Regulations on assessment of the quality and genuineness of VOO. The volatile compounds are primarily involved in the flavor of EVOOs and include the principal components responsible for the positive fruity attribute, characteristic of an oil obtained from healthy, fresh fruits, both ripe or unripe (Angerosa et al., 2004). Phenolic substances

affect bitter taste and pungent perception (Bendini et al., 2007) and they also play a very important role in product stability against oxidative modification (Carrasco-Pancorbo et al., 2005; Gallina Toschi et al., 2005). Recently, the effect of phenolic compounds on the release and perception of volatile compounds of VOO, was studied by Genovese et al. (2015) by adopting simulated *in vitro* mouth conditions; such investigation lead to interesting findings about a possible "physicochemical trapping effect" performed by specific phenolic compounds on some defined aroma compounds. Several studies on the possible correlation between the sensory attributes and the qualitative and quantitative profile of phenolic compounds in VOOs have been carried out. In particular, bitterness and pungency perceptions have been linked to the content of specific secoiridoids (Andrewes et al., 2003; Bendini et al., 2007; Gutierrez-Rosales et al., 2003; Mateos et al., 2004). On the other hand, numerous volatile compounds formed by the lipoxygenase (LOX) pathway and chemically divided into different classes (aldehydes, alcohols, ketones, esters and penten dimers) are known to be responsible both for the fruity attribute and secondary pleasant olfactory notes in VOO, such as green notes (Kalua et al., 2007). On the contrary, the main off-flavours (sensorial defects) originates from sugar fermentation (winey), anaerobic microorganisms (muddy), branched amino acid production (fusty), enzymatic activities of molds (musty) and to auto-oxidative process (rancid) (Bendini et al., 2012).

Consumers are not always able to recognize, understand or appreciate the intrinsic attributes that define the quality of a specific food product such as EVOO: this is not due to their reduced sensory acuity, but to different traditions, culinary habits and nutritional education, which are all factors that may influence consumer behavior (which is not always directed to the highest quality products) (Tuorila et al., 1998). Some investigations have highlighted how positive sensory attributes for EVOO such as bitter and pungent are actually negative drivers of liking (Delgado and Guinard, 2011; Recchia et al., 2012; Valli et al., 2014). On the other hand, consumers defined bitter and pungent as the most appropriate attributes to describe this product (Caporale et al., 2006) and as drivers of their preferences (Hassine et al., 2015). Different attitudes towards bitterness, pungency and fruitiness are also seen in Italian consumers (Predieri et al., 2013) and these can be explained by different levels of familiarity with EVOO and eating habits; on the other hand, neither the involvement or the predilection for this product are able to guarantee

consumer recognition of high quality products (Recchia et al., 2012). For example, even if most Californian consumers considered EVOO to be a 'healthy' food, most were also unaware of the bioactive components of EVOO or their specific health benefits (Santosa et al., 2013).

Many authors have emphasized the importance on several types of information on consumer behavior, especially those related to the geographical origin, brand, health, packaging, production method and processing technologies. Differences between ratings of satisfaction when expressed without (blind) or with information (informed) on the product have been reported by different authors, confirming that the perception of quality is strongly influenced by the expectations created by such information (Caporale et al., 2006; Cardello, 2003; Carrillo et al., 2012; Laureati et al., 2013; Varela et al., 2010). Recently, Caporaso et al. (2015) found particularly high polyphenols contents in Italian EVOOs covered by Protected Designations of Origin (PDOs), thus permitting, for some of them, also the inclusion in the label of the health claim that ''olive oil polyphenols contribute to the protection of blood lipids from oxidative stress'' (EU Reg. 432/2012).

Based on the above considerations, this study investigated selected EVOOs present on the Italian market and was performed to: (*i*) evaluate the influence of information concerning organic or conventional production methods of extra virgin olive oils (EVOOs) on consumers behavior; (*ii*) investigate the factors that can lead to product acceptability; (*iii*) verify the relationship between the presence of minor compounds (volatile and phenols) with the associated sensory perceptions.

5.2 MATERIALS AND METHODS

5.2.1 Samples

Eight samples (coded as "S1-S8") sold as EVOOs were purchased from an Italian supermarket. Table 1 summarizes coding and information on the samples. These EVOOs were selected in order to represent the variety of EVOOs available on the Italian market, according to the following screening criteria: balanced number of conventional and organic samples; two samples belonging to an Italian protected designation of origin (Italian P.D.O.) and one monocultivar EVOO (*cv* coratina, Apulia); samples sold in three price ranges, at high (> 8 €/L), medium (5-8 €/L) and low price (< 5 €/L) and presence of

samples characterized by different intensities of fruitiness, bitterness and pungency (a preliminary sensory analysis on a larger set of samples was performed as described in the paragraph below). All samples were stored at 12°C in the dark before analysis.

SAMPLE CODE	SAMPLE INFORMATION	GEOGRAPHICAL ORIGIN	PRICE RANGE
S1	EVOO (organic)	ITALY	M
S2	EVOO (conventional)	ITALY	M
S3	EVOO (organic)	ITALY	M
S4	EVOO (organic)	ITALY	M
S5	EVOO (conventional)	European Union	L
S6	EVOO P.D.O. (conventional)	ITALY (Sicily)	H
S7	EVOO P.D.O. (conventional)	ITALY (Emilia-Romagna)	H
S8	EVOO *cv* Coratina (organic)	ITALY (Apulia)	M

Table 1. Information, features and coding of extra virgin olive olis (EVOOs) samples. P.D.O., EVOOs produced according to Protected Denomination of Origin; price range: L, low price (< 5 €/L); M, medium price (5–8 €/L); H, high price (> 8 €/L).

5.2.2 Analytical sensory evaluation by a trained panel

The IOC (International Olive Council) Panel test method was carried out by a group consisting of nine trained assessors of the Professional Committee of DISTAL (Department of Agricultural and Food Sciences of University of Bologna, recognized by the Ministry of Agricultural, Food and Forestry Policies). Positive and negative descriptors were selected and adopted according to the official procedure (EC Reg. 640/2008). Moreover, evaluation of green notes and other positive attributes was carried out with reference to the list of descriptors established for P.D.O. EVOOs, according to the IOC standards (IOC/T.20/Doc. no 22., 2005). The level of intensity of each descriptor was

graded by judges using a continuous unstructured line scale of 10 cm. Each 15 mL sample was tasted in a normalized cup (Menietti Enologia snc, Italy) at 28 ± 2°C in a tasting booth, regulated in terms of shape and equipment (IOC/T.20/Doc. no 5., 2007). Results were expressed as the median values of the tasters' sensory perceptions. The robust coefficients of variation were calculated and validated (acceptable values ≤ 20%), according to EC Reg. 640/2008.

5.2.3 Hedonic sensory evaluation by consumers

The samples were subjected to an acceptance test carried out in an Italian supermarket (Liguria region) by a group of 60 consumers. Participants were recruited and selected using predetermined screening criteria based on purchasing frequency of organic food consumption, gender and age. In particular, they were split into two subgroups based on high (heavy users) or low frequency (light users) of organic food consumption (according to their answer about frequency of consumption as "several times a week or more" and "several times a month or less", respectively). The consumer group consisted of 70% heavy users and 30% light users; regarding sex, 57% were female and 43% male; the main age groups were from 20 to 50 years (20–30 years old, 30%; 31–40, 30%; 41–50, 28%), whereas consumers older than 50 years old were less represented (51–60, 7%; 61–80, 5%). EVOOs were served at room temperature (±20°C) in plastic cups; white bread was provided as a carrier. Consumers were asked to express their judgment on the degree of overall acceptability of each sample (appearance, smell, taste, mouth-feeling) and on the intensity of selected attributes among those used by the Panel of experts (see supplemntary material S1), using a 9-point hedonic scale ranging from 1 to 9 (1 = do not like at all and 9 = like very much). All evaluations, except for the degree of overall liking and the intensity of negative attributes, were also assessed using a 5-point just about right (JAR) scale from 1 to 5 (1 = way too little, 2 = too little, 3 = just about right, 4 = too much, 5 = way too much). The central location consumer test was realized in two sessions (blind and informed conditions) on two days to test if product information affected the consumer purchase decision. During the first tasting day, participants performed the blind test; the day after, the same participants were invited to perform the informed test (information on the production method were available during their evaluation). In the blind test, each consumer evaluated all the samples, in order to have 60 judgments for each of the 8 samples. In the informed test, consumers were asked to taste 10 samples

with information about the organic/conventional farming system: actually, on the basis of the blind test results, the most liked conventional and organic EVOO were resubmitted for evaluation (during the second tasting day), but information on their production methods (organic or conventional) was inverted. For data collection, eight PCs with the FIZZ software ver. 1.31 (Biosystemes, Couternon, France) installed, were used.

5.2.4 Chemical solvents and reagents

Methanol and water for HPLC analysis (respectively purity ≥99.9% and non-volatile matter ≤0.0003%), clorophorm (purity >99%), acetic acid (purity ≥99.7%), ethanol (purity ≥99.9%), isooctane for spectrophotometry (purity ≥99.9%), diethyl ether (purity >99%), sodium thiosulfate (purity ≥98%), potassium iodide (purity ≥99%), Folin-Ciocalteu reagent, sodium carbonate anhydrous (purity ≥99.9%), sodium molybdate dehydrate (purity ≥99 %), potassium hydroxide (purity ≥98 %), phenolphthalein solution 2% in ethanol were all purchased from Sigma-Aldrich (St. Louis, MO, USA).

5.2.5 Basic quality parameters

Basic quality parameters of samples, such as free acidity (FA) calculated as the percentage of oleic acid, peroxide value (PV) expressed as meq of active oxygen per kg of oil (meq O_2 kg^{-1}), spectrophotometric indices (K_{232}, K_{270} and ΔK) were evaluated according to official methods (EU Reg. 61/2011). All analyses were determined in triplicate for each sample.

5.2.6 Fatty acid composition

The fatty acid composition was determined as fatty acid methyl esters (FAMEs) by gas chromatography (GC) analysis, after alkaline treatment according to the official method (EU Reg. 61/2011). FAMEs were analyzed by using a Clarus 500 gas chromatograph from Perkin Elmer (Shelton, CT, USA) equipped with a flame ionization detector (FID), according to Bendini et al. (2006). For each chemical determination, three replicates were analyzed for each sample. FAMEs were identified by comparing the retention time of compounds with a Nu-Check GLC – 463 standard mixture (Nu Check, Elysian, MN, USA) injected in the same analytical conditions. Results were determined in triplicate for each sample and expressed as percentage of each fatty acid of the total.

5.2.7 Extraction of polar phenolic extracts

A liquid-liquid extraction, performed according to Carrasco-Pancorbo et al. (2007), was used to extract the phenolic compounds from EVOOs. The dried extract was dissolved with 5 mL of methanol/water (50:50, v/v) and an aliquot was filtered through a 0.45 μm filter (VWR, West Chester, PA, USA) before HPLC analysis. For spectrophotometric determinations, the extract was further diluted 1:5 (v/v) using the same mixture mentioned above. Three replicates were analyzed for each sample. The extracts for spectrophotometric assays were stored at - 18°C before use.

5.2.8 Determination of total phenols and ortho-diphenols by a spectrophotometric method

The total phenolic content was spectrophotometrically determined at 750 nm by the Folin-Ciocalteu reagent following the protocol described by Bendini et al. (2006). The content of spectrophotometric *o*-diphenol was evaluated at 370 nm using the sodium molybdate dihydrate reagent, according to Mateos et al. (2001). Both assays were measured with a UV-VIS 1800 Shimadzu spectrophotometer (Shimadzu Corporation, Tokyo, Japan).

The total phenol and *o*-diphenol concentrations were quantified using two specific calibration curves built by using gallic acid (Fluka, Buchs, Switzerland) as standard. Data were expressed as mg of gallic acid per kg of oil and the analysis was carried out in triplicate for each sample.

5.2.9 Determination of bitterness index

Evaluation of bitterness index (BI K_{225}) in polar extracts was carried out spectrophotometrically at 225 nm according to Gutièrrez et al. (1992), with some modifications. The phenolic extract, obtained as described previously, was diluted (1:250) with methanol/water (50:50, v/v) solution and the absorbance at 225 nm was measured against a solvent reference in a 1-cm quartz cuvette. Three replicates were measured out for each sample.

