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Valorization of food, food waste and by-products by means of sensory evaluation and volatile compounds analysis

Presentata da: Dott.ssa Federica Tesini

Coordinatore Dottorato Prof. Giovanni Dinelli
Relatrice Prof.ssa Tullia Gallina Toschi

Esame finale anno 2017
A Maurizio, Daniela, Luca, Marisa e Camilla
“Siamo esploratori pronti per nuove partenze”

Giorgio De Chirico
Assessment for admission to the final examination
for the degree of PhD in Agricultural, Environmental and Food Science and Technology

CANDIDATE: Federica Tesini
CURRICULUM: Food Science and Biotechnology
TUTOR: Prof. Tullia Gallina Toschi
THESIS TITLE: Valorization of food, food waste and by-products by means of sensory evaluation and volatile compounds analysis

RESEARCH ACTIVITY:
This PhD thesis dealt with the valorization, through characterization and suggestion of use, of food matrices (virgin olive oil, salami, faba beans, fresh cheese, cooked ham and tomato by-product of seeds and skin) by means of sensory evaluation (both with consumers and trained judges of a panel) and volatile compounds analysis.

In particular, to achieve the objectives, a series of specific research issues were faced:

- Rapid direct analysis to discriminate geographical origin of extra virgin olive oils (EVOOs) by Flash Gas Chromatography Electronic Nose, sensory analysis and chemometrics;
- Chemical and sensory characterization of olive oil enriched in lycopene from tomato by-product: sensory evaluation, chromatographic profile of volatile compounds and other chemicals;
- Identification and percentual composition of volatile compounds in differently processed faba beans and relation with sensory aspects;
- Peculiar attributes of a typical Italian salami from the Mora Romagnola pig breed: an integrated sensory and instrumental approach (chromatographic profile of volatile compounds and image analysis);
- Sensory and rapid instrumental methods for the quality evaluation of cooked ham;
- Children preferences of coloured cheese prepared during an educational laboratory: sensory evaluation of visual preference in cheese, relations with gender and age variables.
- Exploring influences on food choice in a large population sample: The Italian Taste project

COMPULSORY COURSES:

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<td>Research financing and project design in agricultural sciences (Progettazione e finanziamento della ricerca in agricoltura)</td>
<td>D. Viaggi</td>
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<td>Statistical methods in agriculture and data analysis (Metodologie statistiche applicate all'agricoltura con applicazioni informative)</td>
<td>A. Berardinelli</td>
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ABROAD PERIOD:
(March-July 2015 “University of Helsinki – Department of Food and Environmental Sciences”).
The aim of the work was the study of off-flavours produced during processing (different types of heat-treatments) of faba beans. In order to identify compounds responsible for this unpleasant characteristic, both sensory and chromatographic profile of volatile compounds were studied. The professor in charge of the exchange at the University of Helsinki was Hely Tuorila.

TUTOR JUDGMENT:
During this three years of PhD, the student has developed good skills in the fields of sensory analysis (affective and descriptive methods), volatile compounds analysis and statistical analysis of data for the characterization and definition of aromatic profile in food. Further comment concerns
her ability to work in group and to carry on an autonomous research activity. She has reached a very good competence in project planning, writing, in delivering results within given deadlines and in the organization and coordination of specific dissemination initiatives. Her work has already resulted in four publications on international scientific journals with impact factor, for one of which she’s the first author, that are part of her thesis work.

I fully endorse the proposal of a PhD by the PhD School in Agricultural Sciences of the University of Bologna, in recognition of the work carried out under my supervision.

LIST OF PUBLICATIONS:

- Food Quality and Preference "Exploring influences on food choice in a large population sample: The Italian Taste project” Vol. 59, P. 123-140 (2017); Erminio Monteleone, Sara Spinelli, Caterina Dinnella, Isabella Endrizzi, Monica Laureati, Ella Pagliarini … & Federica Tesini.
- Helyion “Sensory and rapid instrumental methods as a combined tool for quality control of cooked ham” Vol. 2; Issue 11, e. 00202 (2017); Sara Barbieri, Francesca Soglia, Rosa Palagano, Federica Tesini, Alessandra Bendini, Massimiliano Petracci, Claudio Cavani, Tullia Gallina Toschi.
- Food Chemistry “Rapid direct analysis to discriminate extra virgin olive oils geographical origin by Flash Gas Chromatography Electronic Nose and Chemometrics” Vol. 204, P. 263-273 (2016); Dora Melucci, Alessandra Bendini, Federica Tesini, Sara Barbieri, Alessandro Zappi, Stefania Vichi, Lanfranco Conte, Tullia Gallina Toschi.
- Italian Journal of Food Science “Children preferences of coloured fresh cheese prepared during an educational laboratory” Vol. 27, Issue 4, P. 521-526 (2015); Federica Tesini, Monica Laureati, Rosa Palagano, Mara Mandrioli, Ella Pagliarini, Tullia Gallina Toschi.

LIST OF PUBLICATIONS IN CONGRESS ACTS:

- XXI Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology “Valorization of food, food waste and by-products by
means of sensory evaluation and volatile compounds analysis”, **Oral presentation**, Federica Tesini, Portici (NA), 14-16 Settembre 2016.

- 7th European Conference of Sensory and Consumer Research “Peculiar attributes of a typical Italian salami from the Mora Romagnola pig breed: an integrated sensory and instrumental approach”, Federica Tesini, Enrico Valli, Federica Sgarzi, Francesca Soglia, Massimiliano Petracci, Alessandra Bendini, Claudio Cavani, Tullia Gallina Toschi, Dijon, Francia, 10 - 14 Settembre 2016.


The Board unanimously agrees that Dr. Federica Tesini is qualified to sit the final exam for the doctorate degree in Agricultural, Environmental, Food
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1. Aim of the work

This PhD thesis dealt with the valorization, through characterization, of different food matrices: virgin olive oil, salami, faba beans, fresh cheese, cooked ham and tomato by-product of seeds and skin.

In particular, products object of this study were analysed with an integrated approach by means of sensory evaluation (both with consumers and trained judges of a panel) and volatile compounds analysis.

The detection of the aromatic profile of a food is relevant for the comprehension and the definition of the volatile fraction of a product. Additionally, the identification of the molecules responsible for the sensory, specifically olfactory (direct and indirect), food perception is relevant to define its acceptability and, in some cases (e.g. olive oil), its quality. Sensory analysis is a scientific discipline used to evoke, measure, analyze and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste and hearing. The Quantitative Descriptive Analysis (QDA®) approach is the most widespread method for the sensory profile definition and it has been used from the 70' up to nowadays. To study the relationship between volatile compounds and sensory characteristic of food, faster separative methods, such as gas-chromatography, are needed, in order to characterize complex matrices, both quantitatively and qualitatively, and to define the correlation between instrumental data and human perception. Combining data obtained from sensory evaluation and volatile compounds analysis is relevant for the definition of a product fingerprint, useful not only to describe the product itself but also to highlight its strengths and to emphasize many characteristics like a certain level of typicality (e.g. products defined by a strong connection with a geographical area) or novelty (products developed by using food waste or by-products).

Thus, sensory and chemical characterization of products could be useful for investigating food, food waste and by-products taken into account during this PhD research plan. In particular, to achieve the previous objectives, a series of specific research topics were faced:

1) Rapid direct analysis to discriminate geographical origin of extra virgin olive oils by Flash Gas Chromatography Electronic Nose, sensory analysis and chemometrics. In
Aim of the work

particular, this 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.

2) Chemical and sensory characterization of olive oil enriched in lycopene from tomato by-product: sensory evaluation, chromatographic profile of volatile compounds and other chemicals. This work dealt with the production of an olive oil naturally enriched with antioxidants, recovering carotenoids, in particular lycopene, using an industrial by-product of tomato seeds and skin. For this purpose, a co-milling of olives and tomato by-product was carried out.

3) Identification and quantification of volatile compounds in differently processed faba beans and relation with sensory aspects. The aim of this investigation was the definition of aroma profiles (both from sensory analysis and volatile compounds analysis) of different samples of faba beans, in order to determine the volatile compounds responsible for off-flavour produced during bean processing.

4) Characterization of typical Italian salami from Mora Romagnola pig breed: an integrated sensory and instrumental approach. In this work, a sensory and instrumental analytical approach for characterizing a typical Italian salami, manufactured from an autochthonous pig breed, was investigated. The aim was to highlight the importance of an integrated approach as a tool for supporting and ensuring the authenticity of traditional food products: in this case study, the sensory profiles, color differences (with electronic eye analysis), volatile compounds and texture properties were taken into account.

5) Sensory and rapid instrumental methods for the quality evaluation of cooked ham. 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.

6) Children preferences of coloured cheese prepared during an educational laboratory: sensory evaluation of visual preference in cheese, relations with gender and age
Aim of the work

variables. The aim of this study was the investigation of sensory visual preferences for a fresh and naturally coloured cheese, produced during an educational laboratory. In particular, preferences were studied among young consumers, taking into account variables as gender and age.

7) The Italian Taste project: exploring influences of food choice in a large population. The aims of this study were twofold: firstly, to illustrate the variables selected to explore the different dimensions of food choice and to report the experimental procedure adopted for data collection. Secondly, the paper aimed to show the potential of the Italian taste dataset on the basis of data collected in the first year of study on 1225 individuals.

Contribution of the author to papers 1 to 7:

1) The candidate contributed to this work in analyzing the samples with SPME-GC-MS and was part of the sensory panel used for Panel Test. Additionally, she contributed to the work writing and revision, after peer review.

2) The candidate contribution to this work consisted in volatile compounds and basic chemical parameters analysis. She was also part of the testing panel used for Panel test and she helped in proof writing and revision.

3) The candidate conducted all the experiments presented in this work, except for the quality control of rapeseed oil; she additionally defined the sensory profile sheet and elaborated data of this study. She also wrote the manuscript, thanks to the help of the other authors.

4) The candidate largely contributed to this work analyzing data from volatile compounds analysis and image analysis. She defined the sensory profile sheet and elaborated the data; she finally wrote the paper thanks to the contribution of the other authors.

5) The candidate contributed to this work being part of the panel for sensory evaluation of the cooked ham samples; she additionally helped in writing the final paper and revising it after comments of the peer reviewer.
Aim of the work

6) The candidate contribution to this work consisted in designing the questionnaire and projecting the educational laboratory. She was also part of the team for practical application of the laboratory and administration of it to participants. She elaborated the data and wrote the paper, thanks to the contribution of the other authors.

7) The candidate had the responsibility to enrol people in the project. Furthermore, she conducted the tests and contact more than 120 participants; since the project will finish at the end of 2017, the candidate will continue with this activity till December 2017.
2. Introduction

2.1 The aromatic profile of food, food waste and by-products

Food analysis is important for the quality control, shelf life study, characteristics definition, and description of products. In this context, the analysis of flavour, defined as a combination of olfactory and taste perceptions, is strictly connected to compositional and enzymatic modifications of food. In fact, the flavour of a product is sensitive and related to modifications naturally happened or induced in the product (Kataoka et al., 2000). Volatiles are low molecular weight (MW) compounds (<300 Da) with high vapour pressure that vaporize at room temperature. Odorants are volatile chemical compounds that are carried by inhaled air to the olfactory epithelium, where they reach and bond with specific proteins of olfactory receptors to give an odor sensation (Morales et al., 2013). These molecules should be characterized by a boiling point from 20 to 300 °C, and a MW lower than 300 Da, in order to be picked up by olfactory receptors. The binding between a volatile molecule and its receptor can result from different types of strength (Moret et al., 2014):

- Dipole-dipole interaction: characteristic of molecules presenting functional groups;
- Van der Waals interaction: weaker than the previous, this interaction is typical of perfect match between receptor and its substrate.

Since 1985, Pelosi purpose a classification of volatile compounds, in relation to the already mentioned characteristics, in:

- Molecules without functional groups (e.g. saturated, unsaturated and aromatic hydrocarbons);
- Molecules with only one functional group;
- Molecules with two or more functional groups (Pelosi, 1985).

The total number of volatiles present in a sample can vary from few ng/kg to many mg/kg; additionally, it can happen that molecules only present in traces have a higher sensory contribution if compared to others, eventually present in bigger amount. This is related to the fact that only a part of volatile compounds responsible for the aromatic profile of food can be really perceived by human senses: these compounds are named as charactering or
impact compounds. To characterize aromatic molecules, the knowledge of their olfactory threshold, the lowest concentration of the volatile needed in order to make a human nose able to perceive it, is needed. In fact, the perceived odour of any material is composed of one or more volatile compounds that are present in concentrations above the sensitivity threshold (Delahunty et al., 2006). Many compounds have been identified in food products, whose importance for the flavour and/or taste of foods as well as their contribution to off-flavours can be assessed if their concentrations and threshold values are known. Two types of flavour thresholds, the absolute (which includes detection and recognition threshold) and the difference threshold, can be distinguished. The detection threshold could be defined as the minimum concentration which can be detected without any requirements to identify or recognize the stimulus, while the recognition threshold is the minimum concentration at which a stimulus can be identified or recognized. On the other hand, difference thresholds are the smallest changes in concentration of a substance required to give a perceptible change (Van Gemert, 2003).

The odorant must possess certain molecular characteristics in order to produce sensory perceptions. It must have some water solubility, a sufficiently high vapour pressure, low polarity, some ability to dissolve in fat (lipophilicity), and surface activity. Odorous substances have in common that they are either gases or volatile liquids. This is the form in which the odorant reaches the sensory epithelium, either through the nostrils with inspired air or by the back door through the mouth and throat. The receptor structures for olfaction are covered with mucus so that aqueous solubility is an asset to an odorant.

Since volatile molecules are responsible for the aromatic fraction in food, food waste and by-products, the aromatic profile represents a chemical “fingerprint” of the product, and the nature and the relative amount of the compounds present in the volatile fraction are distinctive features of the product itself. For example, considering olive oil, volatile compounds are mostly produced because of fatty acid oxidation; the endogenous plant enzymes, by the lipoxygenase pathway, carry on the genesis of secondary products, mainly volatiles, responsible for positive attributes. On the other hand, the presence of microbial activity, responsible for exogenous enzymes and oxidative phenomena, is related with the origin of negative attributes (Kalua et al., 2007). Thus, the aromatic profile of an olive oil is extremely complex as well as strictly linked with several sensory (olfactory and taste)
Introduction

perceptions. Considering meat fermented product, as salami, aroma compounds can arise from a complex pattern of chemical reactions involving the components of the matrix, like oxidation of unsaturated fatty acids and microbiological metabolism of lipids, proteins and carbohydrates (García-González et al., 2009, Bianchi et al., 2007).
Introduction

2.2 Sensory analysis of food

2.2.1 Anatomy and physiology of the five senses

Senses are physiological capacities of organisms that provide data for perception. The senses and their operation, classification, and theory are overlapping topics studied by a variety of fields, most notably neuroscience, cognitive psychology (or cognitive science) and sensory analysis. The nervous system has a specific sensory system or organ, dedicated to each sense.