5.2.10 Determination of phenolic compounds by HPLC-DAD/MSD

High performance liquid chromatography (HPLC) analysis was carried out using a HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA), equipped with a

binary pump delivery system, degasser, autosampler, HP Diode Array UV-VIS Detector (DAD) and HP Mass-Spectrometer Detector (MSD). A Zorbax Eclipse XDB-C_{18} (Phenomenex, St. Torrance, CA, USA) column (5 µm particle size, 25 cm × 3.00 mm ID) was used. All analyses were carried out at room temperature. The wavelengths were set to 280 nm and 330 nm. Quantification of phenolic compounds (tentatively identified by comparing retention times, UV-VIS and mass spectra with pure standards and data present in literature) was performed using calibration curves of 3,4-dihydroxyphenylacetic acid for compounds with maximum absorption at 280 nm (Fluka, Buchs, Switzerland) (5–1000 mg L^{-1}) and caffeic acid for compounds having maximum absorption at 330 nm (Fluka) (5–1000 mg L^{-1}, r^2 = 0.9995). The gradient elution was carried out using the conditions described by Carrasco-Pancorbo et al. (2007). The detection was made using quadrupole MS with an electrospray (ESI) interface operating in positive ion mode within m/z 50-800 range and adopting the following conditions: drying gas flow, 9 L min^{-1} at 350°C; nebulizer gas pressure, 50 psi; capillary voltage, 3000 V. Nitrogen was used as both nebulizer and drying gas. Three replicates were analyzed for each sample.

5.2.11 Analysis of volatile compounds

Volatile compounds present in the headspace of samples were concentrated by SPME and separated by gas chromatography coupled with quadrupolar mass-selective spectrometry using an Agilent 6890N Network gas chromatograph and an Agilent 5973 Network detector (Agilent Technologies). In particular, a 1.5 g amount of sample was weighed into a 10 mL vial. The oil sample was spiked with 0.15 g of the internal standard 4-methyl-2-pentanone (Sigma Aldrich), prepared in refined olive oil at a concentration of 5 µg g^{-1}. The vial was fitted with a silicone septum, placed in a water bath at 40°C (± 2°C) and here maintained under magnetic stirring for 2 min. Then, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (50/30 µm, 2 cm long, from Supelco Ltd., Bellefonte, PA, USA) was exposed to the sample headspace for 30 min and immediately desorbed for 3 min at 250°C in the gas chromatograph injector port. Volatile compounds were separated on a ZB-WAX column (30 m, 0.25 mm i.d., 1.00 µm film thickness, (Phenomenex). Column temperature was held at 40°C for 10 min and increased to 200°C (held for 2 min) at 3°C min^{-1}; then the temperature increased at 10°C min^{-1} up to 250°C (held for 2 min). The ion source and the transfer line temperatures were set at 230°C and 250°C, respectively. Electron impact

mass spectra were recorded at 70 eV ionization energy in the 30-250 amu mass range at 2 scans s^{-1}. The identification of volatile compounds was first carried out by comparison of their mass spectral data with the information from the National Institute of Standards and Technology (NIST) library (2005 version) and later checked with pure standards. Relative amounts of volatile compounds were expressed as mg of internal standard (4-methyl-2-pentanone) per kg of oil, according to the analytical protocol described by Baccouri et al. (2008). Quantification of volatile compounds was carried out as a sum of specific classes and single volatile compounds associated with flavor (aldehydes C_6, alcohols C_6, esters C_6, ketones C_5, alcohols C_5, pentenic dimers, hydrocarbons, terpenes) and off-flavor compounds that mainly contribute to sensory defects (winey: methyl acetate, ethyl acetate, methanol, ethanol and acetic acid; fusty-muddy: 3-methyl-1-butanol acetate, 1-butanol, 2-methyl-1-butanol; musty: octanoic acid, octane, 1-octanol; rancid: sum of saturated aldehydes, unsaturated aldehydes, furans, 6-methyl-5-hepten-2-one, butanoic acid and hexanoic acid). All determinations were carried out in triplicate.

5.2.12 Statistical analysis

The software XLSTAT 2011.1.03 version (Addinsoft, USA) was used to elaborate chemical and sensory data by analysis of variance (ANOVA), principal component analysis (PCA) and preference mapping (PREFMAP). Student's *t*-tests ($p < 0.05$) were also carried out in order to establish if there was a significant difference for the hedonic ratings between the blind and informed tests and between heavy (frequent) and light (infrequent) consumers of organic food.

5.3. RESULTS AND DISCUSSION

5.3.1 Sensory evaluation by the trained Panel

None of the samples included in this study presented any sensorial defects (EC Reg. 640/2008), and thus all samples were classified as EVOO. The intensity of the most important positive sensory attributes (fruity, bitter, pungent) evaluated by the Panel showed some differences among the analyzed samples (Fig. 1) and allowed to describe EVOOs with the optional terms that could be used for labeling (EC Reg. 640/2008): with regard to fruity, there was a first group with medium intensity of this attribute (S2, S3, S4, S6, S7, S8) and a second one with light intensity (S1, S5). S6 showed the highest intensity of fruity (6.0), which is the limit value to define medium and intense levels. In

terms of bitter and pungent intensities, samples showed a similar trend: S1 and S6 were characterized by light intensities of these attributes, while samples S3, S4, S5 and S7 were judged to have medium intensity. The exceptions were samples S2 and S8, which showed an intense perception of bitter taste (6.1 and 6.4, respectively). In summary, S6 was characterized by the highest intensity of fruity, green and other positive attributes perceived by smell but, on the other hand, this sample was low in bitter and pungent taste; S8 was characterized by the highest intensities of bitter and pungent taste and had medium intensities of fruity and green by smell. S5 and S1 were balanced for the taste attributes, but low in green notes and other positive attributes (median values < 2).

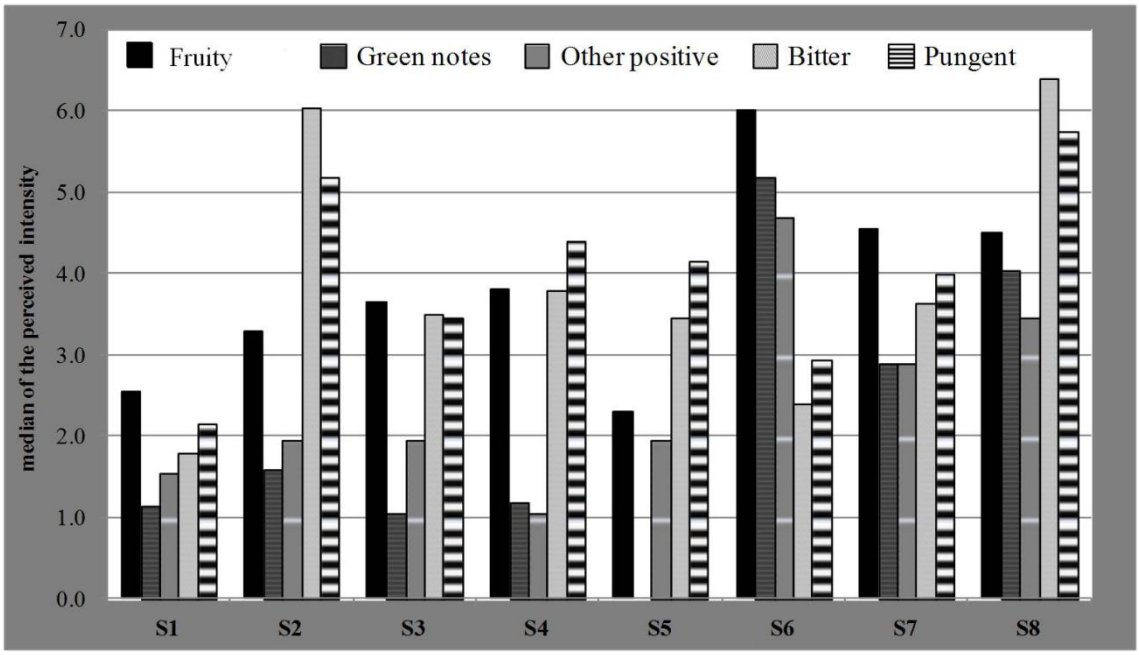

Fig. 1. Positive attributes and their intensity (median values) estimated by the recognized professional committee DISTAL (Department of Agricultural and Food Sciences of University of Bologna).

5.3.2 *Hedonic sensory analysis by consumers*

Considering data related to consumer preferences expressed in the blind session (Fig. 2a), S6 (conventional) and S1 (organic) were significantly more liked than S2 and S8, which were the least liked. The overall liking registered for S7 (conventional) was not significantly different from the mean value obtained for S6. S1 and S7 were again proposed in the informed test with the opposite information (the organic S1 was indicated as conventional and the conventional S7 was passed off as organic). Significant differences were found for overall liking in the blinded versus informed test: S6 and S1

were characterized by significant lower values of overall liking in the informed test compared with the blind one; S3, S2 and S8 showed the opposite trend, so that the overall liking was higher in the informed test. When S1 was labeled as conventional it was significantly better liked than S7 when it had been labeled as organic. For the other samples, no significant differences were found.

Observing the mean of the overall liking scores given by the judges with different frequency of organic food consumption (heavy or light users), a slightly tendency of light users to score less than heavy users in both conditions (blinded and informed) was seen (Fig. 2b). In the informed test, when S1 was labeled as conventional, both heavy and light users scored higher. On the other hand, when S7 was labeled as organic there were no clear difference concerning overall liking. Also while the number of interviewed consumers was limited, the results indicated that organic farming information did not affect the judgment of consumers surveyed, who, however, differentiated and rewarded only the products that best met their expectations concerning the sensory characteristics of the products. It is possible that this result may also be due to the test situation in which consumers are forced to evaluate sensory quality of the products, whereas in the real purchase conditions at the grocery or supermarket they may be more influenced by information contained on the labels.

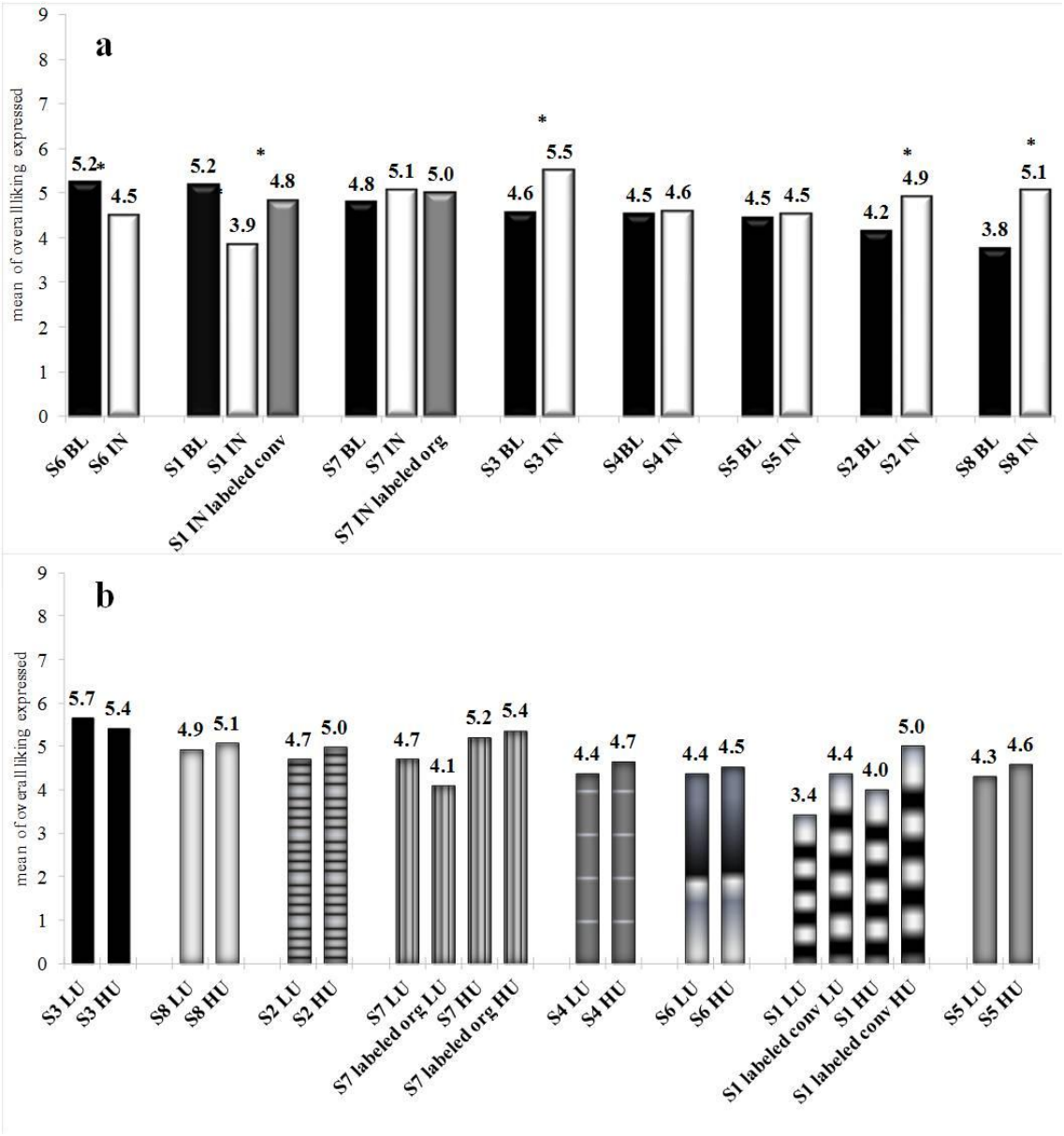

Fig. 2. (a) Comparison of overall liking results (n = 60): blinded versus informed consumers data. Samples are shown in decreasing order according to the degree of overall liking expressed during the blinded session. BL = blind test; IN = informed test; (b) comparison of overall liking results: heavy (n = 42) and light users (n = 18) both in informed test. HU = heavy users; LU = light users. Conv = conventional; org = organic. Results marked with an asterisk differ significantly, Fisher LSD, $p < 0.05$.

Considering the information of the JAR scales in the consumer test, the results related to the intensity of bitter (Fig. 3) were particularly interesting: all samples were rated as "just about right" by 30-40% of consumers; only S8 and S2 were perceived as "too much" or "way too much" bitter by about 50% of consumers. On the other hand, concerning the intensity of pungent, all samples were perceived as "too little" or "way too little" pungent by about 50% of consumers (data not shown).

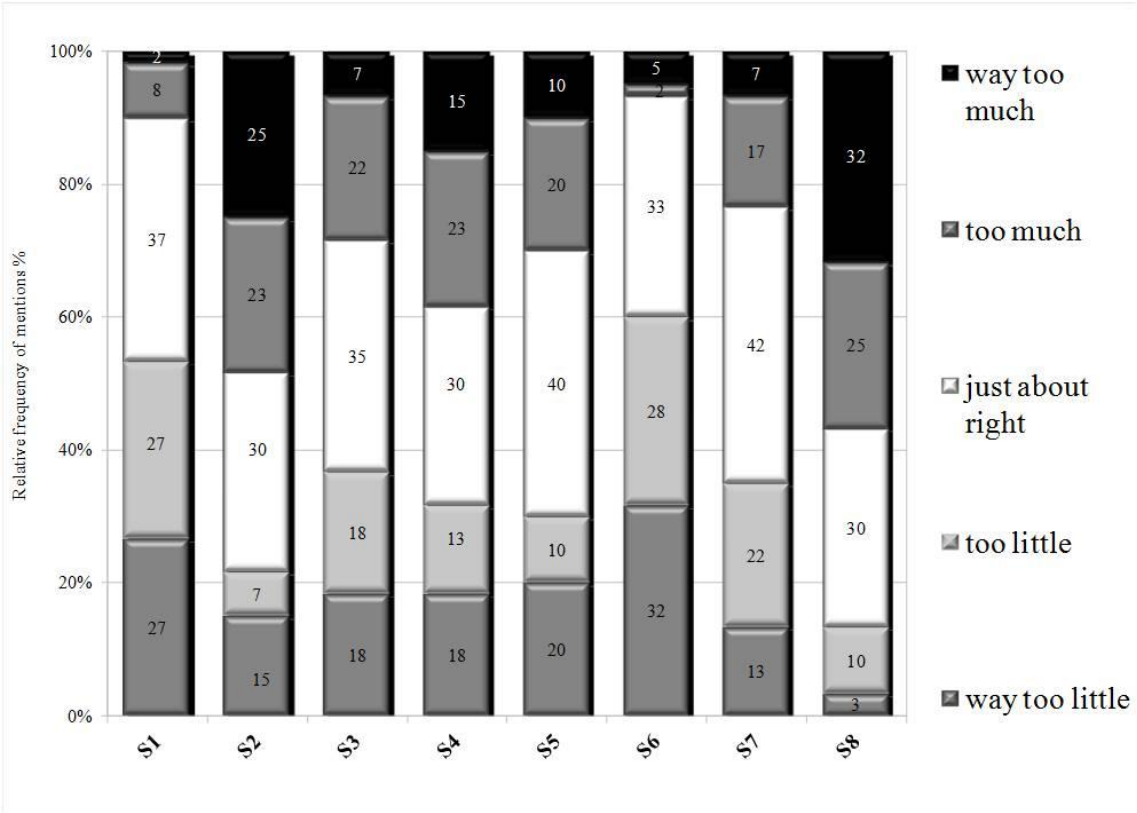

Fig. 3. Results of the JAR scale consumer test (n = 60) regarding intensity of bitter attribute in the blind test. S1 (organic); S2 (conventional); S3 (organic); S4 (organic); S5 (conventional); S6 (conventional); S7 (conventional); S8 (organic).

5.3.3 Quality indices

The values of chemical quality parameters are presented in Table 2. Concerning the FA, K_{232}, K_{270} and ΔK, all samples showed values under the respective limits fixed for EVOOs (EU Reg. 61/2011); PV were also generally under the limits, except for S4 which presented a higher content (around 28 meq O_2 kg^{-1} oil) than the limit for EVOOs, suggesting poor oxidative status. Fatty acid composition (Table 3) of all EVOOs was generally characterized by a high percentage of monounsaturated fatty acids (MUFA, 73.8-75.6%) and relatively low percentage in saturated fatty acids (SFA, 15.6-16.6%) and

polyunsaturated (PUFA, 8.6- 9.8%), according to the typical range for EVOOs (EU Reg. 61/2011).

Quality indices	FA%	PV	K_{232}	K_{270}	TP	*o*-DPH	BI K_{225}
Sample	Mean	Mean	Mean	Mean	Mean	Mean	Mean
S1	0.3_{bc}	15_{c}	1.74_{b-d}	0.14_{ab}	197.7_{e}	57.1_{e}	0.28_{c}
S2	0.5_{a}	17_{b}	2.01_{ab}	0.18_{a}	254.0_{c}	80.0_{c}	0.34_{b}
S3	0.3_{c-e}	17_{b}	2.34_{a}	0.17_{ab}	231.3_{d}	90.2_{b}	0.35_{b}
S4	0.2_{e}	28_{a}	2.29_{a}	0.19_{a}	327.9_{b}	46.6_{f}	0.43_{a}
S5	0.4_{b}	14_{d}	1.38_{d}	0.19_{a}	218.3_{d}	68.9_{d}	0.33_{bc}
S6	0.3_{bc}	13_{e}	1.81_{b-d}	0.14_{ab}	94.7_{g}	47.2_{f}	0.20_{d}
S7	0.3_{b-d}	11_{f}	1.46_{cd}	0.11_{b}	159.6_{f}	61.0_{de}	0.32_{bc}
S8	0.3_{de}	13_{d}	1.89_{a-c}	0.15_{ab}	428.1_{a}	114.0_{a}	0.48_{a}
EU Reg. 61/2011	≤0.8	≤20	≤2.50	≤0.22	np	np	np

Table 2. Chemical data (mean values, three replicates) of samples. Free acidity, FA (expressed as g oleic acid per 100 g of oil); peroxide values, PV (expressed as meq of active oxygen per kg of oil); K_{232}, K_{270} (expressed as specific extinctions); total phenols, TP and *o*-diphenols, *o*-DPH (both expressed as mg gallic acid per kg of oil) and bitter index, BI K_{225} (expressed as specific extinction); not provided = np. Different letters in the same column indicate significant differences (Fisher LSD, $p < 0.05$).

Sample	S1	S2	S3	S4	S5	S6	S7	S8
Phenolic compounds								
HY	30.9±1.6	9.7±0.3	35.7±1.3	9.7±0.1	28.5±0.7	4.9±0.2	4.6±0.3	10.3±0.1
TY	12.1±0.8	10.8±0.4	21.7±0.9	10.8±0.1	16.9±0.5	9.5±0.3	3.9±0.2	7.4±0.2
VA	2.9±0.2	2.0±0.2	2.2±0.1	1.7±0.3	1.5±0.1	2.8±0.2	1.6±0.2	2.9±0.1
SY	4.8±0.6	2.4±0.1	2.2±0.1	4.4±0.4	3.2±0.1	7.9±0.3	2.7±0.2	3.3±0.2
DOA	45.7±5.5	85.3±4.2	48.0±1.4	64.2±2.5	61.3±2.5	23.8±3.5	47.0±2.2	143.8±8.9
LUT	3.7±0.2	0.8±0.2	2.1±0.5	2.9±0.3	1.6±0.3	0.5±0.0	3.1±1.0	2.4±1.0
DLA+Acpin	54.0 ±5.9	130.0±11.4	88.4±4.4	134.0±2.2	69.7±4.8	49.5±6.4	79.7±2.0	135.1±12.9
API	1.9±0.1	0.7±0.0	1.1±0.2	1.6±0.1	0.8±0.1	0.5±0.1	1.8±0.6	1.1±0.4
Oagl	30.8±2.2	64.7±8.1	52.4±2.1	67.5±1.1	87.7±6.5	24.4±1.0	36.1±0.3	66.5±3.0
Lagl	5.9±0.6	20.4±2.8	12.6±0.7	28.4±0.2	27.0±3.9	4.4±0.2	9.0±1.2	14.3±1.1
TOT	192.7±17.7	339.1±4.7	266.4±8.9	325.1±5.9	298.3±19.4	128.2±3.3	189.8±5.3	387.0±27.0
Fatty acids composition (%)								
SFA	15.3±0.09	14.3±0.13	13.5±0.02	16.3±0.10	14.4±0.10	17.8±0.10	16.6±0.15	15.0±1.23
MUFA	75.3±0.08	77.8±0.10	77.0±0.06	76.4±0.16	79.3±0.16	70.9±0.08	76.8±2.86	76.8±0.14
PUFA	9.5±0.05	7.9±0.03	9.5±0.05	7.4±0.05	6.4±0.06	11.4±0.03	6.6±0.29	8.2±1.09
OA/LA	8±0.05	11±0.03	9±0.06	11±0.10	14±0.11	7±0.01	12±0.17	10±1.12
Volatile compounds (flavour and off-flavour)								
Aldehydes C_6	4.3±0.2	11.0±1.5	4.1±0.6	8.6±1.3	2.1±0.4	4.7±0.4	21.4±0.1	22.5±3.0
Alcohols C_6	3.6±0.2	3.0±0.4	1.9±0.3	2.2±0.4	1.6±0.3	4.9±0.2	2.0±0.1	5.9±0.8
Esters C_6	0.9±0.1	0.4±0.1	0.3±0.1	1.0±0.3	0.5±0.1	0.5±0.0	0.7±0.0	0.7±0.1
TOT C_6 LOX	8.9±0.4	14.5±2.0	6.4±0.8	11.9±2.0	4.2±0.7	10.1±0.7	24.1±0.1	29.1±3.9
Ketones C_5	0.7±0.1	0.5±0.1	0.5±0.0	0.7±0.1	0.4±0.0	0.7±0.0	1.0±0.0	1.3±0.1
Alcohols C_5	0.4±0.0	0.4±0.0	0.3±0.0	0.4±0.1	0.1±0.0	0.5±0.0	0.3±0.0	0.9±0.1
Pentenic dimers	1.5±0.2	1.2±0.2	0.9±0.2	0.7±0.0	0.3±0.0	0.9±0.1	1.7±0.1	2.3±0.3
TOT C_5 LOX	2.6±0.3	2.1±0.3	1.7±0.2	2.0±0.4	0.8±0.2	2.1±0.1	3.0±0.1	4.6±0.5
Hydrocarbons	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.0	0.8±0.4	0.2±0.0	0.4±0.1	0.4±0.1
Terpenes	0.2±0.0	0.2±0.1	0.1±0.0	0.1±0.0	0.2±0.0	0.3±0.0	0.2±0.0	0.4±0.1
Winey	9.1±0.7	3.4±1.2	4.2±0.8	3.3±0.2	10.0±1.3	0.8±0.1	1.8±0.1	7.9±0.7
Fusty/Muddy	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	n.d.	0.1±0.0
Rancid	0.6±0.1	0.8±0.1	0.5±0.1	0.8±0.1	0.8±0.2	0.5±0.0	0.7±0.1	0.7±0.2

Table 3. Percentages of the fatty acids grouped as SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids). OL/LA (oleic acid/linoleic acid). Phenolic compounds were determined by HPLC-DAD/MSD and expressed as mg I.S. per kg of oil. HY, hydroxytyrosol; TY, tyrosol; VA, vanillic acid; SY, syringic acid; DOA, decarboxymethyl oleuropein aglycon; LUT, luteolin; DLA+Acpin, decarboxymethyl ligstroside aglycon (oleocanthal) + acetoxypinoresinol; API, apigenin; Oagl, oleuropein aglycon; Lagl, ligstroside aglycon. Volatiles responsible for flavor (C_5-

LOX and C_6-LOX) and off-flavors (sum of compounds that mainly contribute to sensory defects, see the related paragraph in Materials and Methods), expressed as mg of 4-methyl-2-pentanone per kg of oil. Not detected, n.d. (LOD for volatile compounds = 0.01 mg of 4-methyl-2-pentanone per kg of oil). All results are reported as the mean of three replicates.