2.2.1a The vision

Sight or vision is the capability of the eye(s) to focus and detect images of visible light on photoreceptors in the retina of each eye that generates electrical nerve impulses for varying colors, hues, and brightness. There are two types of photoreceptors: rods and cones. Rods are very sensitive to light, but do not distinguish colors. Cones distinguish colors, but are less sensitive to dim light. There is some disagreement as to whether this constitutes one, two or three senses. Neuroanatomists generally regard it as two senses, given that different receptors are responsible for the perception of color and brightness. Some argue that stereopsis, the perception of depth using both eyes, also constitutes a sense, but it is generally regarded as a cognitive (that is, post-sensory) function of the visual cortex of the brain where patterns and objects in images are recognized and interpreted based on previously learned information. This is called visual memory.

Figure 1. Eye anatomy, lateral section (image source: La Valle S.M., 2015).
Introduction

Figure 1 shows the anatomy of a human eye. The shape is approximately spherical, with a diameter of around 24 mm and only slight variation among people. The cornea is a hard, transparent surface through which light enters and provides the greatest optical power. The rest of the outer surface of the eye is protected by a hard, white layer called the sclera.

All vision is based on the perception of electromagnetic rays. These rays pass through the cornea in the form of light; the cornea focuses the rays as they enter the eye through the pupil, the black aperture at the front of the eye. The pupil acts as a gatekeeper, allowing as much or as little light to enter as is necessary to see an image properly. The pigmented area around the pupil is the iris. Along with supplying a person's eye color, the iris is responsible for acting as the pupil's stop, or sphincter. Two layers of iris muscles contract or dilate the pupil to change the amount of light that enters the eye. Behind the pupil is the lens, which is similar in shape and function to a camera lens. Together with the cornea, the lens adjusts the focal length of the image being seen onto the back of the eye, the retina. Visual reception occurs at the retina where photoreceptor cells called cones and rods give an image color and shadow. The image is transduced into neural impulses and then transferred through the optic nerve to the rest of the brain for processing. The visual cortex in the brain interprets the image to extract form, meaning, memory and context (Crescitelli, 1960).

In food products, especially meats, fruits and vegetables, the consumer often assesses the initial quality of the product by its color and appearance; thus, appearance and color are the primary indicators of perceived quality. The visual characteristics of a product can affect the consumers’ perceptions of other sensory modalities in that food as well (Lawless et al., 2010). Together with touch and smell senses, the sight is responsible for texture attributes perception (Lawless et al., 2010) that has been defined as “the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing and touch” (Szczesniak, 2002).
2.2.1b The smell

The human sense of smell has often been regarded as the least refined of all the human senses and far inferior to that of other animals. In fact, Aristotle (384–322 BC) blames this lack of finesse on the ducts in the human nose and claims that people who have noses with narrower ducts have a keener sense of smell, but he cites no experimental evidence for this assertion (Aristotle in Problemata XXXIII, and in De Sensu et Sensibili in Parva Naturalia). Moreover, the Roman philosopher Lucretius (99–55 BC) focused on the shape of the particles as conveying the quality of the odour and speculated on human olfaction by considering the nature and role of the odorant particles (Lucretius in De Rerum Natura). Also, the sense of smell is intimately linked with our emotions and aesthetics, but, despite the importance of odour, there is a lack of a suitable vocabulary to describe odours with precision. This is recognised by Plato in Timaeus: “the varieties of smell have no name, but they are distinguished only as painful and pleasant” (Brattoli et al., 2011). The identification of primary olfactory submodalities has been attempted many times. The first serious effort at identification was made by Linnaeus, the Swedish botanist, in 1756.

The anatomical characteristics of the smell apparatus are showed in Figure 2.

![Nose anatomy, lateral section](image source: McGraw-Ill Company)
Chemicals transported by the inhaled air are trapped and dissolved into the olfactory epithelium, a small region of both nasal cavities where odorants stimulate an electrical response of the olfactory nerves: the olfactory signal is thus transmitted to the brain, where the final perceived odour results from a series of neural computations. Odours are recognized thanks to the memory effect of previous experienced smells, thus accounting for the high subjectivity of the odour perception (Pearce et al., 1997).

The olfactory receptors are located in two small portions of epithelium, very high in the nasal cavity. This remote location may serve some protective function against damage, but it also means that only a small percentage of the airborne substances flowing through the nose actually reach the vicinity of the sensory organs. There are several million receptors on each side of the nose and they have a terminal knob protruding into the mucus with about 20-30 very fine cilia which “float” into the mucus layer. One function of these cilia is to increase the surface area of the cell, exposing the receptor cells inside the epithelium and they each send a thin axon into the olfactory bulbs. The olfactory receptors are true nerve cells; they are unusual neurons in that they have a limited life span and are usually replaced in a month. The mechanism of odor receptors counts about 350 receptor types, that are G-protein coupled receptors with a sequence indicating seven transmembrane segments connected by intracellular and extracellular loops and have short N-terminals. Each odor receptor cell expresses only one type of receptor protein; thus, different odors are represented by activation of different segments of the olfactory bulb. However, the matter is complicated by the fact that receptors are tuned to multiple odor molecules, and, conversely, many odor molecules can stimulate a wide array of receptors (Lawless et al., 2010).

The largest contribution to the diversity of flavors comes from the volatile airborne molecules sensed by the olfactory receptors. Whether sniffed through the external nares, the vast diversity of what could be defined as a food flavor is mediated by smell. Due to the tendency to localize aromatics from foods in the mouth, many people do not realize that the olfactory sense is responsible for sensing most flavors other than simple five tastes. Olfaction has a dual role as both an external sensory system and an internal one (Rozin, 1982): compounds that arise in the mouth pass up into the nasal cavity from the rear direction, opposite to that from sniffing; this is defined as a retronasal perception.
**Introduction**

Together with the visual characteristics of a product, the smell is responsible of the first reaction of human being to a food (Pagliarini, 2002).

**2.2.1c The taste**

Taste is the ability to respond to dissolved molecules and ions called tastants. The sensation of taste includes five established basic tastes: sweetness, sourness, saltiness, bitterness, and umami (Korsmeyer, 2002). The taste system consists of 3 types of taste papillae (Figure 3), on which taste buds are located. Fungiform papillae, which are mushroom shaped structures, are located towards the front of the tongue. Each fungiform papillae usually contains 3-5 taste buds. Circumvallate papillae are located towards the back of the tongue, and unlike fungiform papilla, they each contain more than 100 taste buds. The ridges and grooves located along the sides of the tongue are foliate papillae. Like circumvallate papillae, foliate papillae also contain more than 100 taste buds each. A fourth type of papillae, filiform, also exists, but does not contain any taste buds. Each taste bud consists of 30-100 taste receptor cells. Taste receptor cells are long, thin cells oriented perpendicular to the surface of the tongue. One end of the taste each taste receptor cell is exposed to the oral cavity and has microvilli on its surface to increase contact with stimuli. The opposing end of the taste receptor cell contacts nerve fibers which feed into the glosopharyngeal nerve, chorda tympani or vagal nerve, depending on the location of the taste bud (Da Conceitao et al., 2007).
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When stimuli enter the oral cavity, they may bind to the taste cell membrane receptors, pass through specific channels, or activate ion channels. These processes then trigger the taste cell to release neurotransmitters, sending a signal to the brain. The way in which different types of stimuli generate taste responses is still not fully understood. Sweet and bitter taste are thought to operate by way of specific G-protein coupled receptors (GPCR), T1R and T2R, respectively. The T1R GPCR for sweet taste has been shown to have multiple binding sites, used by sugars, artificial sweeteners, and sweet taste antagonists. Bitter taste can be elicited by a far greater number, and more diverse set, of compounds than sweet taste. The great variety of bitter compounds indicates that no single receptor could be responsive to all bitter compounds. Indeed, this has been shown, with more than 20 bitter T2Rs identified. It has also been shown that each bitter taste cell does not express all of the bitter T2Rs, but only a few (Roper, 2007). Salt taste reception studies have pointed to the presence of cation channels. As the concentration in the oral cavity increases, cations flow into salt receptor cells, resulting in depolarization, and eventually the release of neurotransmitters. Varied responses to similar concentrations of different salty compounds indicate that there may be more to salt taste than cation channels on the taste cell surface. The reception of sour taste was originally linked to the concentration of hydrogen ions. However, it has since been shown that there is no direct relationship between pH, titrable acidity, and sour taste. Solutions of organic acids at the same pH elicit differing sour taste responses.
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Likewise, solutions of organic acids of the same normality also result in difference sour taste responses. It is obvious that undissociated acids play a role in sour taste, but the mechanism is unclear. Umami is an oral sensation stimulated by salts of glutamic or aspartic acids, roughly translated by japanese as “delicious taste”, and attributes to the taste of monosodium glutamate (MSG) and ribosides such as salts of inosine monophosphate and guanine monophosphate (Lawless et al., 2010).

2.2.1d The touch

Sensations of touch arise by the activation of sensory receptors located in the skin that are responsive to mechanical stimuli. The sensory receptors for touch and proprioception are complex in structure, but the basic organization is that of a neuron that has an ending, endings responsible for mechano-electric transduction. Once the mechanical stimulus is transduced into an electrical impulse, the neuron transmits this information very quickly to the spinal cord and then to the brain. Information arising from the mechanoreceptors of the body and face goes to specific regions within the brain that interpret the signals in terms of tactile perceptions. The cortical regions devoted to this function have many independent representations of the body surface. The anatomical aspect of skin is showed in Figure 4.

Figure 4. Anatomy of skin (Image source: Pearson education).
Introduction

The sense of touch is responsible for the perception of textural characteristics of products. The textural properties of a product are linked with its compositional and mechanical properties; the evaluation of these aspect is defined as “all the mechanical, geometrical and surface attributes of a product perceptible by mechanical and tactile receptors” (ISO 5492).

2.2.1e The hearing

There are three components to the ear: the outer ear, the middle ear and the inner ear. The outer ear is composed of the pinna, or ear lobe, and the external auditory canal. Both structures funnel sound waves towards the ear drum or tympanic membrane allowing it to vibrate. The pinna is also responsible for protecting the ear drum from damage. Modified sweat glands in the ear canal form ear wax.

The middle ear is an air-filled space located in the temporal bone of the skull. Air pressure is equalized in this space via the Eustachian tube which drains into the nasopharynx or the back of the throat and nose. There are three small bones, or ossicles, that are located adjacent to the tympanic membrane. The malleus, incus, and stapes are attached like a chain to the tympanic membrane and convert sound waves that vibrate the membrane into mechanical vibrations of the three bones. The stapes fills the oval window which is the connection to the inner ear. The anatomy of the ear is reported in Figure 5.

![Anatomy of the human ear (image source: Study Blue).](image)

Figure 5. Anatomy of the human ear (image source: Study Blue).
The ear canal acts as a resonating tube and actually amplifies sounds at between 3,000 and 4,000 Hz adding to the sensitivity (and susceptibility to damage) of the ear at these frequencies. The ear is very sensitive and responds to sounds of very low intensity, to vibrations which are hardly greater than the natural random movement of molecules of air. To do this the air pressure on both sides of the tympanic membrane must be equal. The Eustachian tube provides the means of the pressure equalization. It does this by opening for short periods, with every 3rd or 4th swallow; if it were open all the time one would hear one's own every breath. Because the lining membrane of the middle ear is a respiratory membrane, it can absorb some gases, so if the Eustachian tube is closed for too long it absorbs carbon dioxide and oxygen from the air in the middle ear, thus producing a negative pressure. This may produce pain (as experienced if the Eustachian tube is not unblocked during descent of an aeroplane). The middle ear cavity itself is quite small and the mastoid air cells act as an air reservoir cushioning the effects of pressure change. The outer and middle ears serve to amplify the sound signal. The sense of hearing is involved in sensory evaluation of food for all of the attributes related with vibration: during biting, man vibrations are produced and can be associated with attributes like crunchy (e.g. biscuits or chips) (Vickers, 1991).
2.2.2 Sensory analysis: definition and methodologies

Sensory analysis is “a scientific discipline used to evoke, measure, analyse and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste and hearing” (Stone et al., 2012).

The definition represents a conscious to be as inclusive as is possible within the framework of food evaluation, with the word “food” used as global, indicating an ingredient is a food, a beverage is a food, and so forth (O’Sullivan, 2016). Sensory characterization provides a representation of the qualitative and quantitative aspects of human perception, enabling measurement of the sensory reaction to the stimuli resulting from the use of a product and allowing correlations to the other parameters (Varela et al., 2012; Lawless et al, 2010; Murray et al., 2001). Globally, sensory analysis represents the definition and scientific measurement of a product perceived by the five senses. This definition fits both with qualitative and quantitative methods and does not discriminate the assessors according to their capability and awareness of the methods or the test in relation with the final objective of a study. In fact, only a correct pre-selection of the method and the judges, as a function of the real objective of a sensory investigation, is the correct way to proceed with sensory analysis, giving it the real significance of a scientific discipline. Sensory analysis answers to questions that embrace quality from different points of view: description, preference and discrimination: all of these answers are useful to communicate and provide an input in decision making (Carpenter et al., 2012). In general, three types of sensory testing are commonly used: i) affective, hedonic tests that involve consumers and concern subjective and acceptance issues, for these tests judges should be untrained; ii) discrimination, these are analytic tests that deal with the investigation product perceptible differences and request judges that could be trained and screened for their sensory acuity; iii) descriptive, analytical tests oriented to the description of specific sensory characteristics. This last category needs trained or highly trained judges, previously screened for their acuity and motivation (Lawless et al., 2010).
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2.2.2a Discrimination methods

Discrimination tests should be used when the sensory specialist wants to determine whether two samples are perceptibly different (Stone et al., 2004). Analysis is usually based on the statistics of frequencies and proportions (counting right and wrong questions). From the results of these tests differences based on the proportions of persons who are able to choose a test product correctly from a set of similar products can be inferred. A classic example of this test was the triangle procedure, where two products are equal and the third one differs, for example, in the amount of one ingredient but it’s produced with the same recipe and in the same way. Judges would be asked to pick the different sample from among the three. Ability to discriminate differences would be inferred from consistent correct choices above the level expected by chance. A product preference test can determine if consumers prefer one product when compared to another product. Another multiple choice different test was developed by Peryam and Swartz (Peryam et al., 1950) for purposes of quality control, and it’s named duo-trio test. In this test, two test samples and a reference samples are given to untrained assessors. One of the test samples matches the reference while the other one comes from a different product/batch/process. The participant try to match the correct sample to the reference, with a chance of probability of one-half. Another widespread test is the paired comparison, where participants are asked to choose which, among two samples, is the strongest or more intense in a given attribute. Partly due to the fact that panelist’s attention is directed to a specific attribute, this test is very sensitive to differences (Lawless et al., 2010). Simple difference tests have proven very useful in application and are in widespread use today. Typically, a discrimination test will be conducted by 25-40 participants who have been screened for their sensory acuity to common product differences and who are familiar with the test procedures. A replicate test is often performed while the respondents are present in the sensory test facility. The popularity of these tests is also due the simplicity of data analysis: statistical tables of binomial distribution give the minimum number of correct responses needed to conclude statistical significance as a function of the number of participants.
2.2.2b Affective methods

These tests have the main objective of quantify the degree of linking or disliking of a product. The most straightforward approach to this method is to offer people a choice among alternative products and see if there is a clear preference from the majority of respondents. The problem with these tests is that they do not give information on the magnitude of liking or disliking from respondents. The hedonic scale, frequently used for these tests, was developed in 50’s (Jones et al., 1955) and was constituted by a 9-point scale for liking with a centered neutral category and attempted to produce scale point labels with adverbs that represents psychologically equal steps or changes in hedonic tone. Typically, a hedonic test involves 75-150 participants, that have to be regular consumers of the product tested. Consumers are given several alternative versions of the product and the large number of interviewed compensates the effect of individual preferences, ensuring statistical power and test sensitivity. This also provide an opportunity to look for segments of people who may like different styles of a product, for example, different colors or flavors. This approach with consumers has the aim to indagate their preferences, asking them to indicate their most liked product. Although these tests appear straightforward and simple, several complications are encountered in the methods, notably how to treat replicated data and how to analyse data that include a “no-preference” option as a response (Lawless et al., 2010).
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2.2.2c Descriptive analysis (DA)

Classic or generic descriptive analysis is the gold standard technique in sensory science (Lawless et al., 2010). Sensory characterization is extensively applied in the industry for the development and marketing of new products, the reformulation of existing products, the optimization of manufacturing processes, the monitoring of sensory characteristic of the product present on the market, the implementation of sensory quality assurance programs, the establishment of relationship between sensory and instrumental methods and for estimating sensory shelf life (Varela et al., 2014).