5.3.4 Phenolic compounds

The amount of phenolic compounds is fundamental to assess the quality of EVOO due to their involvement in protection from oxidation and their contribution to bitter and pungency (Bendini et al., 2007). The concentration of total phenols, *o*-diphenols (calculated using gallic acid calibration curves, respectively with r^2 = 0.997 and r^2 = 0.994, see Section 2.8) and bitter index (BI K_{225}) are presented in Table 2. Samples S2, S3, S4, S5 and S8 can be considered as "medium rich" in phenolic compounds with values higher than 200 mg kg^{-1} but lower than 500 mg kg^{-1} (according to the range proposed by Montedoro et al., 1992); in particular, it should be noted that the sample S8, obtained from olives of the Coratina variety (typical of the south of Italy), was characterized by the highest presence of these compounds (428.1 mg gallic acid kg^{-1}). On the other hand, S6 showed the lowest concentration of total phenolic compounds and, according to the sensory results from the trained Panel, was rated as one of the least bitter EVOO (Fig. 1). By evaluating the total phenolic content of a set of 30 samples of EVOO purchased in the Italian market, similar results were recently obtained by Caporaso et al. (2015). The *o*-diphenols content showed a trend similar to total phenols and ranged from 46.6 (S4) mg kg^{-1} to 114.0 (S8) mg kg^{-1}. Bitter index (BI K_{225}) values followed the same pattern as total phenol and *o*-diphenol, confirming that the phenolic fraction of EVOO is mainly responsible for the bitter taste.

Five different classes of phenolic compounds were tentatively identified and quantified in samples: phenolic acids (especially derivatives of benzoic acids), flavones (luteolin and apigenin), lignans [(+)-pinoresinol and (+)-acetoxypinoresinol], phenyl-ethyl alcohols (hydroxytyrosol, tyrosol) and secoiridoids (aglycon derivatives of oleuropein and ligstroside); the quantification was performed using calibration curves built as described in Section 2.9 (r^2 = 0.9987 for the one related to 3,4-dihydroxyphenylacetic acid at 280 nm and r^2 = 0.9995 for the other related to caffeic acid at 330 nm). With regards to the

secoiridoid derivatives, DOA concentration ranged from 23.8 to 143.8 mg Kg^{-1}. The most interesting aspect observed was the highest concentration of decarboxymethyl oleuropein aglycon (DOA) in S8, which was considered the most bitter in sensory tests: this compound showed an average value that was significantly higher than that of the other seven samples (Table 3). The concentration of decarboxymethyl ligstroside aglycon (DLA), previously associated with the sensory perception of pungent (Andrewes et al., 2003), was quantitatively - even if in co elution with Acpin- more relevant in S2, S4 and S8 with values significantly higher than other EVOOs. These latter samples were the most pungent of the set (Fig. 1). DLA, more commonly known as oleocanthal, appears to be responsible for the burning sensation in the back of the throat when consuming EVOOs and has anti-inflammatory properties similar to ibuprofen (Beauchamp et al., 2005).

5.3.5 Volatile profile

The volatile compounds identified and quantified in the headspace of EVOOs are reported in Table 3, and divided into positive flavors and off-flavors compounds. Among the C_6-LOX aldehydes, generally associated with positive sensory notes like "green", "almond" and "cut grass" (Aparicio and Morales, 1998; Morales et al., 1996), the most representative were (*Z*)-3-hexenal and (*E*)-2-hexenal. Sample S5, judged by the Panel as the EVOO with the lowest intensity of fruity and devoid of green notes (Fig. 1), showed the lowest content in the C_6-LOX aldehydes, and in particular (*E*)-2-hexenal. Sample S7, showing a high value for C_6 aldehydes, was one of the EVOOs of the set with the highest intensity of fruity (together with S6) according to the Panel results. Sample S8 was the richest in (*E*)-2-hexenal and characterized by a medium intensity of fruity (Fig. 1) but with poor acceptability by consumers (Fig. 2a) who perceived it as too bitter (Fig. 3). On the other hand, the concentration of C_6-LOX alcohols (hexanol, (*Z*)-3-hexenol (*E*)-2-hexenol) and C_6-LOX esters (hexyl acetate, (*E*)-2-hexenyl acetate, (*Z*)-3-hexenyl acetate), both related to several positive notes of EVOOs (Kalua et al., 2007), were quantitatively low in all the examined samples. Considering the C_5-LOX ketones, the content in 3-pentanone (data not shown) was significantly higher in the samples that were more liked by consumers (S1, S6 and S7), but also in S8, that was the least well accepted in blind tests (Fig. 2a). As already explained, the low value of the overall-liking of S8 was due to its high intensity of bitterness. Sample S5, characterized by a poor sensory quality, was very low in molecules that are enzymatically produced by the LOX pathway and showed greater amounts of

typical off-flavor compounds. Although in almost all samples there were components which contribute to off-flavor, it is necessary to keep in mind that volatile molecules, even if perceived in small amounts (μg kg^{-1} or ppb), do not all show the same contribution to the global aroma of VOO; which is influenced both by their concentrations and by their sensory threshold values (Angerosa et al., 2004; Kalua et al., 2007).

5.3.6 Principal component analysis

Phenolic compounds, the volatile molecules responsible for pleasant notes, and the positive attributes assessed by trained tasters were elaborated by principal component analysis (PCA) and showed as vectors in a plane composed of four quadrants (Fig. 4a and b). The first two components were responsible for 76.5% of variance (45.7% for F1 and 30.8% for F2). As seen in Fig. 4a, it is possible to highlight that Oagl, DOA, DLA, hexanal and 1-penten-3-one, as well as bitter and pungent perceived by trained judges (IOC Panel test) were distributed in the first quadrant. In the second quadrant, fruity, green and positive sensations and the main volatile compounds related to positive flavors of EVOOs can be found: C_6-LOX compounds ((*E*)-2-hexenal, hexyl acetate, 1-hexanol, (*E*)-2-hexenol) and C_5-LOX compounds (3-pentanone, 1-penten-3-ol, (*Z*)-2-penten-1-ol). In the third quadrant, opposite to the first, (*Z*)-3-hexen-1-ol was present, while (*Z*)-3-hexen-1-ol acetate and Lagl was placed in the fourth quadrant. Fig. 4b shows a projection on the plane of all samples. The approximate position of the product near sensory attribute/chemical parameter vector(s) allows for the assumption that the product expresses these attribute/chemical substances. Therefore, S8 is located between the first and the second quadrant and it is characterized by the richest content in phenolic and "positive" volatile compounds (Tables 2 and 3) as well as by high intensity of bitter, pungent, fruity, green and other positive notes perceived by odor (Fig. 1). The position of sample S6, between the second and third quadrant, reflects the high content in (*Z*)-3-hexen-1-ol and the low content of phenolic compounds responsible for bitter and pungent. The presence of sample S5 in the fourth quadrant is mainly due to the low intensities of positive olfactory sensations.

Moreover, positive correlations exist between DOA and DLA with the attributes bitter (0.910, $p < 0.05$) and pungent (0.899, $p < 0.05$) while, considering the volatile compounds, a positive correlation (0.712, $p < 0.05$) between green notes and 1-penten-3-ol was found.

The PCA results also show that the considered parameters (phenolic compounds, volatile molecules responsible for pleasant notes and positive sensory attributes) were not effective to discriminate EVOOs produced by different agricultural methods (organic and conventional).

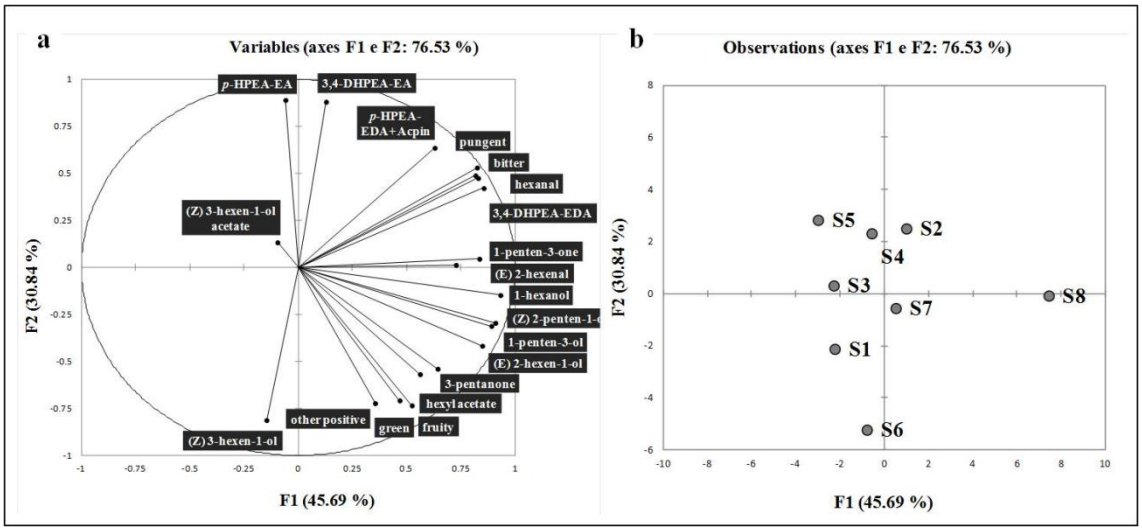

Fig. 4. (a) PCA loadings; (b) PCA score plot. Oagl, oleuropein aglycon; Lagl, ligstroside aglycons; DLA+Acpin, decarboxymethyl ligstroside aglycon + acetoxypinoresinol; DOA, decarboxymethyl oleuropein aglycons.

5.3.7 Preference map

The results of sole sensory analysis, obtained by both trained judges and consumers (overall liking evaluated in blind tests), can be summarized in a preference map (Fig. 5) that clearly showed that S1, the least bitter and pungent sample, was also the most liked, and that S2 and S8 (the most bitter and pungent) were the least liked by the consumers: according to other authors (Delgado and Guinard, 2011; Recchia et al., 2012), consumers preferred EVOOs characterized primarily by sweet taste and low intensity of bitterness and pungency. It is highly likely that they are unaware that these attributes are linked to richness in phenolic compounds, which are responsible for some of the healthy characteristics of EVOO (EU Reg. 432/2012). Such a lack of knowledge about high quality EVOOs was also confirmed considering samples S6, S8 and S5. In fact, only a moderate degree of appreciation for the pleasant olfactory notes was demonstrated by the consumers' preference. Sample S6 was characterized by the highest intensity of fruity, green and other positive olfactory sensation, but poor in bitter and pungent taste (Fig. 1); it was positioned where the majority of consumers (60-70%) have a preference and

acceptability above average. On the other hand, sample S8 was one of the most bitter and pungent and showed medium intensities of fruity and positive olfactory sensations; it was placed in an area where only 20-30% of consumers have a preference above average. Moreover, sample S5, which was characterized by low intensities of all positive attributes, was appreciated by consumers, reaching 50-60% of above average overall liking.