These methods are used for quantifying the perceived intensities of different attributes detected in a sample. In the late 1940s the first method to do this with a panel of trained judges was established and named Flavor Profile® (Caul, 1957). This first approach to descriptive analysis represents a flexible tool, useful to solve problem of off-flavors in nutritional capsules and questions about the sensory contribution of sodium glutamate in different processed foods. This method enabled panellists to characterize all of the notes by means of a category scale and noting their order of appearance. Subsequently, in the 1970s, a refined method, known as Texture profile, to quantify food texture, much as the flavour profile had enabled the quantification of flavour properties (Szezesniak et al., 1975). Using Texture Profile, rheological and tactile properties of foods, and how these change every time during biting, could be characterized, by means of a fixed set of force-related and sharpe-related attributes. After that, other approaches were proposed for descriptive analysis of food. In 1974, the method named Quantitative Descriptive Analysis (QDA®) enlarged the possibility of description, in terms of attributes, to all the sensory perception, and not only to taste and texture (Stone et al., 2012). To the best of our knowledge, descriptive analysis has proved to be the most comprehensive and informative sensory evaluation tool. In fact, this method can be applied to a wide variety of product changes and research questions in food product development. Additionally, the information acquired can be related to consumer acceptance information but also to instrumental measurement, by means of statistical techniques such as regression and correlation. In fact, sensory descriptive analysis acts like a bridge between different areas of research, product development and consumer science, providing a link between the products characteristics and consumer perception.
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Before performing a DA, few keypoint should be fixed: the selection of judges (and their numerosity), the training phase, the selection of attributes and references have to be pointed out, togheter with a certain number of replicates when real samples are evaluated.

**Panel selection.** The panellists must be motivated and interested on the research activity in which they are going to be involved in. A number of ten to twelve judges is recommended. Regarding the previous screening of panellists, researchers are divided: many encourage this (Barcenas et al., 2000; Noronha et al., 1995), while others found that the screening seems to decrease panel performance, especially when the process is onerous and protracted (Nachtsheim et al., 2012).

**Term generation and reference standard.** In this process the panellists are enrolled in determining, through consensus, the attributes that discriminate among the samples. On the first day, judges are served many samples, chosen to be as different as possible, in order to define, individually and quietly, a list of attributes that must be actionable; thus, reference standard could be selected for each of them. After this first moment of individual work, the panellists start a discussion, guided by the panel leader, and reading the attributes they selected. The panel leader works as a communication facilitator without involvement and interference with panel discussions. In this way, terms that refer to the same meaning can be grouped and only one among each group will be selected, in order to avoid redundancy.

At the next training session, panellists are given another subset of samples and the process is repeated; additionally, potential reference standards (selected from literature or previous experiences) are showed to the judges, to anchor the attributes. References can be used for generating sensory terminologies, especially when panellists are confused and disagree with each other on some sensory attributes. This process is repeated as many time as it is needed, in order to ensure that all the potential terms have been listed. Once the attribute list has been completed, the training session involve making sure that the entire panel is comfortable with the specific reference standards; subsequently, panellists are shown the profile sheet set up with all the terms selected.

**Evaluation of samples.** Once the panel has been trained and tested, the actual evaluation of samples can start. It is usual that this process occurs in individual, temperature and light controlled booths (Lawless et al., 2010) and data collection could be done by means of
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paper profile sheet data acquisition or by computerized system. Panelists must be made to feel welcome and appreciated during the data acquisition phase, to ensure continued motivation and interest. It is not unusual to serve them some snacks as a token of appreciation after they complete their sensory sessions. In certain situations, it may also be appropriated to pay panellists (Varela et al., 2014).


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2.3 Methods for volatile compounds analysis

In general, the analytical methods used for the evaluation of volatile compounds involve all
the steps of the general analytical process such as sampling, sample preparation, separation,
identification, quantification and data analysis (Morales et al., 1992).

Methods for volatile compounds determination reported in literature can be divided in two
different groups, according to their eventual requirement for a pre-concentration of
compounds, in order to have volatiles in a concentration higher than the quantification limit
of the method itself (Angerosa et al., 2004; Angerosa, 2002).

This additional requirement of pre-concentration phase is related to the complexity of
headspace in many food matrix (e.g. olive oil); moreover, volatiles can contribute to the
aromatic profile of a sample even being present in a very low concentration (Escuderos et
al., 2007).

The main techniques that do not necessary need a pre-concentration of compounds are: the
direct injection, the static headspace analysis (SHS) (Escuderos et al., 2007) and the thermal
desorption (TD). The latter is represented by the direct desorption of analytes from an
appropriate adsorbent, avoiding intermediate extraction and concentration steps prior to
analysis. As a consequence, TD methods are characterised by a higher sensitivity, which
may provide shorter sampling times or lower sampling volumes.

On the other hand, techniques related to an enrichment/pre-concentration of the samples are
frequently used and represented by (Vichi et al., 2010; Escuderos et al., 2007):

- **Dynamic Headspace Analysis (DHS)**: volatile compounds are dragged by an inert gas
  flow, previously gurgled through the heated sample, and subsequently trapped in an
  adsorbent suitable material (active carbon, polymers, solvents, cryogenic traps).
  Molecules are then desorbed by elution with a suitable heated solvent and gas
  chromatographically analysed;

- **Supercritical Fluid Extraction (SFE)**: this tecnique advantages are time reduction and
  high efficiency extraction;

- **Stir Bar Sorptive Extraction (SBSE)**: based upon sorption, which is a form of
  partition based upon the analyte's dissolution in a liquid-retaining polymer from a
  liquid or vapor sample, thus, originating a bulk retention (Bicchi et al., 2000).
Solid-Phase Micro Extraction (SPME): SPME techniques was developed as an alternative to the dynamic headspace analysis and is based on the use of a holder, comparable to a syringe that contains a steel needle enclosing a retractable chemically inert silica fibre. The fibre is coated with an adsorbent material (stationary liquid or solid phase) which is exposed, for a predefined time, in the sample headspace. Molecules are then desorbed and subsequently gas chromatographically analysed.
2.3.1 Gas chromatographic analysis of volatile compounds

The chromatographic technique was applied for the first time by Tswett, a Russian-Italian botanist, during his research on plant pigments, in 1903. He used liquid-adsorption column chromatography with calcium carbonate as adsorbent and petrol ether/ethanol mixtures as eluent to separate chlorophylls and carotenoids.

Actually, the chromatographic system could be schematically illustrated in 4 “blocks”, as reported in Figure 1: the mobile phase is flown through the column (stationary phase); the samples to be analysed is introduced in the system by the injector port; in this way, the sample is inserted into the mobile phase and passes through the column; at the exit point of the column, an analite detector is located and used to define the composition of the analysed mixture.

![Figure 1. Blocks diagram for a chromatographic system.](image)

When the mobile phase is a gas, the technique is defined as gas chromatography (GC). In this technique, the substances to be separated, nevertheless they are liquids, solids or gasses, have to be carried to a temperature useful to transform them to gasses, or to move them to vapor state. The state of gas is obtained by heating the substances in the moment of the introduction into the instrument and maintaining the high temperature.

The essential parts that compose a gas chromatograph are (McNair et al., 2009):

1. supply for carrier gas;
2. sample injection system (or injector);
3. chromatographic column housed in the oven of the instrument;
4. detector;
The equipment used in gas chromatography is reported in Figure 2.

In GC, the gas used as mobile phase is an inert gas that does not compete with the stationary phase, but which has simply the task of "carry" (carrier gas) the solutes to the end of the column. The differential migration of different molecules is due to the different interactions they have with the stationary phase (Mentasti et al., 1990).

The analysis of a sample starts when the syringe lays the solution into the injector device which contains a soft septum that provides a gas-tight seal but can be penetrated by a syringe needle for sample introduction. There are several different designs of injection ports according to the various sample and instrumental requirements (e.g. type of column) (Qian, 2010):

- Split mode: the sample is injected into a vaporization chamber glass (glass liner) in which it rapidly evaporates. The flow of gas is relatively high and follows 3 different
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ways: a part (purge line) touches and cleans the silicone septum, a part (sample line) carries the sample vapor into the column and a part (split-line) carries the sample vapor to the output of the separator (splitter), regulated by a needle valve (split valve). The relationship between the separator flow (split flow) and the column flow is called "split ratio" and is what is regulated by the split valve; it determines the amount of sample that actually enters the chromatographic column.

• Splitless mode: the sample is injected into a glass liner chamber with the splitter valve closed. The process of volatilization of the sample, therefore, is progressive from the point of injection and the transfer to the column of the volatilized solutes is slow; additionally, the solutes are mixed with solvent. After a certain time (splitless period), during which only the purge line remains open, the split line is opened both to split a part of the solutes and to make the other part flows towards the column. To minimize the loss of the sample components by condensation on the top of the injector, the temperature is maintained constant from the septum down thanks to an additional heater.

• On-column mode: the "on-column" injectors could be equipped with a manual introduction valve (by rotation) or an introduction septum that accommodates the syringe needle into a needle channel. The mixture of solute enters the glass liner with the injector maintained at room temperature by a flow of cold air; subsequently, the injector is heated to obtain the evaporation of the solvent and vaporization of the sample components, which are transported by the gas inside the column.

The GC column may be classified either as packed or capillary (open tubular). The second type may be viewed as an alternative for the packed column, being the most popular. In the capillary column, the stationary phase could be a thin film of liquid that coats the inside wall (wall-coated capillary column – WCOT), an adsorbed layer of a very small solid support coated with a liquid phase (support-coated open-tubular column – SCOT) or a porous layer of a solid adsorbent (porous layer open-tubular column – PLOT).

The detectors are able to return both a full signal or a differential signal. The integral signal produces a cumulative chromatogram, represented by a series of ramps which correspond to the solutes separated by the column, with an order that corresponds to their elution order. The differential signal produces a chromatogram represented by a series
of peaks which correspond to the solutes separated by the column as a function of their retention time. The detectors generally used for the GC analysis are:

- **Thermal Conductivity Detector (TCD)**: is based on the principle that a gas, flowing out of a heated filament reduces the filament temperature by removing part of the heat. The amount of heat removed depends on the flow and the thermal conductivity of the gas flowing. In a gas mixture, therefore, it depends on the thermal conductivity of each component (solute, solvent, carrier gas) and the partial pressure of each of them. The TCD body dual cell is a metal block of mass and high thermal inertia in which two cells are obtained. It is thermostatically controlled to a temperature of about 50 °C higher than the maximum of the column, so as to avoid condensation phenomena.

- **Flame Ionization Detector (FID)**: this detector is one of the most commonly used. In this case the detection is based on the ions formed during combustion of organic compounds in a hydrogen flame. The generation of these ions is proportional to the concentration of organic species in the sample gas stream. The formation of positive ions and electrons produces their migration towards the cathode and towards the anode respectively, with the production of a stream of charged, as an electric current capable of providing a signal. The FID response doesn’t depend on the concentration of the solute, but on the number of carbon atoms present in the molecule.

- **Electron Capture Detector (ECD)**: this detector is based on the primary ionization of the substances for emission of β- particles (fast electrons) by a weak emitter, such as the radioactive isotope 63Ni. The carrier gas carries the solutes containing electronegative groups (halogens, peroxides, quinones, nitro, etc.) to the detector. The anions that are formed have a reduced mobility to the anode (than that of the electrons towards the anode) and, therefore, a modest electrical conductivity, in order to not alter the value of the background.

- **Nitrogen Phosphorous Detector (NPD)**: is based on a FID in the presence of alkaline salts (silicate of Rb or Cs). The flame vaporizes and ionizes the Rb of the silicate, with formation of Rb⁺ ions and electrons. The Rb⁺ ions migrate to the cathode, where it will discharge and re-form the salt, while the electrons are attracted by the anode. If the difference of potential between the two electrodes and the flame temperature are
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constant, there is a balance which corresponds to a constant electric current between the two electrodes. When the solutes that elute from the column reach the detector, if some of them contain the atoms of N, P or halogen, they are decomposed by the flame and form of radical that selectively reacts with Rb$^+$ ions present in the flame, removing them from the balance of ionization of Rb. To restore this balance, the system comprises further vaporization and ionization of the silicate Rb, with the formation of new electrons. The net balance of these processes is, therefore, an increase of the electric current in the circuit, i.e. the signal that reveals the solutes.

Additionally, one of the most diffused detector, frequently coupled to gas chromatographic system, is the mass spectrometer (MS). This application is able to convert individual molecules into ions, so that they can be moved about and manipulated by external electric and magnetic fields. Because ions are very reactive and short-lived, their formation and manipulation must be conducted in a vacuum. The pressure under which ions may be handled is roughly $10^{-5}$ to $10^{-8}$ torr (less than a billionth of an atmosphere). Each of the three tasks listed above may be accomplished in different ways. In one common procedure, ionization is effected by an high energy beam of electrons, and ion separation is achieved by accelerating and focusing the ions in a beam, which is then bent by an external magnetic field. The ions are then detected (qualitatively and quantitatively) electronically and the resulting information are shown in a mass spectrum (Figure 3). In GC–MS systems, the analytes may be ionized in a number of ways but, most often, electron ionization (EI) is used especially for automated screening analysis. Cations formed by the electron bombardment are pushed away by a charged repeller plate (anions are attracted to it), and accelerated toward other electrodes, having slits through which the ions pass as a beam. Some of these ions fragment into smaller cations and neutral fragments. A perpendicular magnetic field deflects the ion beam in an arc whose radius is inversely proportional to the mass of each ion. Lighter ions are deflected more than heavier ions. By varying the strength of the magnetic field, ions of different mass can be focused progressively on a detector fixed at the end of a curved tube (also under a high vacuum). When an high energy electron collides with a molecule it often ionizes it by knocking away one of the molecular electrons (either bonding or non-bonding). Residual energy from the collision may cause the molecular ion to fragment into neutral pieces and smaller fragment ions. The molecular ion
is a radical cation, but the fragment ions may either be radical cations or carbocations, depending on the nature of the neutral fragment. The mass spectra obtained is representative of the molecules fragmentation into single ions (Figure 3).

Figure 3. Example of mass spectrum (image source: teaching material of Prof. G. Bonaga)

The most intense ion is assigned an abundance of 100, and it is referred to as the base peak. Most of the ions formed in a mass spectrometer have a single charge, so the mass-to-charge ratio \((m/z)\) value is equivalent to mass itself. Modern mass spectrometers easily distinguish (resolve) ions differing by only a single atomic mass unit (amu), and thus provide completely accurate values for the molecular mass of a compound. The highest-mass ion in a spectrum is normally considered to be the molecular ion, and lower-mass ions are fragments from the molecular ion, assuming the sample is a single pure compound.
2.3.2 Solid-Phase Micro-Extraction Gas-Chromatography coupled to Mass Spectrometry (SPME-GC-MS)

SPME coupled to GC and MS, is considered the election technique for the separation, identification and quantitation of food typical volatile compounds (Romero et al., 2015; Damerau et al., 2014). Since 2000’, the use of SPME-GC-MS represents an alternative to the use of Dynamic Headspace Analysis method, as in this case the analite present in the sample is directly extracted in the coated fiber; samples are prepared without the addition of any solvent. This technique makes use of a fused-silica fiber coated with different stationary phases (Cavalli et al., 2003). The fiber part is made up of a modified syringe with a holder and a needle; the fiber is preserved inside the needle and exposed only during the absorption phase, in the sample headspace. For this reason, the fused silica fiber is covered with a thin film of polymers that act like a sponge, absorbing the volatiles (Figure 4) (Kataoki et al., 2000).

![Figure 4](image-source-Sigma-Aldrich). After the absorption, compounds should be desorbed from the fiber. Normally, the desorption, separation, identification and quantification of volatiles are carried out by gas-chromatography coupled to mass spectrometry (GC-MS), with its well-known advantages
as an efficient tool for analyses of volatile and semi volatile compounds. After a suitable
extraction time, the fiber is reinserted inside the needle and subsequently placed in a GC
injection port; then the analytes are desorbed in the injector by heating. The sample solution
is injected into the GC inlet where it is vaporized and swept onto a chromatographic column
by the carrier gas (usually helium). The sample flows through the column and the
compounds comprising the mixture of interest are separated by virtue of their relative
interaction with the coating of the column (stationary phase) and the carrier gas (mobile
phase, usually helium). The final part of the column passes through a heated transfer line
and ends at the entrance to ion source where compounds eluting from the column are
converted to ions by electron ionisation (EI): a beam of electrons ionise the sample
molecules resulting in the loss of one electron. A molecule with one electron missing is
called the molecular ion and is represented by $\text{M}^+$(radical cation). The next component is a
mass analyser, which separates the positively charged ions according to various mass
related properties depending upon the analyser used. Several types of analyzers exist:
quadrupoles, ion traps, magnetic sector, time-of-flight, radio frequency, cyclotron resonance
and focusing to name a few. The most common are quadrupoles and ion traps. After the ions
are separated they enter a detector, the output from which is amplified to boost the signal.
When the resulting peak from this ion is seen in a mass spectrum, it gives the molecular
weight of the compound. Due to the large amount of energy imparted to the molecular ion it
usually fragments producing further smaller ions with characteristic relative abundances that
provide a 'fingerprint' for that molecular structure. This information may be then used to
identify compounds of interest and help elucidate the structure of unknown components of
mixtures (Figure 5). The detector sends information to a computer that records all the data
produced, converts the electrical impulses into visual displays and hard copy displays. In
addition, the computer also controls the operation of the mass spectrometer.
Figure 5. Diagram of gas chromatograph with mass spectrometer (image source: Leckerman Law, LLC).

One main concern of analytical laboratories is to reduce the extraction time of compounds from matrices by the minimum use of organic solvents. A parallel target also is to reach satisfactory levels of detection apart from the acceptability in terms of recovery. In this frame SPME is a complementary tool that can comply with the above criteria (Tzanetou et al., 2013).
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2.3.3 Odour detection methods: olfactometry and chemical sensors

An odour is a mixture of light and small molecules, also at very low concentrations in the inhaled air, which, upon coming in contact with the human sensory system, is able to stimulate an anatomical response: the experienced perception is the odour (Craven et al., 1996).

Among the most innovative methods for the analysis of volatile compounds the application of so-called "electronic noses" (E-noses) can be mentioned, as a system developed with the aim to mimic the human olfaction in the discrimination of odors (Röck et al., 2008).

The discovery of materials with chemo-electronic properties has provided the opportunity for the development of artificial olfactory instruments mimicking the biological system (Craven et al., 1996). In the last ten years, a large field of scientific research has been devoted to the development of E-Nose that are sensor-based instruments, capable of discrimination between a variety of simple and complex odours.

Instrumental approaches to the characterization of odorants are based on the evaluation of the air chemical composition. First, the odorous air needs to be collected for subsequent analysis: the traditional volatile organic compounds (VOCs) sampling methods, like adsorbers or metal canister and polymer bags. According to the principle of the system used, electronic noses can be divided in different generations.

2.3.3a Sensor-based electronic noses

The first generation of electronic nose was constituted by metal oxide sensors with different selectivity; these sensors send electrical signals in the presence of volatile molecules (Ragazzo-Sanchez et al., 2005). The main advantage of this method is represented by the rapid identification of the sample smell, without the necessity of identifying every single compound responsible for the smell itself (Xia et al., 2015; Wilson et al., 2009). Several studies, related to the application of electronic nose to different food matrices, have already been published (Messina et al., 2015; Cimato et al., 2006; Cosio et al., 2006a; Cosio et al., 2006b). Like human olfaction, E-Noses are based on “an array of electronic-chemical sensors with partial specificity to a wide range of odorants and an appropriate pattern recognition system” (Gardner et al., 1994). In contrast to the ideal gas sensors, which are required to be highly specific to a single chemical species, sensors for E-
Nose need to give broadly tuned responses like the olfactory receptors in the human nose: in both cases the odour quality information and recognition is ensured by the entire pattern of responses across the sensors array, rather than the response of any one particular sensor. Furthermore, mimicking the data processing in the biological systems, the incoming chemo-electronic signals are processed through the use of data reduction techniques (Principal Component Analysis - PCA); in both human and electronic noses, the function of odour recognition is finally achieved by means of some form of associative memory for the storage and recall of the previously encountered odours.

A wide variety of competing sensor technologies (conducting polymers, piezoelectric devices, electrochemical cells, metal oxide sensors (MOX) and metal-insulator semiconductor field effect transistors (MISFETs)) are currently available: independently of the considered device, sensor elements have to show fast, reproducible and reversible responses to odour samples (Ampuero et al., 2003; Pearce, 1997).

Chemical sensors comprise an appropriate and chemically-sensitive material interfaced to a transducer; hence, the solid-state sensors are essentially constituted by a chemically sensitive interface (sensitive material) and a transducer able to convert a chemical input (gas concentration or ions concentration) and/or physical input (temperature, pressure, acceleration, etc.) into an output, generally an electrical signal, by means of a conditioning and/or signal processing electronics. The input magnitudes include chemical and/or biological magnitudes such as concentration and identity of unknown species in gaseous or liquid phase, other than physical general magnitudes such as temperature, pressure, speed, acceleration and force. A transduction process is realized by converting the input-event into an output electrical signal (analogue voltage or current, digital voltage) correlated to the measurand that generates it. The output electrical signal is properly conditioned, processed and stored for analysis (Gardner et al., 1991). Gas sensors, based on the chemical sensitivity of semiconducting metal oxides, are readily available commercially and have been more widely used to make arrays for odour measurement than any other single class of gas sensors. On the other hand, the various categories of solid-state chemical sensors are differentiated by the physical principle of the signal transduction by distinguishing the following transducers: conductometric (resistive), optical, electrochemical, mechanical/acoustic or ultrasonic, thermal and MISFET.
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2.3.3b GC-based electronic noses

**Dynamic olfactometry.** As occurring in the industry (i.e., food, beverages, perfumes, etc.) for many years, the sensory evaluation of smells by means of panels of sensory trained evaluators has been the main odour assessment and quantification tool: the so-called dynamic olfactometry is the standardized method used for determining the concentration of odours and evaluating odour complaints (Schulz et al., 1996).

This methodology is based on the use of a dilution instrument, called olfactometer, which presents the odour sample diluted with odour-free air at precise ratios, to a panel of human assessors. The examiners are selected in compliance with a standardized procedure performed using reference gases; only assessors who meet predetermined repeatability and accuracy criteria are selected as panelists. The odour concentration, usually expressed in odour units (ou/m$^3$) is numerically equal to the dilution factor necessary to reach the odour threshold, that is the minimum concentration perceived by 50% of population (EN13725).

According to European standardization, 1 ou/m$^3$ is defined as the amount of odourant that, when evaporated into 1 m$^3$ of gas air at standard conditions, causes a physiological response from a panel (detection threshold) equivalent to that of $n$-butanol (reference gas) evaporated into 1 m$^3$ of neutral gas. The perception of odours is a logarithmic phenomenon (Stevens, 1960); for this reason, in this kind of measurements it is necessary taking into account that odour concentration is associated to odour intensity though a defined logarithmic relation.

The opportunity of using sensory perception for the development of conventional instruments for chemical analysis is represented by gas chromatography-olfactometry (GC-O) technique that couples the traditional gas chromatographic analysis with sensory detection, in order to study complex mixtures of odorous compounds (Leland et al., 2001). The gas chromatographic separation of an odorous air sample could be useful for identifying specific odorant components: GC-O, thus, allows a deeper comprehension of the odorant composition as concerns the compounds’ identification and quantification, offering the advantage of a partial correlation between the odorant chemical nature and the perceived smell (Friedrich et al., 1998). The schematic representation of a GC-O equipment is reported in Figure 6.
Each separated compound, eluted by the GC, can be detected by a human assessor (odor present or not), who is able to measure the duration of the odor activity (start to end), to describe the quality of the odor perceived and to quantify its intensity. GC-O in combination with a mass spectrometer (GC-O-MS) not only enables the evaluation of odor compounds, but also their identification with mass spectral information.

In particular, the flow of the eluate is split so that the analytes reach both detectors simultaneously, permitting a comparison of both signals. The retention times of the analytes might differ for the two detectors (typically shorter for the mass spectrometer), due to the fact that the mass spectrometer works under vacuum conditions while the olfactometric detector works under atmospheric pressure conditions.

The design of all commercially available olfactometric ports is very similar. The eluate reaches the port through an uncoated transfer line (deactivated silica capillaries) and is sniffed in a glass or a PTFE conical port, fitted to the shape of a nose. The transfer line is heated to prevent the condensation of semi volatile analytes on the walls of the capillary. Auxiliary gas (moist air) is added to the eluate to prevent the drying of the assessors' nose mucous membranes, which could cause discomfort especially in longer analyses. The transfer line length can vary, but it must be long enough to ensure a comfortable sitting position and to avoid discomfort due to the vicinity of hot chromatograph components during detection. Each port is also equipped with an electric push-button to generate a signal of 1 V when pressed. If the extract analyzed is sufficiently concentrated, the eluate stream
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can sometimes be separated into several streams and delivered to more olfactometric ports, for the simultaneous detection by several assessors (Brattoli et al., 2013). There are several factors that determine the quality of the data collected by GC-O. The method used to extract volatile compounds from the samples determines the composition of the extract, and therefore the quality of the eluate available for perception. The set-up of the GC instrument and of the separation conditions affects the quality of chromatography and the response of the human detector. The peak shape affects the perception of odor intensity and the calculation of detection thresholds. Chromatographic behavior of odor substances varies with the compounds and the stationary phases of the GC column.

Flash-GC E-nose. Nowadays, a new approach is represented by the new generation electronic noses, also known as Flash Gas Chromatography Electronic Nose. An example of this new generation of electronic noses is represented by the HERACLES Electronic Nose (Alpha MOS, France – Figure 7), that features two metal columns of different polarities mounted in parallel and coupled to 2 Flame Ionization Detectors (FID); it allows headspace or liquid injection modes. Therefore, two chromatograms are obtained simultaneously, allowing a double identification of the chemical compounds. The integrated thermoregulated solid adsorbent trap allows an efficient pre-concentration of light volatiles and shows a great sensitivity (in the pg range). With fast column heating rates (up to 600°C/min), results are delivered within seconds and the analysis cycle time is around 5 to 8 minutes (Xiao et a., 2014).

Figure 7. HERACLES II Electronic Nose apparatus (image from Alpha MOS)
The software for the instrument management and control can also perform multivariate statistical analysis as Principal Component Analysis (PCA), Partial Least Square (PLS), Discriminant Factor Analysis (DFA), Statistical Quality Control (SQC) and Soft Independent Modelling of Class Analysis (SIMCA). The compounds are detected using FID detector and the software is able to “tag” every single molecule by comparison of its Kovats retention index saved in the database of the instrument. Several studies regarding the use on new generation electronic noses have been already published, concerning different food matrices as: virgin olive oil with different geographical origin (Melucci et al., 2016), plum and cherry spirits differently produced and with different geographical origin (Śliwińska et al., 2016a and 2016b), meat produced with porks differently fed (Wojtasik-Kalinowska et al., 2016), slimming pills produced with the addition of prohibited substances (Xia et al., 2015), sorgum distilled with different geographical origin (Peng et al., 2015), odour nuisance from landfill (Gębicki et al., 2014), quality evaluation of agricultural distillates (Dymerski et al., 2014).