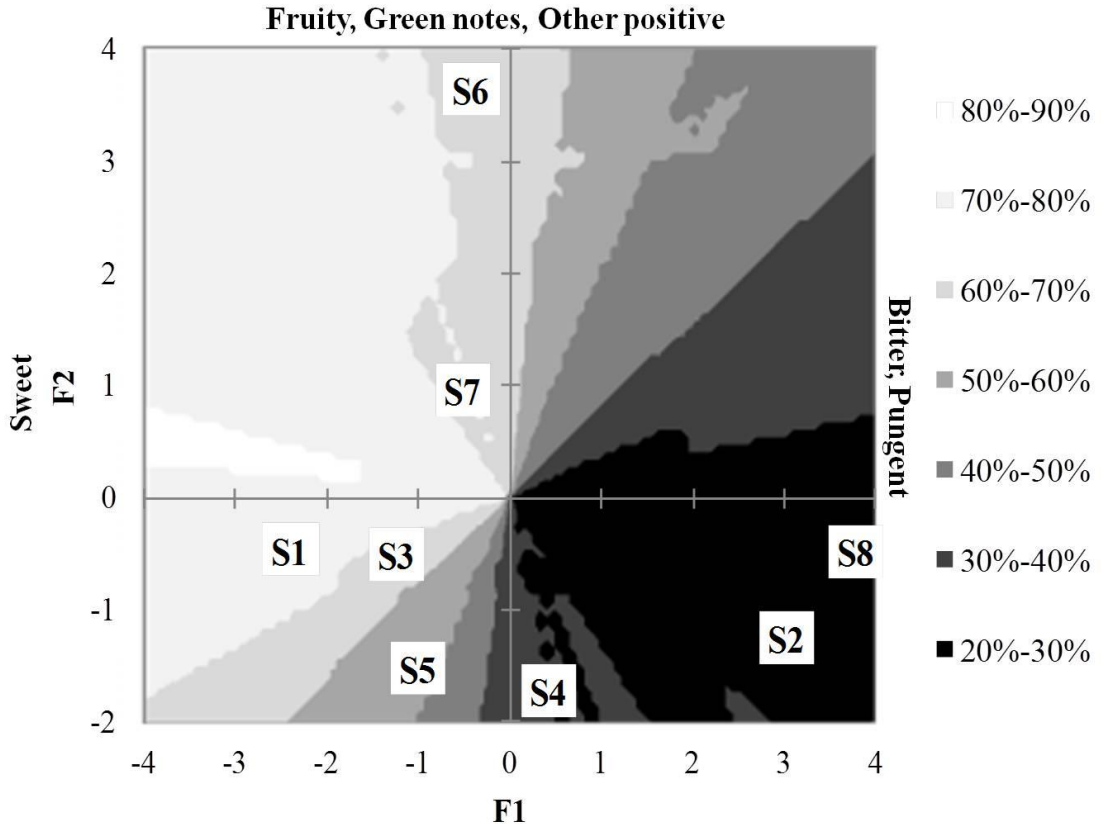

Fig. 5. Preference mapping resulting from the elaboration of IOC Panel test and consumer preference data (blind session).

5.4 CONCLUSIONS

The results obtained from the judges interviewed in this study, allow to observe a gap between consumers subjective preference and consumers knowledge (objective) about EVOO consumption.

No significant impact of the information dealing with the agricultural method used (organic and conventional) for EVOO production on consumer preference, was showed.

This could indicate that the consumers interviewed do not have a specific image linked to an organic EVOO in terms of expectations concerning its sensory characteristics.

More in general, it is well known that specific composition of EVOOs, related with the qualitative and quantitative profiles in minor compounds and linked to several factors (e.g., olive variety, ripeness degree, technological and related to the oil storage factors), rather than the agricultural system alone, can influence their sensory profiles.

The composition in specific minor compounds (phenols and volatiles) of the selected EVOOs effectively differentiated samples belonging to the same commercial class but having different sensory characteristics. The well-known relationships between phenolic components and bitterness and pungency in EVOOs were also confirmed. Good correlations were found between these sensory attributes and the content of phenolic compounds as determined by spectrophotometric methods and HPLC. In particular, considering the single compounds analyzed in HPLC, it appeared clear how the attribute of bitter was mainly related to the dialdehydic form of oleuropein aglycone, while the pungent sensation was related to the presence of the dialdehydic form of ligstroside aglycone. With regards to the determination of volatile compounds, the positive correlation between green notes and 1-penten-3-ol was highlighted.

As regards the consumers, a preference map allowed the identification of drivers of liking and disliking. Consumers appreciated the fruity attribute and, in part, the pungent sensation, but did not recognize bitterness as a positive attribute. This could be related to the common aversion reaction towards the majority of bitter substances or to the degree of familiarity with this kind of sensation due to food habits. In the olive oil sector, it is well known among scientists and experts that bitterness and pungency are positive attributes for EVOO due to their close link with the phenolic substances responsible for healthy properties (in particular in protection of blood lipids from oxidative stress) and antioxidant activity towards the lipid matrix. In the years to come, future efforts should be addressed towards dissemination of accurate information about the relationship between EVOO composition and sensory characteristics, for example in terms of labeling, in order to improve consumer awareness, introducing more relevant factors that may help them to properly appreciate this peculiar vegetable oil.

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Supporting material

ATTRIBUTE	SCALE	QUESTION	ANCHORING-POINT
odor liking	9-point-hedonic	How much do you like the odor of this product?	very little/neither nor/very much
fruity	JAR	The intensity of the fruity is:	way too little/too little/just about right/too much/way too much
taste liking	9-point-hedonic	How much do you like the taste of this product?	very little/neither nor/very much
bitter	JAR	The intensity of the bitter is:	way too little/too little/just about right/too much/way too much
pungent	JAR	The intensity of the pungency is:	way too little/too little/just about right/too much/way too much
sweet	JAR	The intensity of the sweet is:	way too little/too little/just about right/too much/way too much
overall liking	9-point-hedonic	How much do you like this product?	very little/neither nor/very much

Supplementary material S1. List of attributes, scales, questions and anchoring-points used for the consumer test. JAR: just about right.

Chapter 6

How the addition of spices and herbs to virgin olive oil to produce flavored oils affects consumer acceptance

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ABSTRACT

With the aim to expand the olive oil market to a larger number of consumers who are not familiar with the sensory characteristics of virgin olive oil, the use of novel products known as "flavored olive oils", obtained by adding different kind of spices and aromatic herbs, is spreading in many countries. In order to test consumer acceptability for this type of product, in a country (Tunisia) in which virgin olive oil is regularly consumed, flavored olive oils were prepared by adding aromatic extracts of thyme, oregano, a mix of herbs (used as pizza seasoning), rosemary, and basil to a monovarietal Chemlali virgin olive oil and consumer test on 206 subjects was performed.

Selected quality parameters (free acidity, peroxide number, oxidative stability, specific absorption at K_{232} nm and K_{270} nm) were also measured and no significant variations were detected. Slight differences were found concerning the content of minor compounds (chlorophylls, carotenoids and total phenols). On the other hand, notable differences were seen in the profiles of volatile compounds, which appeared to be responsible for the observed variability in consumer acceptance. Although the unflavoured oil was more appreciated than the flavored ones, among the latter thyme flavored olive oil was the most appreciated.

Keywords: flavored olive oil; aromatic extracts; physical-chemical composition; consumer acceptance; volatile compounds.

6.1 INTRODUCTION

Spices and herbs are widely used in Mediterranean cuisine, i.e. garlic and onion in seafood and meat plates and sauces, rosemary and thyme in barbecued meat and chicken, oregano and basil on pizza. In addition to palatability, herbs and spices provide some healthy effects, extend the shelf life, and improve the safety of prepared food. These effects are mainly due to well-known antioxidant and antimicrobial properties of herbs and spices [1-5].

Virgin olive oil is a basic culinary ingredient of the Mediterranean diet and cuisine and generally is highly appreciated for its characteristic taste and odor [6]. Wholesome and healthy effects of virgin olive oil have been also reported [7-8] as well as their cosmetic uses as oil phase ingredient and odour fixative of perfumes and essential oils.

Recently, a new set of products known as “flavored olive oils”, with many different tastes, has been introduced into the market [9].

The main strategy is to expand the olive oil market to a larger number of consumers by acquiring new those not yet familiar with the uses and properties of olive oil, that might be tempted by an olive oil enriched with other ingredients of the Mediterranean diet [10].

Furthermore, given that the strong and peculiar olfactory notes of extra virgin olive oil can be difficult to be familiarized for consumers having the attitude of using mild oils [13], the chance to reduce the intensity of “fruity” by masking it with spicy could be a good strategy to enlarge the olive oil market.

According to the definition of the European Union Commission [11-12], an extra virgin olive oil is a liquid lipid matrix that conforms to a series of chemical and sensorial parameters, is free of sensory defects and possesses an impeccable aroma and flavor of olive fruit (fruity>0). An extra virgin olive oil must be extracted “*only from olives with a superior quality, cannot undergo any treatment other than washing the fruits, and decanting, centrifuging and filtering the extracted olive oil. It excludes oils obtained from seeds by chemical or mechanical methods or the use of solvent extraction or re-esterification methods, and those mixed with oils from other sources*”. Based on this clarification, a flavored olive oil obtained using an extra virgin olive oil (EVOO) cannot be called "extra virgin olive oil" in the label, but can be defined [13] as an olive oil that has been processed with vegetables, herbs, spices or

other fruits to improve its nutritional value, enrich the sensory characteristics and increase its shelf-life. In the same way, and after lengthy discussion in May 2014 on the issue of "flavored extra virgin olive oils and infused olive oils", the committee of the international olive council (IOC) [14] specified that extra virgin olive oil is the juice of the olive and nothing else. Olive oil is defined solely as the blend of refined olive oil and virgin olive oil without the addition of any other product.

Flavored olive oils are very popular in the US, UK, and Australia, none of which is an IOC member and there are no laws that forbid the commercialization of these kind of products in those countries. Because of the success of these oil dressings, the California Olive Oil Council (COOC) is trying to establish a meaningful labeling standard [13]. A strong demand for flavored oils in the UK and other not heavy consumer countries was noteworthy for the last decade; this may be explained by the attitude of flavor from species, plants, and essential oils to camouflage the strong attribute of olive oil that can be unpleasant for those who are unfamiliar [13].

Many procedures of oil flavored production are available and the choice is fundamental since the extraction method affects both acceptability and oxidative stability of the oil preparation. Maceration is the oldest method of oil aromatization: herbs, spices, and fruits are mixed with oil and left at a room temperature for a defined time. The mixture is then filtered to remove turbidity and solid parts [10, 13, 15]. Co-milling the olives with herbs, spices, or fruits as lemons or bergamots [16] is a new approach for preparing clear and safe flavored olive oils [13]. Recently, another approach commonly used is the addition of essential oil to the virgin olive oil [13] that presents advantages in terms of high safety. In fact, many spices and herbs can carry spores produced by *Clostridium botulinum* [13] and this latter procedure permits more flexibility of production because it is not necessary to have the added flavor (herbs, spices, or fruits) available during milling.

In the present study, a set of five flavored olive oils obtained using thyme, oregano, herbs (a mixture used as pizza seasoning), rosemary, and basil was prepared and compared with an unflavored one. The main aims were to: (i) study the possible influence of aromatization process on the quality of the product, (ii) characterize the volatile fraction of different samples, and (iii) test consumer acceptance.

6.2 MATERIALS AND METHODS

6.2.1 Samples

EVOO produced from Chemlali olives by a three-phase continuous extraction system was used for the preparation of flavored oils. It belonged to the EVOO commercial class according to the basic parameters [11-12]. Flavored olive oils and oily preparations of flavours used in this study were produced by the Mills of "Rivière d'or" localised in Monastir, Tunisia. Specifically, oily preparations of flavors were obtained by mixing the essential oils into an organic sunflower oil. The flavoured olive oils were prepared by mixing the EVOO and a percentage of five commercial oily preparations of flavors (thyme, S1; oregano, S2; mix of herbs for pizza, S3; rosemary, S4; basil, S5). According to the appropriate intensity of the flavor (preliminarily tested), we used 0.7% of the flavour of rosemary and 1% of the other flavors. All chemical and sensory tests were also carried out on the EVOO control sample (T).

6.2.2 Physical-chemical parameters

Basic quality parameters of EVOO such as free acidity (FA) given as the % of oleic acid, peroxide value (PV) expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg), and spectrophotometric indices (K_{232}, K_{270}) were evaluated according to official methods [11-12]. All analyses were performed in triplicate for each sample.

6.2.3 Oxidative stability evaluation

The sensitivity to oxidative phenomena was evaluated by the Rancimat apparatus (Mod. 743, Metrohm Ω, Switzerland). Briefly, 3 g of each sample was heated to 120°C and submitted to an air flow of 20 L h^{-1}. Stability was expressed as induction time (hours).