2.4 Data Analysis

All measurements were carried out in triplicate (if not stated otherwise), and the results are expressed as mean values (± standard deviations). The analysis of variance and multivariate analysis of data were performed with XLSTAT version 2011.1.03 software, when other statistical softwares were used, they are specified in the following sections. In the first study, PCA, partial Least Square analysis and pairwise t-test were used for the evaluation of volatile compounds data. Statistical analysis of sensory data was carried out calculating median values. In the second study, the significant differences in terms of chemical compounds amounts were calculated with Fisher Least Significant Differences (Fisher LSD) and a value of p ≤ 0.05 was considered to be statistically significant. In the third study, the peak areas were area normalized and mean centred before the PCA and SPME-GC-MS data were explored with ANOVA and subsequently with PCA (IBM – SPSS) and combined to sensory mean values by means of multifactorial analysis (MFA). The significant differences in terms of chemical compounds amounts were calculated with Fisher Least Significant Differences (Fisher LSD) and a value of p ≤ 0.05 was considered to be statistically significant. In the fourth study, peak areas were area normalized and mean centred before the PCA and SPME-GC-MS. The analysis of variance was carried out and data were
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analysed with PCA; sensory, chemical and instrumental (analysis of image and textural properties) data were combined with MFA. In the fifth study, data from sensory and instrumental (analysis of image and textural properties) analysis were explored with PCA with Unscrambler® X (v.10.1; CAMO Software AS, 2011, Norway). The precision and the predictive capabilities of the models were evaluated by the coefficients of determination (R2) and root-mean square error estimated by cross-validation (RMSECV). In the last study, preference data were analysed according to chi square test (p>0.05). Statistical analysis of data was carried out using the SAS/STAT statistical software package version 9.3.1. (SAS Institute Inc., Cary, USA).
2.5 References


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EN13725: Air Quality—Determination of Odour Concentration by Dynamic Olfactometry; Committee for European Normalization (CEN), Brussels, Belgium, 2003.


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

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Authors: Melucci D\textsuperscript{a}, Bendini A\textsuperscript{b}, Tesini F\textsuperscript{b}, Barbieri S\textsuperscript{b}, Zappi A\textsuperscript{a}, Vichi S\textsuperscript{c}, Conte LS\textsuperscript{d}, Gallina Toschi T\textsuperscript{b}

\textsuperscript{a} Department of Chemistry Ciamician, University of Bologna, Via Selmi, 2, 40126 Bologna, Italy
\textsuperscript{b} Department of Agricultural and Food Sciences (DiSTAL), University of Bologna, P.zza Goidanich 60, 47521 Cesena, Italy
\textsuperscript{c} Department of Food Science and Nutrition, University of Barcelona, Food and Nutrition Torribera Campus, Av. Prat de la Riba, 171, S.ta Coloma de Gramenet, Spain
\textsuperscript{d} Department of Food Science, University of Udine, Via Sondrio 2/a, 33100 Udine, Italy

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3.1.1 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.
**3.1.2 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. 29/12 (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., geographical 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, et al., 2015; Azizian et al., 2015; Mendes et al., 2015; Sinelli et al., 2010; Diraman et al., 2009; Dibeklioğlu, 2009). In this context, adulteration of EVOOs has been studied by liquid chromatography (HPLC), GC, and linear discriminant analysis (LDA) using fatty acids (FA) and triacylglycerols (TGs) as markers (Jabeur et al., 2014; Ollivier et al., 2006). HPLC-mass spectrometry (MS) and LDA allowed determination of the phenolic profile for discrimination of geographical origin (Taamalli et al., 2012). In particular, specific volatile compounds or their classes (e.g., terpenoid compounds) have been used to discriminate EVOO samples according to geographic origin (Cecchi et al., 2013; Ben Temime et al., 2006; Zuninet al., 2005; Vichi et al., 2003). 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 et al., 2013; García-González et al., 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 et al., 2013); principal components analysis (PCA) (Cimato et al., 2006); LDA (Pouliarekou et al., 2011); PCA and hierarchical clustering analysis (HCA) (Procida et al., 2005). Among the chemical species in EVOO, many volatiles have been related with specific sensory characteristics (Aparicio et al., 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 et al., 2015). Moreover, the electronic nose results have been successfully correlated to those obtained with other techniques (sensory, GC, and GC–MS) (Lerma- García et al., 2010; Mildner-Szkudlarz et
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al., 2008). 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 flash 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.
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3.1.3 Materials and Methods

3.1.3a 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.

3.1.3b Volatile compounds analysis

Flash Gas Chromatography Electronic 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 Italia 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 (H2) 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.

**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 analysed 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
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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 the 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 x0.25 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.
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3.1.3c Sensory analysis

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).

3.1.3d 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).

3.1.3e 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 preprocessed 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 (HCA), 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 (LDA) 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.
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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 (yi) and the degree of belonging to class M (yM). 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 preselection 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).
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3.1.4 Results and discussion

3.1.4a 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.

3.1.4b 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 Figure 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 C6 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”.

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Figure 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);
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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 E-Nose (Set A). Samples and peak numbers according to the (A).

Moreover, a larger peak of a compound tentatively identified as dodecene could be observed (see also Figure 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. C5 molecules and pentene dimers). Volatile data obtained from SPME-GC-MS were elaborated by PCA to compare the profile of volatile compounds (Figure 2).

![Figure 2](image.png)

Figure 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).

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, in red) 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 in blue) 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.

**3.1.4c PLS-DA from FGC E-nose full chromatograms of Set A**

Figure 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 Figure 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 RMSECV (0.15) with high correlation (R2= 0.980; R2 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.
3.1.4d 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 13 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 Figure 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 Enose 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 Figure 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
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from four suppliers who declared that they were from EU countries, but did not contain Italian oil.

**Figure 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.

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.

**3.1.4e 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 paragraph 3.1.4b, although in this case there is a much higher number of objects. The outputs relevant to the PLS-DA model are reported in Figures 4A to 4C. 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
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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.
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**Figure 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.
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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 14 chromatograms, 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 paragraphs 3.1.4c and 3.1.4e 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 (yI or yM) was obtained. Predicted values for yI or yM 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”.

3.1.4f 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 paragraph 3.1.4d, 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 subdataset was obtained. The scores plot obtained from I-M dataset is reported in Figure 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.

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

It must be pointed out that neither the gas chromatographic conditions nor the headspace conditions, employed for SPME-GC-MS and FGC E-nose respectively, 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; γ -coppaene; γ -murolene; γ -pinene; benzeneethanol (2-Phenylethanol);
benzenemethanol; citronellal; decanal; decane; dimethylnonadienal; (E,E)-γ-farnesene; (E)-2-heptenal; (E)-2-hexenal; (E)-2-hexenol; (E)-2-pentenal; (E)-2-pentenol; (E)-b-ocimene; ethanol; ethylacetate; formic acid; hheptanal; 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. 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 morepolar 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 Figure 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 (Figure 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 16 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 Figures 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
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correlation loadings plot, respectively reported in Figures 6A and 6B, shows that I samples are characterized by negative PC1 scores and M samples are characterized by positive PC1 scores; this suggests that molecules identified by MS spectra and characterized by negative PC1 loadings and positive PC1 loadings may be related to I and M samples, respectively.

Figure 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).
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3.1.4g 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 paragraphs 3.1.4d and 3.6f 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 paragraph 3.1.4d) 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 characteristic, 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.

3.1.5 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 seconds of acquisition time and 18 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.

3.1.6 Acknowledgements

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3.1.7 References


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study bacteria and fungi in biofilms used for bioremediation. Current Drug Targets, 14, 1023-1033.


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3.2 Chemical and sensory characterization of olive oil enriched in lycopene from tomato by-product: sensory evaluation, chromatographic profile of volatile compounds and other chemicals

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Authors: Bendini A\textsuperscript{a}, Di Lecce G\textsuperscript{a}, Valli E\textsuperscript{a}, Barbieri S\textsuperscript{a}, Tesini F\textsuperscript{a}, and Gallina Toschi T\textsuperscript{a,b}

\textsuperscript{a} Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, U.O.S. Cesena (FC), Italy
\textsuperscript{b} Food Waste Innovation Center, Alma Mater Studiorum, University of Bologna, Bologna, Italy

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\textbf{3.2.1 Abstract}

The aim of this investigation was to produce an olive oil (OO) naturally enriched with antioxidants, recovering carotenoids, in particular lycopene, using an industrial by-product of tomato seeds and skin. For this purpose, a technological process in a low-scale industrial plant to co-mill olives and tomato by-product in defrosted or freeze-dried forms was applied and studied with respect to control samples. Preliminary results obtained from two different experiments were carried out by 40 kg of cultivar Correggiolo olives and 60 kg of olive blends from different cultivars. In both the experiments, the co-milling showed significant enrichment in carotenoids, especially in lycopene (mean values of 5.4 and 7.2 mg/kg oil from defrosted and freeze-dried by-products, respectively). The experimental results demonstrated the possibility to obtain a new functional food naturally enriched in antioxidant compounds, which might be marketed as “OO dressing enriched in lycopene” or “condiment produced using olives and tomato by-product”.

\textbf{Keywords:} Antioxidants; co-milling; lycopene; olives; tomato by-product.


3.2.2 Introduction

Carotenoids are important lipid-soluble plant pigments involved in photosynthesis and photo-protection from excessive light. Among these, lycopene, an acyclic carotenoid which contains conjugated double bonds arranged linearly in situ typically in the all-trans form, has numerous biological properties, mainly linked to its antioxidant (single oxygen quenching and radical scavenging) and anti-inflammatory properties (Kaulmann et al., 2013), that are beneficial for human health. Lycopene is found in plasma and tissues of the human body, including skin, where it seems to exert an effective action in detoxifying free radicals and protecting from damage caused by UV and photo-aging (Cazzola, 2012). Several epidemiological studies have suggested that an adequate intake of lycopene may reduce the risk of cancer and chronic diseases (Rao et al., 1999; Stahl et al., 1996), even if the EFSA Panel in 2011 concluded that a clear cause and effect relationship has not been established yet (EFSA, 2011). Specific research has shown that an increase in dietary lycopene produces an increase in serum lycopene irrespective of the amount of fat intake. Moreover, a diet high in olive oil (OO) and rich in lycopene may decrease the risk of coronary heart disease by improving the serum lipid profile (Ahuja et al., 2006). The role of antioxidants in human nutrition and their associated health-beneficial effects for a number of chronic diseases, including certain types of cancer and cardiovascular diseases, have gained increased attention. There is a particular interest in components of the so-called ‘‘Mediterranean diet’’, such as tomato and OO, which have been associated with a healthier lifestyle (Capanoglu et al., 2010). Tomatoes and OO are rich sources of key antioxidant components such as carotenoids, tocopherols and polyphenols, but their amounts may vary greatly in relation to the adoption of different agronomic, climatic and technological variables (Frusciante et al., 2007; Morello` et al., 2006; Servili et al., 2004; Dumas et al., 2003). These fruits are consumed both as fresh products and after processing (mechanical and thermal treatments for tomato products) or used for cooking. Processing and addition of ingredients can influence the bioavailability of antioxidants originally present in both tomatoes and OO (Kamiloglu et al., 2013). In the industrial processing of tomatoes, 10–30% of their weight is waste constituted by peel and seeds; it is well known that lycopene and phenolic compounds are more concentrated in the peel than in the pulp (Montesano et al., 2006; Knoblich et al., 2005; Toor et al., 2005; George et al., 2004). The predominant form
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of lycopene in tomato products is the all-trans isomer, but, after consumption, lycopene is biologically converted to various cis isomers; this isomerization process also occurs naturally during storage and heating treatments. Lycopene is industrially produced by chemical synthesis or extracted from vegetable sources, mainly tomatoes, using chemical solvents and referred to as natural lycopene. Clinical studies showed that dietary supplements made with natural lycopene are more effective than those containing its synthetic form, probably for the presence of other bio-active molecules co-extracted from tomatoes, as which synergize with lycopene in promoting the positive effects on human health (Cazzola, 2012). However, the use of organic solvents for the extraction of natural lycopene may contaminate the end-product and facilitate the co-extraction of toxic chemicals including fertilizers, pesticides and heavy metals (Rescio et al., 2010). Several studies demonstrated the advantages of the lycopene added in a lipid matrix. Montesano and coworkers (2006) prepared two concentrations (0.5 and 1mg of pure lycopene per 100 g of oil) of lycopene extracted from tomato in extra virgin olive oil (EVOO) and monitored the chemical physical changes of samples for 37 weeks; the main results showed a lower rate of oxidation as well as greater protection of phenolic molecules in oils enriched with pure lycopene than in the control sample. Benakmoum and co-authors (2008), taking advantage of their lipophilic properties, incorporated carotenoids in EVOO and refined OO by preparing mixtures at different proportions (from 5 to 30%) with lyophilized peel thanks to a 24-h diffusion step. In their oil preparations, the authors detected rutin and naringenin as flavonoids coming exclusively from tomato peel and verified enrichment in lycopene and b-carotene. Such positive results have encouraged the marketing of new lycopene containing products sold as functional food, often prepared by adding lycopene extracted from tomatoes to lipid matrices from vegetable or animal origin. Based on previous trials (Cerretani et al., 2008), a low-scale mill to process together olives and tomato by-product (in defrosted or freeze-dried form) has been used in this investigation. Specifically, in the present study, the co-milling of olive paste and tomato by-product was finalized to verify the potential of minor compounds releasing, especially carotenoids, allowing the production of an oil fraction enriched with such antioxidants.
3.2.3 Materials and methods

3.2.3a Samples

Tomato by-product, constituted of skin and seeds, was purchased from an industrial plant that produces tomato sauce. Two aliquots were frozen and freeze-dried and stored until co-milling experiments. In Experiment 1, three batches (each of 40 kg) of cultivar Correggiolo olives were co-milled with tomato by-product using 1 and 0.240 kg of defrosted and freeze-dried tomato by-product, respectively (TB1 and TBL1). In order to evaluate the co-milling process effect on the parameters considered and, in particular, on carotenoid enrichment, a control sample without addition of tomato by-products was obtained (C1). In Experiment 2, three batches (each 60 kg) of olive blends from cultivars Moraiolo, Leccino and Correggiolo were tested in order to confirm the co-milling effect of olives and tomato by-products and to observe if different characteristics of olive paste (for example, in terms of pulp/pit ratio or oil/water ratio) could affect the content of lycopene in naturally enriched OO. All the olives came from Italy and were collected in Emilia Romagna region. The second co-milling process was performed using 1.125 and 0.270 kg of defrosted and freeze-dried tomato by-product to olive paste (TB2 and TBL2), respectively. To verify the enrichment of the carotenoid fraction, a control sample (C2) was also produced. The defrosted and freeze-dried tomato by-products were added in the same proportion with respect to Experiment 1. Experiments 1 and 2 were performed on different days of the oil season, 6th November and 13th November 2013, respectively.

3.2.3b Low-scale plant for co-milling between olives and tomato by-products

For both the experiments (Experiments 1 and 2), a continuous low-scale plant (Oliomio 150, Tavernelle Val di Pesa, Florence, Italy) equipped with a hammer crusher, a vertical malaxator and a two-phase decanter, was used. The same process parameters, such as temperature (27 °C) and time of malaxation (35 min), were set for the production of all samples. Figure 1 shows the flow charts of Experiments 1 and 2.
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**Figure 1.** Flowchart of co-milling processes: Experiments 1 and 2. C: control sample, obtained crushing only the olives; TB: sample obtained by crushing olives with defrosted tomato by-product; TBL: sample obtained by crushing olives with freeze-dried tomato by-product, as explained in “Samples” section.

### 3.2.3c Quality indices determination

Determination of the physicochemical quality parameters (free acidity, peroxide value, UV specific extinction at 232 and 270 nm) was carried out according to the respective official methods (EEC 2568/91 and following amendments).

### 3.2.3d Sensory analysis

The IOC panel test was carried out by eight selected and trained panelists, all members of the Bologna University professional committee (panel DISTAL), which is recognized by the Italian Government. Evaluation of samples was performed under the conditions described in EU Regulation 1348/2013; if secondary positive attributes were perceived, the taster recorded them using the list of sensory descriptors according to the IOC document (IOOC/T.20/Doc. no 22, 2005).
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3.2.3e Extraction of phenolic fraction and spectroscopic determination of ortho-diphenols
Extraction of polar minor compounds followed the protocol described by Pirisi et al. (2000) and modified according to Rotondi et al. (2004). Two aliquots of oil (2 g) were added to 1 ml of n-hexane and 2 ml of a methanol/water (60:40, v/v), mixed and centrifuged for 3 min at 3000 rpm (907.2 x g). The hydroalcoholic extract was collected, and the oil phase was re-extracted twice with 2 ml of methanol/water (60:40, v/v) solution each time. Finally, all the hydroalcoholic fractions were combined, washed with 2 ml of n-hexane to remove the residual oil, then concentrated and evaporated under vacuum at 35 °C. The dry extracts were diluted in 0.5 ml of a methanol/water (50:50, v/v) solution and filtered through a 0.2-mm nylon filter. Extractions were performed in three replicates, and extracts were stored at -18 °C before analysis. The content of total o-diphenols (o-DIPH) was spectrophotometrically evaluated, according to Mateos et al. (2001) at 370 nm and against a reference prepared with the same procedure, but without adding the phenolic extract. A specific calibration curve ($r^2=0.993$) was built to express the data as mg gallic acid kg$^{-1}$ of oil.

3.2.3f HPLC analysis of individual phenolic components
Analysis of the phenolic profile was performed according to the COI/T.20/Doc No. 29 (2009) protocol, with some modifications related to the chromatographic analysis. The method provides for direct extraction of the phenolic fraction from OO using a methanol solution and subsequent quantification by HPLC coupled to an UV detector at 280 nm. Syringic acid was used as the internal standard, while the content of the individually identified phenolic compounds was expressed as tyrosol (mg kg$^{-1}$). The chromatographic analysis was performed using a 1260 series HPLC instrument equipped with a quaternary pump (Agilent Technologies, Waldbronn, Germany) and a reverse phase C18 100A Kinetex column (2.6 mm, 100x3.00mm I.D., Phenomenex, Torrance, CA). The elution gradient was carried out with a solvent system of water/formic acid (99.5:0.5 v/v) as mobile phase A and acetonitrile as mobile phase B; the total runtime was 13 min and the gradient elution was as follows: from 0 to 3 min solvent B increased from 5 to 20%, at 4 min solvent B reached 40%, at 9 min solvent B reached 60%, and finally at 10 min solvent B at 100%; at 13 min, 5% solvent B was restored. The column was thermostated at 30 °C and equilibrated for 5 min prior to each analysis; an injection volume of 5 ml and a flow rate of 0.7 ml min$^{-1}$ were
Results

used. The main phenolic compounds were tentatively identified based on mass spectra using a mass spectrometer (MS, Agilent, Phoenix, AZ) in electrospray ionization mode. The MS working conditions were: nebulizer gas pressure, 0.24 MPa; drying gas flow, 7l min\(^{-1}\) at 300 °C; capillary voltage, 2.5 kV; voltages of skimmers 1 and 2, -41.0 and -6.0V, respectively. Nitrogen was used as a nebulizer and drying gas. The MS was scanned within the m/z 100–900 range in the negative and positive ion mode.

3.2.3g Carotenoid extraction from tomato by-products

Aliquots of defrosted and freeze-dried tomato waste were subjected to the extraction of carotenoids to define the quantity of tomato by-products to be added during the co-milling experiment and to evaluate the migration of carotenoid compounds. The extraction of carotenoids was carried out in darkness, using an ice bath according to Vallverdú-Queralt et al. (2012) to minimize the oxidation phenomena. Defrosted and freeze-dried tomato by-product (2 g) were homogenized for 1 min with 5ml of ethanol/n-hexane (4:3, v/v) using an Ultra-Turrax at 5000 rpm (2520 x g), sonicated for 5 min and centrifuged at 4000 rpm (1613 x g) for 10 min. The extraction procedure was repeated twice, and the collected supernatants were combined and evaporated under nitrogen flow. Finally, the residue was reconstituted with methanol and methyl-tert-butyl ether (2:1), filtered through 0.45 mm PTFE filter and analyzed by HPLC as described below.

3.2.3h Lutein, β-carotene and lycopene analysis

The carotenoid fraction was extracted and purified from OO samples according to the saponification procedure described for sterol determination in the official method (EEC 2568/91 and following amendments): the oil was saponified with potassium hydroxide in ethanolic solution, and the unsaponifiable matter was then extracted with diethyl ether. The unsaponifiable fraction was recollected in a 5-ml of n-hexane and i-propanol (4:1 v/v) and stored at -18 °C. Before HPLC analysis, 0.5 ml of n-hexane/i-propanol solution was evaporated and dissolved in 0.5 ml of methanol and methyl-tertbuthylether (2:1 v/v). The solution was vortexed and filtered with a 0.45-mm nylon filter. HPLC analysis was performed using an Agilent 1200 Series HPLC system (Hewlett-Packard, Waldbronn, Germany) with a C18 Zorbax Eclipse plus column (4.6 x 250 mm, particle size 5 mm, Agilent, Phoenix, AZ). The elution solvents used were A (methanol/1% ammonium acetate)
and B (methyl-tert-butyl ether), and the targeted compounds were eluted according to the following gradient: starting from 100% A, increased to 20% B in 20 min, and percentage was maintained for 5 min. The flow rate was 1 ml min\(^{-1}\) and the sample injection volume was 20 mL. Detection was at 470 nm. Identification of carotenoids was achieved by comparing the retention times with pure standards of LUT, \(\beta\)-CAR and lycopene (Fluka, Sigma-Aldrich, Steinheim, Germany), and their concentrations in samples were calculated using the respective calibration curves prepared using different concentrations of standard solutions (\(r^2=0.999, r^2=0.995\) and \(r^2=0.998\), respectively).

### 3.2.3i Tocopherol determination

Briefly, 0.5 g of oil sample were added into a 10-ml volumetric flask and brought to volume with i-propanol. The solution was filtered through a 0.45-mm nylon filter paper before HPLC-DAD analysis, which was performed with the same apparatus described previously. Detection was performed at 292 nm. The column was a Cosmosil \(\pi\) NAP 150mm x 4.6 mm, 5 mm particle size (Nacalai-Tesque, Kyoto, Japan). The elution solvents used were: A (methanol/water, 90/10 v/v acidified with 0.2% H3PO4) and B (acetonitrile). The samples were eluted according to the following gradient: 100% A for 22 min; 100% B for 13 min; 100% A maintained for 15 min. The flow rate was 1 ml min\(^{-1}\). The sample injection volume was 10 ml. Identification of \(\alpha\)-TOC (Fluka, Sigma-Aldrich, Steinheim, Germany) was developed by comparing the retention time of a pure standard, and its concentration in samples was calculated using the calibration curve constructed using solutions of \(\alpha\)-TOC at different concentrations (\(r^2=0.999\)).

### 3.2.3l Volatile compounds analysis

Analysis was performed by SPME-GC-MS according to the procedure described by Baccouri et al. (2008); SPME fibers were coated with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) phase (50/30 mm, 2 cm long from Supelco Ltd., Bellefonte, PA), and 4-methyl-2-pentanone (Fluka, Sigma-Aldrich, Steinheim, Germany) was added as an internal standard. Volatile compounds were identified and quantified by GC (Agilent 6890N) coupled with a quadrupolar MS (Agilent 5973N, Agilent Technologies, Phoenix, AZ). Analytes were separated on a column of 30 m, 0.25mm i.d., 1.00 mm f.t. (Phenomenex) coated with polyethylene glycol phase. The
column temperature was held at 40 °C for 10 min and increased to 200 °C at 3 °C min⁻¹. The ion source and the transfer line were set to 180 °C and 230 °C, respectively. Electron impact MS were recorded at 70 eV ionization energy in the 20–250-amu mass range, 2 scans s⁻¹. The identification of the volatile compounds was obtained by comparison of their mass spectral data with the information from the NIST library (2005 version) and MS literature data. Volatile compounds were expressed as mg of internal standard per kg of oil.

3.2.3 Statistical analysis
The software XLSTAT 7.5.2 version (Addinsoft, Belmont, MA) was used to elaborate both the sensory and chemical results by Shapiro–Wilk normality test (α=0.05) to check the normality of distribution of the results, followed by the analysis of variance (ANOVA, with Fishers’ least square difference post-hoc test, p<0.05).

3.2.4 Results and discussion
Free acidity (FA), peroxide value (PV), specific UV extinctions (K₂₃₂, K₂₇₀) and sensory analysis are the basic quality parameters for the classification of different grades of virgin oils produced from olives (EEC 2568/91 and following amendments). Table 1 lists the values obtained for the samples analyzed.

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1</td>
</tr>
<tr>
<td>FA</td>
<td>1.0</td>
</tr>
<tr>
<td>PV</td>
<td>7.6</td>
</tr>
<tr>
<td>K₂₃₂</td>
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</tr>
<tr>
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<td>ß-Carotene</td>
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<td>ß-Diphenols</td>
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<tr>
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<td>Bitterness</td>
<td>2.5</td>
</tr>
<tr>
<td>Pungency</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Table 1. Mean values of basic chemical quality parameters; contents of individual carotenoids and median values relative to sensory evaluation. FA, free acidity (expressed as g of oleic acid per 100 g of oil); PV, peroxide value (expressed as mEq of active oxygen per kg of oil); K₂₃₂, K₂₇₀, specific extinction coefficients at 232 and 270 nm. Contents, expressed as mean values in mg per kg of oil, of individual carotenoids (LYCOP, lycopene; LUT, lutein; ß-CAR, ß-Carotene), α-tocopherol (α-TOC) (expressed as mg of α-tocopherol per kg of oil) and total α-diphenols (α-DIPH) (expressed as mg of gallic acid per kg of oil). Median values relative to the intensities of sensory positive attributes such as fruitiness, bitterness and pungency (FRUITY, BITTER, PUNGENT) and to the main perceived defect (MP DEF). Experiment 1: olives cv. Corregggiolo co-milled with tomato by-
Results

product using defrosted (TB1) and freeze-dried (TBL1) tomato by-product; Experiment 2: olives cvs. Correggiolo, Leccino and Moraiolo co-milled with tomato by-product using defrosted (TB2) and freeze-dried (TBL2) tomato by-product. C1 and C2 are control samples. For each parameter and within the same experiment, different letters indicate statistically significant differences between the mean values (LSD Fisher, p<0.05).

All samples obtained from Experiment 1 showed percentages of FA that were higher than the legal EU limit (≤0.8%, EU Reg. 1348/2013) established for the EVOO category, without significant differences among the control sample (C1) and the oils produced by co-milling of olives and tomato by-product in defrosted and freeze-dried forms (TB1 and TBL1, respectively). Sample C1 showed a clear sensory defect of winey, with an intensity compatible with a virgin OO (median value of 2.0). The winey negative attribute, as well as the FA value, were probably due to the low quality of the olives processed. Unexpectedly, the presence of the winey defect in samples produced by co-milling of olives and tomato byproducts (TB1 and TBL1) was not perceived by assessors. On the other hand, for all samples obtained in Experiment 2, the values of FA, PV and K232 and K270 were within the legal EU limits established for EVOO (EU Reg. 1348/2013), with the only exception of C2 which showed the presence of a winey defect perceived by the sensory panel (median value of 2.0), which caused the declassing of this sample to the VOO category. This defect was not perceived in TB2 or TBL2 samples, as previously mentioned for TB1 and TBL1. This could be explained by the presence of a secondary note resembling tomato recognized by assessors (data not shown) in samples obtained co-milling tomato by-products, which could mask the winey defect. Several volatile compounds were tentatively identified and quantified in the headspace of OO samples and differently grouped in reliance of their enzymatic origin (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th>Experiment 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1</td>
<td>TB1</td>
<td>TBL1</td>
<td>C2</td>
<td>TB2</td>
<td>TBL2</td>
</tr>
<tr>
<td>Mean</td>
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</tr>
<tr>
<td>SD</td>
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<td>0.73</td>
<td>0.15</td>
<td>0.41</td>
<td>0.35</td>
<td>0.15</td>
</tr>
<tr>
<td>ALD C6</td>
<td>3.49</td>
<td>0.11</td>
<td>0.73</td>
<td>4.54</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>EST C6</td>
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<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>KET C6</td>
<td>0.60</td>
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<td>0.00</td>
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<td>0.00</td>
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</tr>
<tr>
<td>ALC C7</td>
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<td>0.10</td>
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<td>0.00</td>
</tr>
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<td>WINEY</td>
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<td>0.00</td>
<td>0.00</td>
<td>4.26</td>
<td>0.58</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Table 2. Mean values of the main volatile classes and of the sum of volatile compounds responsible for the sensory defect of winey. Mean values (expressed as mg of 4-methyl-2-pentanone per kg of oil) of the main volatile classes: aldehydes (ALD C6), alcohols (ALC C6) and esters (EST C6) with
six carbon atoms originating from the LOX pathway; ketones (KET C₅), alcohols (ALC C₅) with five carbon atoms and pentene dimers (P-DIM) originating from the secondary pathway of LOX. Mean values of the sum of volatile compounds considered to be responsible for the main perceived sensory defect of WINEY (methyl acetate, ethyl acetate, methanol, ethanol and acetic acid). Experiment 1: olives cv. Correggiolo co-milled with tomato by-product using defrosted (TB1) and freeze-dried (TBL1) tomato by-product; Experiment 2: olives cvs. Correggiolo, Leccino and Moraiolo co-milled with tomato by-product using defrosted (TB2) and freeze-dried (TBL2) tomato by-product. C1 and C2 are control samples. For each parameter and within the same experiment, different letters indicate statistically significant differences between the mean values (LSD Fisher, p<0.05).