6.2.4 Pigment determination

Amounts of carotenes and chlorophylls were determined as described by Minguez-Mosquera *et al.* [30]. In brief, 7.5 g of oil was weighed, dissolved in cyclohexane, and brought to a final volume of 25 mL. Carotene and chlorophyll pigments were determined by measuring absorbance at 470 and 670 nm, respectively. Results were expressed as mg of pheophytin "a" and lutein per kg of oil, respectively [30].

6.2.5 Extraction of phenolic compounds and determination of total phenols

the phenolic extract was obtained as previously reported by Montedoro *et al.* [31]. Briefly, 10 mL of a solution composed of methanol/water (80:20, v/v) and 20 mg of Tween 20 (2%, v/w) were homogenized with 10 g of olive oil using an Ultra-Turrax T25 apparatus (IKA Labortechnik, Janke & Kunkel, Staufen, Germany) for 1 min at 15,000 g and then centrifuged at 5,000 g for 10 min at 4°C; the extraction was repeated twice. The methanol extract was stored at -20°C for 24 h to eliminate oil droplets. Total phenols were determined colorimetrically and the results were expressed as mg of hydroxytyrosol per kg of oil.

6.2.6 Volatile compound analysis

Solid phase micro extraction (SPME) was applied for headspace sampling. A Supelco SPME fiber coated with polydimethylsiloxane (PDMS, 100 µm) was used; 2 mL of sample was placed into a 5 mL glass vial and, after equilibration (30 min), the fiber was exposed in the headspace of the sample for 50 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred and desorbed in the injection port of the GC-MS system.

GC-EIMS analysis were performed with a Varian CP 3800 gas-chromatograph equipped with a DB-5 Capillary column (30 m x 0.25 mm, 0.25 µm coating thickness) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperature were 250°C and 240°C, respectively; oven temperature was programmed from 60°C to 240°C at 3°C min^{-1}; carrier gas, helium at 1 mL min^{-1}; splitless injection. Identification of compounds was based on comparison of the retention times with those of pure standards, comparing their linear retention indices relative to the series of *n*-hydrocarbons, using the information from the National Institute of Standards and Technology library (NIST 98 and ADAMS) and homemade library mass spectra built from pure substances and components of known mixtures and MS literature data [32-37]. Moreover, the molecular weights of all identified substances were confirmed by GC-CIMS using MeOH as CI ionizing gas. The relative proportions of the volatile constituents were expressed as percentage (%) by peak-area normalization.

6.2.7 Acceptance test

A total of 206 subjects participated in the study. Specifically, 103 females and 103 males were interviewed to investigate the overall liking of samples. The group of participants came from different regions of Tunisia and was selected using predetermined screening criteria based on level of education (high or incomplete), purchasing and consumption frequency as well as familiarity with the typical EVOO of Tunisia. Participants were asked to evaluate, according to their preference, the six samples (T and S1-S5) by smell and taste and to express their degree of liking using a 9-point hedonic scale (scores: like extremely: 9; like very much: 8; like moderately: 7; like slightly: 6; neither like nor dislike: 5; dislike slightly 4; dislike moderately: 3; dislike very much: 2; dislike extremely: 1) [38].

The test was realized in blinded conditions and each consumer had to complete a questionnaire on personal data and other information (age, gender, region of origin, socio-professional category, and consumption frequency of EVOO). Samples were served at room temperature in plastic glasses coded with three-digit numbers and presented to consumers by randomization. The amount of each sample served was 20 mL with no obligation to finish the glass. During the test, unsalted bread, apples and water were provided to each judge.

6.2.8 Statistical analysis

All parameters were determined in triplicate for each sample. Data were processed by SPSS statistical package (Version 12.00 for Window, SPSS Inc. Chicago, Illinois, 2003). The significance of differences at a 5% level among means was determined by one-way ANOVA, using Tukey's test. For from the acceptance sensory test, in order to check if a difference between samples existed, we used ANOVA and the F-test. Duncan's multiple range test was used to obtain all pair wise comparisons among sample means. Correlation analysis was performed employing Person's test. Principal components analysis (PCA) was performed to verify the possible relationship between variables. PCA was performed with XLSTAT for Windows release 6.0 (Addinsoft, New York, NY).

6.3 RESULTS AND DISCUSSION

6.3.1 Quality indices determination

The addition of oily flavored preparations (S1-S5) to EVOO (T) had no effect on its basic quality parameters. These results were in good agreement with our previous study [15] and other recent studies [17].

6.3.2 Oxidative stability evaluation

The oxidative stability index (OSI) of EVOO (T) was 5.0 hours (Table 1). The addition of oily flavored preparations did not significantly increase oxidative stability, in agreement with previous observations [15]. However, a slight ability to better counteract oxidation was seen for samples flavored with oregano (S2), partially confirming the results of Sousa et al. [18].

Table 1. Mean values of oxidative stability (OSI), pigments (chlorophylls and carotenoids), and total phenols of extra virgin olive oil (EVOO: T) and flavored olive oils (EVOO + thyme: S1; EVOO + oregano: S2; EVOO + mix of herbs: S3; EVOO + rosemary: S4; EVOO + basil: S5). Values in the same row with different subscript letters represent significant differences between samples at $p < 0.05$ by Tukey's test (n = 3).

Samples / Compounds	T	S1	S2	S3	S4	S5
OSI (hours)	5.0 ^{a}	5.7 ^{a}	6.0 ^{a}	5.6 ^{a}	5.3 ^{a}	5.8 ^{a}
Chlorophylls (mg/kg)	8.4 ^{b}	8.1 ^{b}	8.7 ^{b}	8.3 ^{b}	8.1 ^{b}	9.2 ^{a}
Carotenoids (mg/kg)	5.5 ^{a}	5.2 ^{a}	5.2 ^{a}	4.9 ^{a}	5.1 ^{a}	5.2 ^{a}
Total phenols (mg/kg)	452.3 ^{c}	518.6 ^{b}	651.4 ^{a}	427.6 ^{c}	418.7 ^{c}	477.9 ^{c}

6.3.3 Pigment determination

The concentrations of chlorophylls and carotenoids of Chemlali EVOO (T) are reported in Table 1. Values of 8.4 and 5.5 mg kg-1 were obtained for chlorophylls and carotenoids, respectively. The addition of oily flavored preparations (S1-S5) had no appreciable effect on the content of chlorophylls, with the exception of a slight increase ($p<0.05$) in the case

of the basil-flavored olive oil (S5). For carotenoids, no significant variations were noted among samples.

6.3.4 **Extraction and determination of phenolic compounds**

In Chemlali EVOO (T), a concentration of 452.3 mg kg-1 of total phenols was determined and the addition of oily flavoured preparations induced a slight significant increase in the case of oregano (S2) and thyme (S1)-flavored oils (Table 1).

6.3.5 ***Volatile compounds analysis***

The aromatic substances identified in the headspace of oily flavored preparations, EVOO, and flavored olive oils were studied (data not shown). Thymus essential oil and extracts are widely used in pharmaceutical preparations and for flavoring and preservation of several food products. *Thymus*, widespread in Mediterranean area, are well known as medicinal plants due to their biological and pharmacological properties [19]. In the case of the commercial thyme oily preparation, 25 components, which represented 99.6% of total volatiles, were identified. Typically high percentages of the constituents derived from the biosynthetic pathway of the thymol/carvacrol such as *p*-cymene (46.6%) and γ-terpinene (18.8%) were seen, even if the chemical composition can markedly vary in relation with different seasons and species of *Thymus* L. (Lamiaceae) [19].

De Falco et al. [20] reported that oregano essential oils have been shown to have antioxidant, antibacterial, antifungal, diaphoretic, carminative, antispasmodic, and analgesic activities and, among these, the antimicrobial potential is of special interest. In the oily preparation of oregano used in the present investigation, 23 constituents, which represented 99.7% of the total volatiles, were identified. Among the main constituents of the aroma, we detected high amounts 1,8-cineole (36.1%), *p*-cymene (15.6%), β -pinene (6.3%), and γ-terpinene (5.8%).

Fifteen compounds, accounting for 99.5% of total volatiles, were identified in the oily flavored preparations of mix of herbs. More than 40% were represented by α -pinene (42.3%). Other monoterpene hydrocarbons such as β -pinene (19.7%) and γ-phellandrene (11.6%) were detected in high percentages.

Globally, 18 constituents, accounting for 99.7% of total volatiles were identified in the rosemary oily preparation. Its main components were: 1,8-cineole (47.8%), α-pinene

(16.9%), β-pinene (15.1%), and camphor (5.0%). It can thus be stated that flavoring of this commercial solution was obtained from a 1,8-cineole chemotype [21]. Jiang *et al.* [22] demonstrated that the essential oil of rosemary, particularly rich in 1,8-cineole, showed pronounced antibacterial and antifungal activity.

In the flavored oily preparation of basil, 28 volatiles were identified, which accounted for 99.8% of the total composition. The three main constituents were typical compounds of basil essential oil: 1,8-cineole (27.5%), linalool (21.8%), and methyl chavicol (21.0%) [23]. Hussain *et al.* [24] reported that the essential oil of basil had antioxidant and antimicrobial activities, mainly due to the presence of linaool, a typical component of basil.

In the headspace of the EVOO (T), several constituents were identified (Table 2). It was characterized above all by C_6 aldehydes, mainly represented by *(E)*-2-hexenal (41.4%), a volatile with green, sweet, and fruity sensory notes and, secondly, by hexanal (4.1%). Other representative compounds were esters such as *(Z)*-3-hexenyl acetate (3.6%) and 1-hexyl acetate (1.6%). The presence of *(E)*-2-hexenal, *(Z)*-3-hexenyl acetate, and 1-hexyl acetate is usually correlated with freshness of virgin olive oil and normally has a positive effect on consumer preference [25].

In the thyme-flavored olive oil (S1), more than 40% was constituted by *p*-cymene, followed by appreciable amounts of other monoterpenes, such as γ-terpinene (17.9%), thymol (8.3%), and linalool (4.2%). Some of the compounds deriving from the EVOO were still detectable, such as *(E)*-2-hexenal (2.5%), 1-hexanol (0.9%) and *(Z)*-3-hexenyl acetate (0.3%) (Table 2).

Around 27 compounds were identified in the oregano-flavored olive oil (S2) and the main components were 1,8-cineole (36.1%), *p*-cymene (15.6%), α-pinene (6.9%), and β-pinene (6.3%). Among the volatiles of EVOO, *(E)*-2-hexenal (1.0%) was identified (Table 2).

In the herbs-flavored olive oil (S3) the resulting aroma was dominated by α-pinene (27.5%), β-pinene (15.3%), γ-phellandrene (11.6%), carvone (8.2%), and linalool (7.4%). However, some of the aromatic compounds of EVOO were still detectable, even if in lower amounts, such as *(E)*-2-hexenal (7.5%) (Table 2).

In the rosemary-flavored olive oil (S4), 20 compounds were detected. More than 60% of the total aromatic compounds were represented by 1,8-cineole (61.3%), followed by α-pinene (8.9%), β-pinene (8.6%), and camphor (8.3%). Here the typical constituents of EVOO were less detectable: *(E)*-2-hexenal (0.9%) and *(Z)*-3-hexenyl acetate (0.1%) (Table 2).

Taking into consideration the last sample (S5, basil-flavored olive oil), 33 compounds were identified, the most abundant of which was linalool (30.6%), followed by methyl chavicol (26.5%), and 1,8-cineole (22.6%). The aromatic substances of the EVOO were found in very low amounts: *(E)*-2-hexenal (1.3%) and *(Z)*-3-hexenyl acetate (0.3%) (Table 2).

As expected, the transfer of aromatic compounds to the EVOO used as a lipid matrix depended on the type of aroma profile of the added oily preparation. However, it should be noted that some of the aromas (rosemary, thyme, oregano, and basil) had strong flavoring properties, while the mixture of herbs used for pizza less affected the aroma and allowed for perception of the typical aroma notes in EVOO.

In terms of series, our studies showed that aldehydes dominated the total volatile fraction of EVOO, while the headspace fraction of flavored olive oils was dominated by terpenoids fraction, as expected. The present study is in agreement with a previous report [15] which showed that the majority of volatiles belonging to thyme and oregano, such as carvacrol and limonene, were efficiently incorporated into an EVOO matrix. It has to be considered that the percentage of each volatile molecule incorporated into the EVOO depends mainly on the concentration of spices and herbs used to prepare the flavored olive oil.