According to other authors (Angerosa et al., 2004, 2000), C₆ and C₅ volatile molecules coming from the lipoxygenase enzymatic pathway have been primarily considered to be those mainly responsible for the odor notes of EVOO. The group of C₆ compounds derived by the primary LOX pathway have been distinguished in aldehydes (ALD C₆), alcohols (ALC C₆), esters (EST C₆) and the group of C₅ compounds derived by the secondary LOX pathway in ketones (KET C₅), alcohols (ALC C₅) and pentene dimers (P-DIM). Generally, for all samples, the C₆ compounds, and, in particular, ALD C₆, were the most abundant, but, in the case of the two samples judged as defective by sensory analysis (C1 and C2), a higher content in ALC C₆ was found. In a previous study (Bendini et al., 2007), a lower ratio of ALD C₆ to ALC C₆ was detected in samples characterized by lower intensity of green notes and the presence of unpleasant olfactory perceptions. In particular, C2 was also characterized by the highest concentration of compounds that have been related to the sensory defect of winey, such as those linked to sugar fermentation (ethanol, ethylacetate and acetic acid) and overripe olives (methanol and methylacetate). There was no clear evidence of a predominance of specific molecules or of a class of compounds in the volatile profiles of samples produced by co-milling of olives and tomato by-product. Table 3 includes the polar phenolic compounds identified and quantified in all samples by HPLC-DAD-MS. A total of eight compounds belonging to phenolic acids, phenolic alcohols and secoiridoids were tentatively identified. For Experiment 1, the co-milling process did not affect the total phenolic content (TOT PH), but the behavior of selected individual polar phenols showed significant differences depending on the type of sample. The highest concentrations of the oxidized form of decarboxymethyl oleuropein aglycone (ox-DOA) were detected in both the co-milled samples, whereas the content of ligstroside aglycone
(LA) was particularly high in TB1. In contrast, the amounts of decarboxymethyl ligstroside aglycone (DLA) and tyrosol derivative (TY-DER) appeared to increase after the addition of freeze-dried tomato by-product (TBL1). C2 resulted particularly rich in DOA also if the TOT PH was equal to C1 and the co-milling for Experiment 2 showed an increase in the phenolic content more evident for TY-DER and DLA, respectively, for TB2 and TBL2.

Table 3. Contents of individual phenolic compounds and mean values of the sum of all phenolic compounds. Contents, expressed as mean values in mg of tyrosol per kg of oil, of individual phenolic compounds: hydroxytyrosol (HYTY), p-cumaric acid (p-CUM); oxidized form of decarboxymethyl oleuropein aglycone (ox-DOA), derivative of tyrosol (TY-DER), decarboxymethyl ligstroside aglycone (DLA), oleuropein aglycone (OA), ligstroside aglycone (LA). Mean values of the sum of all phenolic compounds (TOT PH). Experiment 1: olives cv. Correggiolo co-milled with tomato by-product using defrosted (TB1) and freeze-dried (TBL1) tomato by-product; Experiment 2: olives cvs. Correggiolo, Leccino and Moraiolo co-milled with tomato by-product using defrosted (TB2) and freeze-dried (TBL2) tomato by-product. C1 and C2 are control samples. For each parameter and within the same experiment, different letters indicate statistically significant differences between the mean values (LSD Fisher, p<0.05).

The total content in o-DIPH (Table 1) showed a significant decrease for both the experiments that was justified by the tendency towards lowering of the main o-diphenols DOA and OA (Table 3) when olives were co-milled with tomato by-product. It is possible that there is an interaction of the enzymatic fraction of the tomato by-product on the hydrolytic and oxidative reactions of the phenolic fraction. Other antioxidant compounds with lipophilic characteristics such as α-TOC and carotenoids were identified and quantified by HPLC (Table 1). In Experiment 1, the content of α-TOC, the most abundant tocopherol in OO, did not change, while in Experiment 2 an increasing trend was registered when tomato by-product was added (TB2, TBL2). The content in β-CAR increased significantly in TB1 and TBL1 compared to the control (Experiment 1) as well as in TB2 and TBL2 with respect to C2 (Experiment 2). A clear transfer of LYCOP from tomato by-product to the
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produced oils was also detected. In Experiment 1, a high percentage of lycopene passed from the by-product to the oils, with recoveries of 80.3% (TB1) and 92.6% (TBL2). Lower percentages were found in Experiment 2, in which the recoveries were 66.3% and 69.8% for TB2 and TBL2, respectively. In general, both the experiments performed by co-milling between olive paste and freeze-dried tomato by-product (TBL1 and TBL2) produced the highest contents of all carotenoids. In this regard, the use of tomato by-product in freeze-dried form, in comparison to the one that was simply de-frosted, may represent a better way to produce a “naturally enriched OO” with a higher concentration of lycopene. On the other hand, differences in terms of quality parameters, volatiles and phenolic compounds could be highlighted between samples in Experiments 1 and 2; this might be related to the quality of the olives themselves and, in particular, to the characteristics that each specific cultivar gave to the OO.

3.2.5 Conclusions

Different factors, mainly the cost of disposing of waste, the appeal of natural additives for the food industry and increased legislative restrictions to protect the environment have contributed over the last few years to the development of improved waste-handling technology. Concerning agro-industrial tomato by-products, many applications for their reuse and valorization have already been proposed, for example, in animal feed thanks to the considerable content of protein and carotenoid (Knoblich et al., 2005; Toor et al., 2005) and for enrichment of low-quality edible oils (Benakmoum et al., 2008). The present investigation suggests the possibility to use the industrial tomato by-product (skin and seeds) for co-milling with olives to obtain a vegetable oil that is naturally rich in antioxidants, especially in carotenoids. Specifically, the presence of lycopene in this functional food takes origin from the tomato by-product thanks to transfer of this lipophilic molecule into the lipid matrix only through a mechanical process, different from maceration and avoiding the use of solvents or chemicals. This new product might be marketed as a “condiment produced using olives and tomato by-products” or “OO dressing enriched in lycopene”. Further research should be carried out to evaluate if the freeze-dried form of tomato by-products is more suitable than the defrosted one considering both the efficacy of antioxidant transfer and economic viewpoints.
3.2.6 References


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EFSA. 2011. 9(4), 2031 “Scientific opinion on the substantiation of health claims related to lycopene and protection of DNA, proteins and lipids from oxidative damage, protection of the skin from UV-induced (including photo-oxidative) damage, contribution to normal cardiac function, and maintenance of normal vision pursuant to Article 13(1) of Regulation (EC) No 1924/2006”.


Results


3.3 Identification and quantification of volatile compounds in differently processed faba beans and relation with sensory aspects

3.3.1 Abstract

Faba bean is one of the world's oldest cultivated plants. Its nutritional and sensory profiles have been studied, but off-flavours, related to the processing of beans, frequently occur. They have to be taken into account in order to optimise sensory characteristics and nutritional value. In the present study, the aroma profiles of native, conventionally oven heated and microwave treated faba bean samples were studied as flour, suspensions, extracts and emulsions with different amounts of oil. The aroma profiles were investigated by volatile compounds identification and quantification and by sensory analysis. The results of this work highlighted how samples produced with thermally treated beans showed the presence of volatile compounds produced during autoxidation processes and perceived by panel as nutty and roasted sensory attributes.

Keywords: faba bean; sensory profile; volatile compounds; combined analysis of data.
3.3.2 Introduction

Faba bean belongs to vetches (*Vicia faba* L.), which is one of the pulse families. In Finland, the cultivation of this beans started in 13th century, but its current cultivation is low. Faba bean seeds are rich in both protein and energy aside from being able to grow in different climatic zones; due to these characteristics faba beans constitute one of the main plant based protein sources in many countries all over the world (Velázquez et al. 2010).

Moreover, in symbiosis with rhizobium bacteria, faba bean has one of the highest nitrogen fixation capabilities; thus, they are not only free of the need for nitrogen fertilizers, but they also contribute to the nitrogen nutrition of the following crops (Stoddard et al., 2008). Additionally, faba bean can be successfully used in crop rotation to reduce the use of nitrogen fertilisers derived from fossil fuel sources.

The sensory profile of faba bean and other pulses has already been studied but only in relation with the addition of pulse flour to other food in processing. Among foods studied are pasta fortified with pea and faba bean flours (Rizello et al., 2017; Rosa-Sibakov et al., 2015; Petitot et al., 2010), biscuits added with chickpea, faba and soy bean flours (Rababah et al., 2006), Panela cheese added with faba bean starch (Escobar et al., 2012), corn chips fortified with faba, soy and chickpea protein isolated (Rababah et al., 2012) and Egyptian bread (Abdel-Kader, 2000). Despite the advantages of faba bean, the use of faba bean flour as a source of protein in food is a challenge, mainly because of an off-flavour known as beany flavour. The formation of off-flavour and aromas is attributed to volatile compounds deriving from linolenic and linoleic fatty acids in lipid oxidation catalysed by enzymes or chemical factors, as suggested by Kinney since 2003 (Kinney et al., 2003). Lipoxygenase (LOX) is an abundant enzyme in legumes that catalyses the oxidation of fatty acids (Scott et al. 1975). The volatiles responsible for beany off-flavour in faba bean products have not been identified yet, although, in the past 30 years, many researchers have carried out studies with beans (Hsieh et al. 1982; Kobayashi et al., 1995; Boatright et al., 1999; Oomah et al. 2007; Kaseleht et al. 2009). Additionally, the volatile profile of soybean (Gao et al., 2017), chickpea, lentils (Shariati-Ievari et al., 2016) and several other pulses have already been investigated; the same cannot be said for faba beans, since the effect of processing on aromatic profile has not been studied. Beany off-flavour is a problem in processing of faba beans and other legumes; lipid modifying enzymes are activated during preparation of
aqueous food applications. In order to prevent the changes caused by enzymes like LOX that catalyses oxidation of fatty acids as linolenic and linoleic acids, beans should be heat-treated prior to milling to inactivate the enzymes (Jiang et al., 2016). However, heating may promote radical-induced lipid autoxidation and therefore heating should not be too intense (Jadhav et al., 1995). If active enzymes are present in aqueous solutions/suspensions, addition of oil that provides substrates for the enzymes may enhance formation of flavour active compounds and change the overall characteristics of off-flavour, which is caused by both enzymatic and non-enzymatic degradation of lipids (McClements et al., 2000). Uncontrolled oxidative and other reactions of lipids lead to off-flavours that impairs perceivable quality of faba bean by consumers. Although beany off-flavour is often recognized in faba bean products, it has not been adequately characterized. Relevant compounds contributing to sensory attributes need to be identified to understand and control beany flavour formation caused by enzymatic (and non-enzymatic) lipid degradation/oxidation reactions (Guichardant et al., 2016; Jiang et al., 2016).

Headspace-solid phase micro-extraction coupled with gas-chromatography and mass spectrometry (HS-SPME-GC-MS) has become a popular technique for analysing the volatile profile of food (Wardencki et al. 2004) because of its sensitivity and selectivity; by HS-SPME-GC-MS volatile compounds responsible for characteristic sensory notes can be identified and quantified. In relation to that mentioned above, further studies on the aromatic profile (sensory and volatile) of faba bean flour, in relation to essential heat treatments (fundamental for the shelf life of the flour itself) are needed.

The aim of this work was to study effects of different heat treatments of faba beans on the sensory and volatile profiles of the flours and food models made from them. Flours were prepared from native (NA, no heat treatment), conventionally oven heated (CO) and microwave (MW) treated beans and studied as: a) flour, b) suspension (water), c) extract and d) emulsions (two levels of rapeseed oil). The volatile profiles of samples were studied using HS-SPME-GC-MS after optimization of the method (Damerau et al., 2014): the appropriate fibers, in order to achieve the most complete profile of faba bean samples were selected, in relation to the different characteristics of models: solid or liquid samples. Additionally, different extraction times were tested. The sensory analysis, by a panel of trained judges, was performed on the food models in order to characterize and quantify the
intensities of selected descriptors among faba bean samples. Finally, results from chemical (volatile) and sensory analyses were compared in order to identify components responsible for off-flavours.

3.3.3 Materials and methods

3.3.3a Samples

Faba beans of Kontu cultivar from the year 2011 were grown in a Finnish experimental field and; once harvested and cleaned, beans were kept in a cool and dry storage room at 10 °C. After storage, beans were thermally treated, de-hulled and milled according to what reported by Jiang et al. (2016). In particular, different heat treatments (Table 1) were applied in order to compare their effect on sensory and chemical properties of beans. Native faba beans (NA), without heat-treatment, were considered as control samples and compared to beans thermally treated by a conventional oven (CO) or by microwave oven (MW).

<table>
<thead>
<tr>
<th>Heat treatment</th>
<th>Type</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>Flour</td>
<td>De-hulled faba beans</td>
</tr>
<tr>
<td></td>
<td>Suspension</td>
<td>Flour mixed with MilliQ water 1.5</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
<td>Overnatant coming from centrifugation of suspension for 15 minutes at 10000g</td>
</tr>
<tr>
<td></td>
<td>Emulsion 3% oil</td>
<td>Extract mixed with 3% of refined rapeseed oil by the Ultra-turrax</td>
</tr>
<tr>
<td></td>
<td>Emulsion 10% oil</td>
<td>Extract mixed with 10% of refined rapeseed oil by the Ultra-turrax</td>
</tr>
<tr>
<td>CO</td>
<td>Flour</td>
<td>De-hulled faba beans heated in conventional oven for 30 minutes at 170 °C</td>
</tr>
<tr>
<td></td>
<td>Suspension</td>
<td>Flour mixed with MilliQ water 1.5</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
<td>Overnatant coming from centrifugation of suspension for 15 minutes at 10000g</td>
</tr>
<tr>
<td></td>
<td>Emulsion 3% oil</td>
<td>Extract mixed with 3% of refined rapeseed oil by the Ultra-turrax</td>
</tr>
<tr>
<td></td>
<td>Emulsion 10% oil</td>
<td>Extract mixed with 10% of refined rapeseed oil by the Ultra-turrax</td>
</tr>
<tr>
<td>MW</td>
<td>Flour</td>
<td>De-hulled faba beans microwaved for 1.5 minutes at 950 Watts</td>
</tr>
<tr>
<td></td>
<td>Suspension</td>
<td>Flour mixed with MilliQ water 1.5</td>
</tr>
<tr>
<td></td>
<td>Extract</td>
<td>Overnatant coming from centrifugation of suspension for 15 minutes at 10000g</td>
</tr>
<tr>
<td></td>
<td>Emulsion 3% oil</td>
<td>Extract mixed with 3% of refined rapeseed oil by the Ultra-turrax</td>
</tr>
<tr>
<td></td>
<td>Emulsion 10% oil</td>
<td>Extract mixed with 10% of refined rapeseed oil by the Ultra-turrax</td>
</tr>
</tbody>
</table>

Table 1. Samples selected for this study and their characteristics. NA: native faba beans without heat-treatment; CO; conventional thermal treated faba beans; MW: microwaved faba beans.
3.3.3b Chemical analysis

Quality of refined rapeseed oil

Oxidative status. Peroxide value status (PV) of refined rapeseed oil, purchased from a retail store in Helsinki, and used for preparing both 3% and 10% emulsions was measured using a ferric thiocyanate method (Lehtonen et al., 2011) in order to check the oil quality. As reported in Table 2, the values obtained were largely inside the quality parameter limit of 10 meq/kg defined by Codex Alimentarius (Codex Stan 2010-199).