Table 2 Volatile compounds[a] of the extra virgin olive oil (EVOO: T) and the flavored olive oils EVOO + Thyme: S1; EVOO + Oregano: S2; EVOO + Herbs: S3; EVOO + Rosemary: S4; EVOO + Basil: S5. Values in the same row with different subscript letters represent significant differences between samples at $p < 0.05$ by Tukey's test, (n = 3).

Volatile compounds (%)	I.r.i*	T	S1	S2	S3	S4	S5
Aldehydes from LOX							
hexanal	800	4.1^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
(E)-2-hexenal	851	41.4^{a}	2.5^{c}	1.0^{d}	7.5^{b}	0.9^{d}	1.3^{cd}
Alcohols from LOX							
1-hexanol	871	0.7^{a}	0.9^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
Esters from LOX							
(Z)-3-hexenyl acetate	1007	3.6^{a}	0.3^{c}	nd^{c}	1.1^{b}	0.1^{c}	0.3^{c}
1-hexyl acetate	1009	1.6^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
Terpenic compounds							
α-thujene	932	nd^{c}	2.0^{a}	0.8^{b}	0.2^{c}	0.2^{c}	nd^{c}
α-pinene	940	nd^{d}	3.2^{c}	6.9^{b}	27.5^{a}	8.9^{b}	0.8^{cd}
camphene	955	nd^{d}	1.7^{c}	2.6^{b}	nd^{d}	5.1^{a}	0.2^{d}
sabinene	977	nd^{c}	nd^{c}	0.7^{a}	nd^{c}	nd^{c}	0.3^{b}
β-pinene	980	nd^{e}	1.5^{d}	6.3^{c}	15.3^{a}	8.6^{b}	1.5^{d}
myrcene	993	nd^{d}	5.0^{a}	3.2^{b}	5.0^{a}	0.3^{d}	1.1^{c}
α-phellandrene	1006	nd^{b}	nd^{b}	0.2^{a}	nd^{b}	nd^{b}	nd^{b}
δ-3-carene	1012	nd^{b}	0.2^{b}	1.7^{a}	nd^{b}	0.2^{b}	nd^{b}
α-terpinene	1019	nd^{b}	0.3^{b}	1.5^{a}	nd^{b}	nd^{b}	nd^{b}
p-cymene	1027	nd^{c}	40.5^{a}	15.6^{b}	0.3^{c}	0.3^{c}	nd^{c}
limonene	1032	nd^{c}	nd^{c}	2.0^{a}	nd^{c}	0.2^{b}	0.3^{b}
γ-phellandrene	1033	nd^{b}	nd^{b}	nd^{b}	11.6^{a}	nd^{b}	nd^{b}
1,8-cineole	1034	nd^{d}	nd^{d}	36.1^{b}	nd^{d}	61.3^{a}	22.6^{c}
(E)-β-ocimene	1051	nd^{c}	0.2^{b}	nd	0.3^{b}	nd^{c}	0.8^{a}
γ-terpinene	1062	0.8^{c}	17.9^{a}	5.8^{b}	0.1^{c}	nd^{d}	nd^{d}
fenchone	1080	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	0.3^{a}
p-mentha-2,4(8)-diene	1088	nd^{b}	nd^{b}	0.3^{a}	nd^{b}	nd^{b}	nd^{b}
p-cymenene	1090	nd^{b}	nd^{b}	nd^{b}	3.0^{a}	nd^{b}	nd^{b}
terpinolene	1090	nd^{b}	0.2^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
linalool	1101	nd^{d}	4.2^{c}	1.7^{d}	7.4^{b}	0.4^{de}	30.6^{a}
(Z)-sabinene hydrate	1070	nd^{b}	0.8^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
terpinolene	1090	nd^{b}	0.2^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
1,3,8-*p*-menthatriene	1112	nd^{b}	nd^{b}	nd^{b}	2.5^{a}	nd^{b}	nd^{b}
camphor	1147	nd^{e}	1.4^{c}	4.0^{b}	0.3^{de}	8.3^{a}	1.1^{cd}

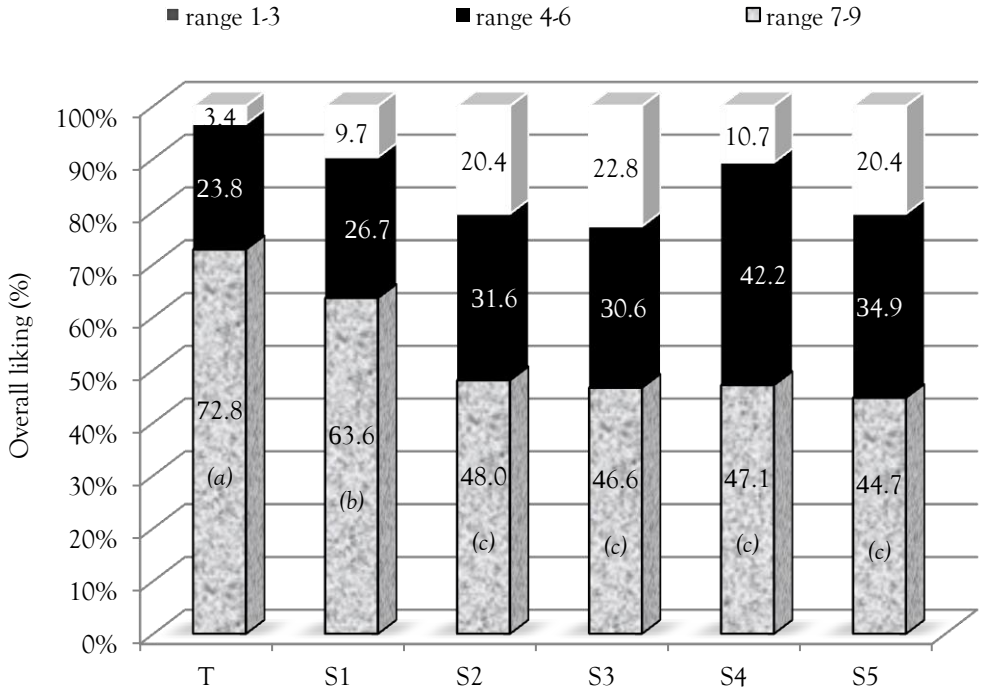
menthone	1154	nd^{c}	nd^{c}	nd^{c}	0.3^{b}	nd^{c}	1.2^{a}
isomenthone	1165	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	0.8^{a}
borneol	1170	nd^{c}	1.0^{b}	0.7^{b}	nd^{c}	1.4^{a}	nd^{c}
menthol	1174	nd^{b}	nd^{b}	nd^{b}	0.1^{b}	nd^{b}	1.0^{a}
4-terpineol	1179	nd^{e}	1.5^{a}	0.6^{b}	0.1^{de}	0.4^{c}	0.2^{d}
p-cymen-8-ol	1184	nd^{b}	nd^{b}	nd^{b}	0.2^{a}	nd^{b}	nd^{b}
γ-terpineol	1191	nd^{c}	0.2^{bc}	0.4^{ab}	0.2^{bc}	0.6^{a}	0.3^{abc}
methyl chavicol	1198	nd^{c}	nd^{c}	nd^{c}	4.1^{b}	nd^{c}	26.5^{a}
endo-fenchyl acetate	1221	nd	nd^{b}	nd^{b}	nd^{b}	nd^{b}	0.2^{a}
methylthymol	1235	nd^{b}	0.2^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
methylcarvacrol	1245	nd^{b}	0.3^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
carvone	1245	nd^{c}	nd^{c}	nd^{c}	8.2^{a}	nd^{c}	3.6^{b}
geranial	1271	nd^{b}	0.2^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
isobornyl acetate	1287	nd^{b}	0.4^{b}	0.4^{b}	0.1^{b}	1.0^{b}	0.9^{a}
thymol	1292	nd^{c}	8.3^{a}	1.5^{b}	nd^{c}	nd^{c}	nd^{c}
menthyl acetate	1296	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	0.1^{a}
carvacrol	1301	nd^{b}	0.6^{a}	3.0^{b}	nd^{b}	nd^{b}	nd^{b}
iso-dihydrocarveol acetate	1330	nd^{b}	nd^{b}	nd^{b}	0.2^{a}	nd^{b}	0.1^{b}
γ-elemene	1340	nd^{b}	nd^{b}	0.2^{a}	nd^{b}	nd^{b}	nd^{b}
eugenol	1361	nd^{b}	nd^{b}	nd^{b}	0.4^{a}	nd^{b}	0.4^{a}
α-copaene	1377	1.2^{a}	0.1^{b}	0.2^{b}	0.1^{b}	0.1^{b}	nd^{b}
γ-bourbonene	1385	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	0.1^{a}
β-elemene	1392	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	0.5^{a}
β-caryophyllene	1418	0.7^{b}	1.5^{a}	1.7^{a}	nd^{c}	0.7^{b}	0.1^{c}
(E)-α-bergamotene	1437	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	1.3^{a}
α-humulene	1456	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	0.1^{a}
germacrene D	1483	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	0.2^{a}
β-bisabolene	1494	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	0.1^{a}
valencene	1494	0.5^{a}	nd^{b}	nd^{b}	nd^{b}	nd^{b}	nd^{b}
(E,E)-α-farnesene	1505	3.4^{a}	0.5^{b}	0.2^{b}	0.2^{b}	0.1^{b}	nd^{b}
(E)-γ-cadinene	1513	nd^{b}	0.2^{a}	nd^{b}	nd^{b}	nd^{b}	0.2^{a}

6.3.6 **Acceptance test**

Sensory analysis plays a major role in market product acceptability [26, 27].

In order to optimize a product, the industry usually applies many sensory methods, mainly affective ones. Among these, acceptance test allows for assessment of the consumer's overall liking [28]. Results of the 9-point hedonic scale are summarized in Figure. 1. It was highlighted that EVOO (T, mean score 7.5) was significantly more liked than flavored olive oils. In fact, for EVOO, about 73% of consumers attributed the highest score for values belonged to the range 7-9 (Figure. 1).

Fig. 1. Percentages of overall-liking for extra virgin olive oil (EVOO: T) and flavored olive oils EVOO + thyme: S1; EVOO + oregano: S2; EVOO + mix of herbs: S3; EVOO + rosemary: S4; EVOO + basil: S5) assessed by 206 consumers. Values in the same row with different subscript letters (a-c) represent significant differences (for 7-9 range) between samples at $p < 0.05$ by Duncan's test with F value (23.45) > F critical (2.21).

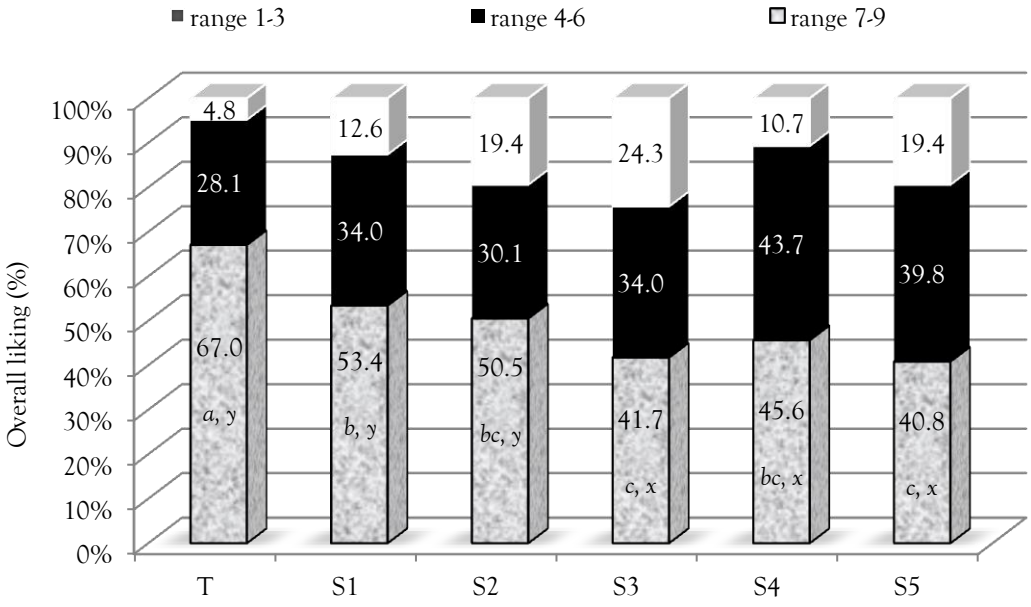


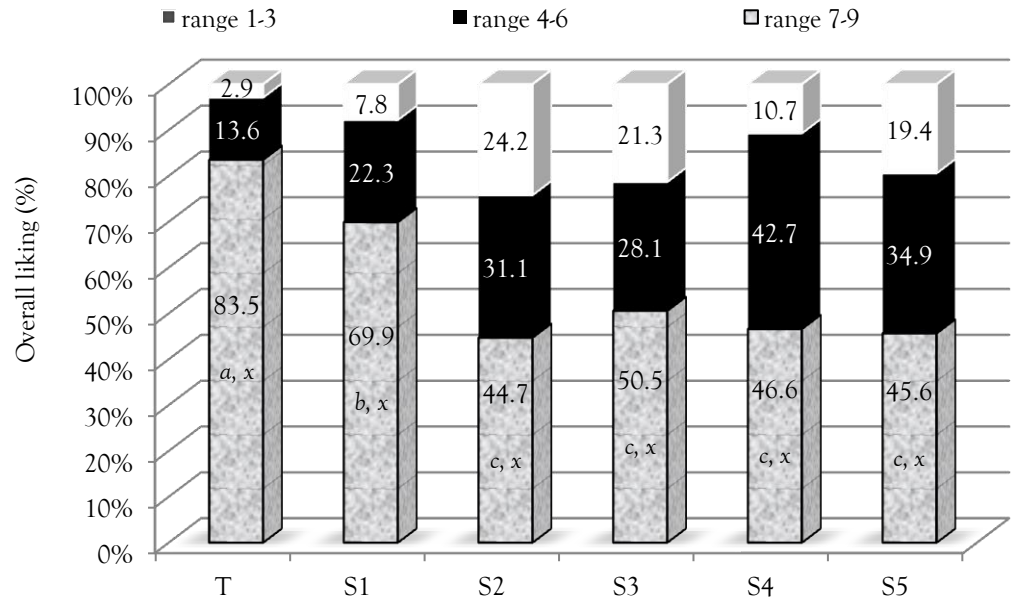
There were also significant differences regarding overall-liking among flavored samples: the thyme-flavored one (S1) was the most appreciated (6.6 mean scores) and 63.6% of consumers gave a positive score (7-9 range), whereas only 9.7% disliked it and assigned lower values of overall liking (Figure. 1). For other samples (S2, S3, S4 and S5), there were no significant differences, but considering the consumers' scores, the rosemary-flavored olive oil (S4) had the least negative judgements among these four oils, with 10.7% of consumers in the range 1-3.

Preference of consumers appeared to correlate with the aromatic fractions of olive oil samples. Specifically, some typical aromatic compounds of EVOO were responsible for the highest appreciation of EVOO. In fact, a close positive statistical correlation was found between *(E)*-2-hexenal, *(Z)*-3-hexenyl acetate, and overall-liking ($r=0.80$ and $r=0.77$, respectively; $p<0.05$). On the other hand, some aroma compounds belonging to oily flavored preparations, appeared to have a negative impact.

Our results are in agreement with various studies reporting on the impact of the incorporation of aromatic preparations on consumer acceptance. Acceptability is not only dependent on the incorporation level, but also on the essential oil composition. In this regard, Antoun and Tsimidou [29] prepared oregano and rosemary gourmet olive oils at several percentages (from 1 to 5% w/w). They found that consumers (32 untrained people randomly depicted) were able to differentiate between levels of addition and preferred samples with the low to moderate odor and flavor, and also claimed that all flavorings were sensory accepted by consumers. In addition, Gambacorta *et al.* [17] evaluated the sensory acceptability of EVOO flavored with oregano and rosemary (prepared by infusion of 10-40 g of herbs and species into one liter of virgin olive oil). According of their studies and as demonstrated by 30 tasters, the addition of herbs and species enhanced the sensory characteristics of the EVOO used as lipid matrix. Observing the overall liking scores given by the judges with different gender (Figure. 2), it is possible to affirm that males agree with the general overall liking, but they liked EVOO (T) and the sample flavored with thyme (S1) more than females. On the other hand, females preferred the oil flavored with oregano (S2).

Fig. 2. Percentages of overall-liking for extra virgin olive oil (EVOO: T) and flavored olive oils EVOO + thyme: S1; EVOO + oregano: S2; EVOO + mix of herbs: S3; EVOO + rosemary: S4; EVOO + basil: S5) assessed by 103 females (graphic at the top) and 103 males (graphic at the bottom) consumers. Values in the same row with different subscript letters (a-c) represent significant differences (for 7-9 range) between samples at $p < 0.05$ by Duncan's test with F value (9.3) > F critical (2.2) for the case of females and F value (18.5) > F critical (2.2) for the case of males. The subscript letters x and y are the differences between males and females for the same sample at $p < 0.05$ by Duncan's test.





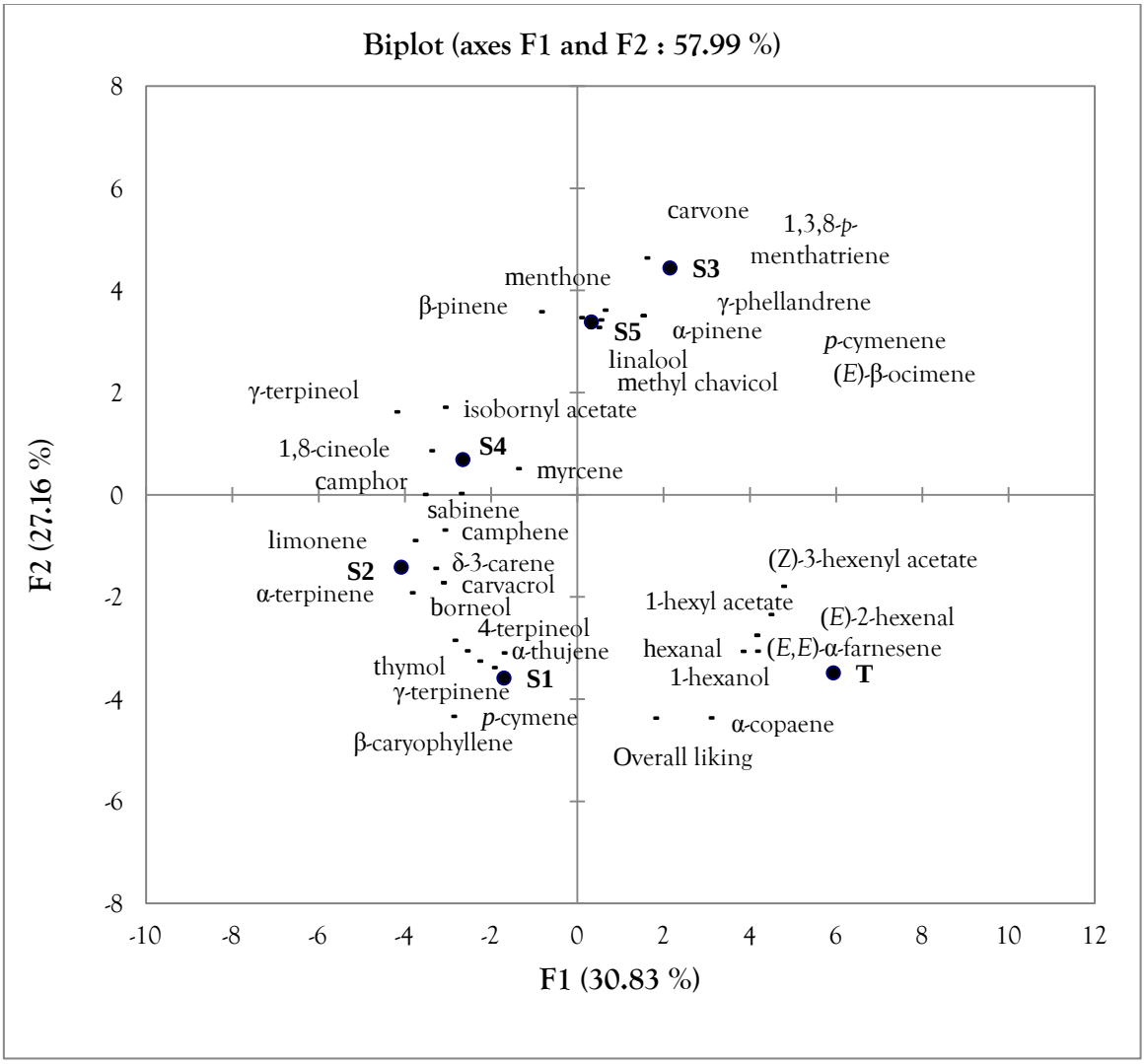
6.3.7 PCA analysis

Volatile molecules and overall liking were elaborated by principal component analysis and shown as vectors in a plane composed of four quadrants to highlight possible correlations (Figure. 3). The first two components were responsible for 57.99 % of variance (30.83 % for F1 and 27.16 % for F2).

In Figure. 3 it can be observed that there was a clear discrimination between unflavored and flavored olive oils; in particular, Extra Virgin Olive oil (EVOO, T) was located in the second quadrant and was characterized by the highest overall liking score as well as by the highest percentage of aroma compounds given by the lipoxygenase pathway; a group characterized by the flavored olive oils with the taste of thyme (S1) and oregano (S2) were placed in the third quadrant and were characterized by the presence of 1,8-cineole, sabinene, γ-terpineol, and myrcene. The rosemary-flavored

olive oil (S4) was located between the third and the fourth quadrant probably because they are affected by variables which characterize both quadrants. Finally, the last group is represented by basil- and herb-flavored olive oils (S3 and S5), which showed similar characteristics in terms of volatile profile.

Fig. 3. Principal component analysis (PCA) obtained for extra virgin olive oil (EVOO: T) and flavored olive oils EVOO + thyme: S1; EVOO + oregano: S2; EVOO + mix of herbs: S3; EVOO + rosemary: S4; EVOO + basil: S5).



6.4 CONCLUSIONS

The results of our study demonstrate that the addition of oily flavored preparations to EVOO at the percentages used, does not generally influence the stability and the concentration of some minor compounds (phenols, chlorophylls, carotenoids) in a significant manner. However, marked changes in the aroma bouquet were noticed. One of the aims of this study was to determine if the addition of spices and herbs to an EVOO used as lipid matrix to produce flavored oils can meet a satisfactory level of consumer acceptability. Tunisian consumers seemed to prefer the smell and taste of the unflavored olive oil over flavoured ones. Considering the different flavors of olive oils, the presence of thyme essential oil was well accepted, whereas the incorporation of oregano, a mix of herbs (used for pizza seasoning), rosemary, and basil oily preparations into the EVOO matrix did not meet an adequate level of liking.

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CONCLUSIONS

CONCLUSIONS

The different studies described in this doctoral dissertation have highlighted the effectiveness in the application of sensory and instrumental methodologies due to the complementary information that they can provide.

In fact, this combined approach allowed reaching a deep knowledge about the product, particularly in the investigation of how product composition, processing and storage can affect the sensory perception and consumer liking.

Different instrumental parameters relevant to the key sensory modalities (appearance, flavour and texture) have been investigated.

Regarding flavor measurements (Chapters 1, 2, 5 and 6), the application of traditional and innovative methodologies to study the volatile fraction of food products in combination with the sensory analysis has been able to:

- identify key components responsible for the characteristic aroma related to their quality and geographical origin;
- identify off-flavours as markers of bad storage and/or processing conditions;
- confirm the close relationships between the chemical compounds presence (volatile and not volatile) and the main positive sensory attributes perceived by smell and taste.

In addition, physical measurements of appearance and texture have been carried out together with sensory analysis in two different studies (Chapter 3 and 4) applying an integrated approach of physical and sensory methodologies.

The obtained results have highlighted specific sensory attributes and physical properties that contribute to better defining product characteristics, the perception of food quality and its typicality. These studies suggest the use of instrumental methods to support the sensory evaluation of physical properties more difficult to assess or to predict sensory

properties. The application of this integrated approach has been also effective for the validation of the existing relationship between sensory and instrumental data.
Finally, some consumer researches have been carried out (Chapter 5 and 6) in order to integrate information from analytical determinations (chemical, physical and sensory) with affective aspects of foods including consumer's overall liking.

These investigations have highlighted the importance of familiarity with the products and eating habits in the definition of consumer behavior: consumers tend to differentiate and reward only the products that best meet their expectations concerning the sensory characteristics but, not always, they are able to recognize, understand or appreciate the intrinsic attributes defining the quality of a specific food product.

Additional efforts should be addressed towards dissemination of accurate information about the relationship between product composition and sensory characteristics. This would improve consumer awareness introducing more relevant factors useful for food product quality recognition that may help them make better buying decisions.