Fatty acid profile. Fatty acid composition of the oil was determined as methyl esters. Afterwards, 1 µl of sample was injected in a GC (HP 5890, Hewlett-Packard) with a DP-FFAP column (30m x 0.32 mm, 0.25 µm, Agilent Technologies); the GC conditions were as reported by Damerau (2014). Results from fatty acid profile analysis are listed in Table 2. The single amounts of palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0) and gondoic acid (20:1) were in line with the fatty acid composition of rapeseed oil (Codex Stan 2010-199).

Tocopherols determination. Tocopherols were measured by NP-HPLC-FLD as described by Schwartz (Schwartz et al. 2007). Tocopherols were further identified by comparison of the retention times with those of authentic standards. The results are presented as µg per g of sample. Consistent with what reported in Codex Alimentarius (Codex Stan 2010-199), and by several authors (Bonvehi et al. 2000; Piironen et al. 1986) γ-tocopherol was the main tocopherol (492.5 7 µg/g), followed by α-tocopherol (252.3 7 µg/g); even 7 µg/g of δ-tocopherol were detected; data are reported in Table 2.

<table>
<thead>
<tr>
<th>Fatty acid methyl esters</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1 (n-9)</th>
<th>18:1 (n-7)</th>
<th>18:2 (n-6)</th>
<th>18:3 (n-3)</th>
<th>20:0</th>
<th>20:1 (n-9)</th>
<th>α</th>
<th>γ</th>
<th>δ</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.6</td>
<td>1.9</td>
<td>58.3</td>
<td>3.2</td>
<td>20.8</td>
<td>8.5</td>
<td>0.6</td>
<td>1.2</td>
<td>252.3</td>
<td>492.5</td>
<td>7.4</td>
<td>0.1</td>
</tr>
<tr>
<td>SD</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Summary of the quality parameters for quality control of refined rapeseed oil. Fatty acid methyl esters are reported as % of total esters; tocopherols in µg/g, the peroxide value is reported in meq/kg.
**Results**

**Lipoxygenase activity determination**

The LOX activity of flours from native beans and heat-treated beans was measured as reported by Jiang et al. (2016). The method was modified from the methods purposed by Axelrod et al. (1981) and Gökmen (Gökmen et al. 2002). For this spectrophotometric assay 0.5 g of NA, CO and MW flours were mixed with 29 ml of MQ-water, for 15 minutes. The mixed samples were centrifuged for 10 minutes at 10000 g at room temperature, then the supernatant was recollected and diluted 1:50. Sodium linoleate substrate solution (10 mM) was prepared by mixing equal amount of Tween 20 and linoleic acid with 4 ml of MQ-water and, in order to clarify the solution, 30 µl of NaOH (1 N) was added. For measuring the lipoxygenase activity, reaction was made adding 200 µl of substrate solution and 200 µl of extract to 2.6 ml of sodium phosphate buffer (pH 6). After incubating the mixture for 3 minutes at 25 °C, the reaction was stopped by adding 3 ml of NAOH (0.1 N) to the mixture. Absorbance was measured at wavelength of 234nm (Lamba 25 UV/Vis, Perkin Elmer Inc., USA). Results were then calculated with molar absorptivity value of conjugated dienes (ε=26000 l/mol cm).

**3.3.3c Volatile compounds analysis**

Volatile compounds present in the samples were analysed by HS-SPME-GC-MS. The HS-SPME-GC–MS method was developed modifying a previously reported method (Damerau et al., 2014).

**Optimization of the method**

Prior to GC analysis for identifying the key aroma-active compounds, optimization of the SPME method to extract the maximum number of the most aroma-active compounds from the sample was carried out. The optimization of the method was carried out using faba beans samples from Kontu cultivar, harvest year 2011. Flours were prepared from native (NA, no heat treatment), conventionally oven heated (CO) and microwave (MW) treated beans and studied as: a) flour, b) suspension (water), c) extract, produced as reported in Samples’s paragraph. Samples were analysed in duplicates.
Results

Volatile compounds were analysed using an HS-SPME injector (combiPAL, CTC Analytics, USA), with a divinylbenzene/carboxen/polydimethylsiloxane fiber (DVB/CAR/PDMS-fiber, 50/30 µm film thickness; Supelco, USA) and a carboxen/polydimethylsiloxane fiber (CAR/PDMS-fiber 85 µm film thickness; Supelco, USA). The SPME fibers were conditioned before the first use according to the instructions of the supplier. The SPME was coupled to a GC (HP 6890 series, Agilent Technologies Inc., Wilmington, DE, USA) with a MS detector (Agilent 5973 Network, Agilent Technologies Inc., Wilmington, DE, USA). The GC was equipped with a capillary column SPB-624 (30 m x 0.25 mm i.d., 1.4 µm film thickness; Supelco, USA). The extraction times tested were 30 and 60 minutes. The instrument and measuring parameters were as reported in section Optimized method for HS-SPME-GC-MS.

Optimized method for HS-SPME-GC-MS

Different fibers were selected for different models: DVB/CAR/PDMS-fiber was used for liquid samples (suspensions, extracts, emulsions) while CAR/PDMS was selected for analysing flours. The extraction time was fixed at 60 minutes and the temperature was set at 50 °C. The fiber was then desorbed for 10 min at 250 °C in the injection port of the GC operating in splitless mode. The GC operating conditions were the following: helium flow rate 0.7 mL/min; oven temperature increasing steps: 40 °C for 5 min, then increased by 5 °C/min to 200 °C and held at 200 °C for 10 min. The ionisation energy of MS was 70 eV and the range of the scan was from 50 to 300 amu. The match between compounds mass spectra and the database Wiley 7N was used for the identification of volatiles (Wiley Registry™ of Mass Spectral Data, 7th Edition, USA). Total ion counts were used for quantifying the amounts of compounds. Peak areas of six and three volatiles of two reference materials, blueberry oatmeal cookies and vanilla soymilk, for flours and liquid samples respectively, were used for monitoring the performance of the HS-SPME–GC–MS. All measurements were done over a time period of 4 weeks. Samples were analysed in triplicates.
3.3.3d Sensory analysis

Samples were evaluated with Generic Descriptive analysis (GDA) (Lawless and Heymann, 2010; Murray et al., 2001). The method provides information about the qualitative as well as the quantitative characteristics of samples. It is widely used for obtaining detailed description of the appearance, aroma, flavour, and texture of food products.

Panel members (n=9, 7 females, 2 males, age from 21 to 44; students and employees of the University of Helsinki) served as panellists. Study protocol followed the ethical guidelines of the sensory laboratory, approved by the University of Helsinki Ethical Review Board in the Humanities and Social and Behavioural Sciences. A written informed consent was obtained from each participant before they entered the screening phase.

Panellists were screened for their sense of smell with Sniffin’ Sticks Screening (SSS), in which odour of 12 felt-tip pens (sticks) is to be identified among four alternative descriptions given for each stick. The number of correct identifications of odorants define the capacity (Hummel et al., 1997). Values of 11-12 are considered normal, while 10 or less need a further examination of the olfactory performance; all the assessors obtained 11-12.

The procedure of selecting optimal descriptors, references and appropriate scales, training and evaluation followed the practical principles of GDA as described by Lawless and Heymann (2010). At the beginning of the experiment, panellists worked individually trying to describe the samples using their own terms: after that, the panel leader guided the discussion through which the attributes were selected. The training phase consisted of three one-hour sessions during which each panellist received all samples to be evaluated; the panel decided whether descriptors were redundant and should be removed from the list or if there were terms that should be added. The training sessions included familiarization with the attributes and references selection: assessors identified possible reference standards on which the rating of the generated attributes was based. Finally, eight olfactory attributes were chosen, paired to their references (Table 3).

Sensory profiling was performed on the 15 samples reported in Table 1. The panelists received seven or eight samples at a time, (15 ± 0.5g) placed in 50 ml dark container closed with plastic cap. Samples were presented coded with three-digit numbers; each panelist worked individually in a panel room, inside singular closed booth, in white light condition.
Results

To prevent the volatile loss, the panelists were instructed to bring the sample close to the nose, remove the plastic cap and sniff carefully, then close the cap immediately and evaluate the intensity of each attribute using a provided unstructured 10 cm length linear scale. A short break between the samples was held. Samples were evaluated in duplicate and the randomized distribution order was applied to the whole set of samples.

Data acquisition, creation of randomized order and sample codes were carried out using Fizz software (Biosystemes, Dijon, France).

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea</td>
<td>Odor reminescent or characteristic of peas</td>
<td>Canned peas</td>
</tr>
<tr>
<td>Beany</td>
<td>Odor mainly associated with beany notes</td>
<td>Canned red beans preserving liquid</td>
</tr>
<tr>
<td>Nutty</td>
<td>Odor reminescent of roasted nuts</td>
<td>Unsalted roasted peanut previously milled</td>
</tr>
<tr>
<td>Grassly</td>
<td>Odor associated with fresh vut grass</td>
<td>0.2 g of Hexanal in 15 g of refined rapeseed oil</td>
</tr>
<tr>
<td>Musty/earthy</td>
<td>Odor reminescent musty/earthy/humid notes</td>
<td>0.5 g of Z-2-hexenol in fresh loam</td>
</tr>
<tr>
<td>Grain/seed</td>
<td>Odor characteristic of seeds or grains</td>
<td>Mixture of bird seeds</td>
</tr>
<tr>
<td>Rancid</td>
<td>Odor characteristic of very old/oxidized oils</td>
<td>Rapeseed oil heated a 60 °C for one week in an opened bottle (half-filled)</td>
</tr>
<tr>
<td>Fermented</td>
<td>Odor reminescent pungent of fermented notes</td>
<td>One week old underratant coming from extract samples centrifugation</td>
</tr>
</tbody>
</table>

Table 3. Odour attributes and references used in the profile sheet to describe the perceived sensations of samples and their definitions.

3.3.3e Statistical analysis

Volatile compounds. The software XLSTAT 7.5.2 version (Addinsoft, Belmont, MA, USA) was used to elaborate both the chemical results by analysis of variance (ANOVA with Fishers’ least square difference post-hoc test, p < 0.05) and principal component analysis (PCA).

Sensory analysis. Three-way repeated measures analysis of variance was carried out on the data from 9 judges to define influence of different heat treatments on each attribute with factors treatment (3), sample type (5) and replicate (2). The significance level p=0.05 was chosen to study the main effects and interactions. Basic analysis (mean, standard deviation) and multivariate tests (test of Within subjects effects-Sphericity assumed) were performed by SPSS software (IBM SPSS Statistic 22).
3.3.4 Results and discussion

3.3.4a Lipoxygenase activity determination

Considering the measurement of LOX activity, both the heat treatments reduced the enzymatic activity. The enzymatic activity in NA sample was 336.8 ± 7.9 µmol of hydroperoxides/g of sample/min. The strongest heat-treatment, in terms of LOX activity reduction, was the conventional thermal treatment. In fact, in CO flour, the enzyme activity was reduced by 81.1% (63.8 ± 5.7), while in MW flour the lipoxygenase activity was reduced by 71.58% (95.7 ± 10.8) with respect to the native sample. As a result, the heat treatments were not strong enough for switching off completely the LOX activity. Thus, the potential of formation of compounds lypoxygenase-catalysed and responsible for off-flavour was reduced but not effectively eliminated.

3.3.4b Volatile compounds analysis

Fiber comparison. After testing CAR/PDMS and DVB/CAR/PDMS fibers on liquid and solid samples, the first was selected for the analysis of flours, while the latter was used for obtaining the volatile profile of liquid models. The quantification of 35 compounds tentatively identified in the volatile profile of flour and extract from NA Finnish faba beans is reported in Figure 1, highlighting that while CAR/PDMS allows to collect greater amounts of volatile compounds in solid samples from flour samples, CAR/DVB/PDMS was more effective in collecting from liquid ones. Taking into account many of the volatiles detected, it can be seen that how the hexanal in flour samples is better detect if the CAR/PDMS fiber is used; on the other hands, octen-3-ol, in extract, gave higher responses with the use of DVB/CAR/PDMS; the same behaviour could be described for limonene.
Figure 1. Volatile compounds analysed with CAR/PDMS and DVB/CAR/PDMS fibers. Comparison of results obtained with the two fibers from flour (a) and extract (b) from native (NA) Finnish beans. Error bars show standard deviation.
Results

**Volatile compounds in different sample matrices.** Twenty-two volatile compounds were tentatively identified, quantified and selected from the 35 initially considered, according to their contribution to the description of the aromatic profile of samples of this study; their amounts are reported in Table 4.

Comparing NA, CO and MW flour samples, it can be assumed that the heat treatment of beans did not have a major impact on volatile compounds, even if some differences could be pointed out. Considering aldehydes, in CO and MW samples, the amounts of hexanal were 2839 and 4449 mean area unit (mau-mean values), while the concentration of this compound in NA samples was 1533 mean area units. Additionally, nonanal level in MW was statistically higher than that in the other samples (1558). These results suggested how thermal treatments may lead to chemical oxidation reactions and need to be optimized.

Considering the suspension from NA flour, it can be seen that many compounds appeared: this results reflected that the addition of water to flour activates the enzymes and that the LOX activity was the highest in untreated beans. Comparison of flours and suspensions showed that a lot of new compounds were produced in suspensions, after the water addition. In particular, alcohols like 1-penten-3-ol, octen-3-ol, aldehydes as nonanal but also furans were formed; these compounds are known to be formed by enzymatic reactions (Kroft et al., 1993). The extracts were characterized by less volatiles with respect to suspension; it could be hypothesized that part of volatiles were retained by solid precipitate, that was removed from suspension to extract during centrifuging. The only exception is represented by the hexanal content, its level was 5912 and 14680 mau in MW suspension and extract, respectively. It could be hypothesized that many enzymes, even LOX, remained in the extract and continued oxidation processes. If extracts are considered as emulsions with 0% of oil added, the effect of the rapeseed oil addition to the samples can be investigated.

Regardless of the nature of beans used to prepare the models, emulsions with 3% and 10% oil showed more volatile compounds than the extract; this could be related to the enzyme activity as the LOX oxidized the fatty acids present in the oil. The emulsions produced with NA flour were characterized by more volatile compounds than the emulsion produced with MW and CO flours: both in the aqueous environment and in the presence of oil, the effect of higher LOX activity was obvious. The amounts of aldehydes and alcohols showed statistically significant differences between NA, CO and MW emulsions: this is the case, for
Results

eexample, of hexanal, hexanol and nonanal. A principal component analysis was performed with data of volatile compounds detected in extracts and emulsions of NA, CO and MW samples and is reported in Figure 2.

Figure 2. Biplot of the variance among extracts and emulsions of samples NA, CO and MW. PC-1 describes the most of the variance (76%) while PC-2 explains the rest (9%).

The figure highlights that while CO and MW samples (extr, 3% oil and 10% oil) seem to be similar with each other. NA emulsions were very different from the other emulsions and extracts, and even from the NA extract. In the NA emulsions, there were much more volatile compounds, which is confirmed by the content of individual compounds as reported in Table 4. Thus, the oil in the emulsions represented a good substrate for the enzyme activities. Moreover, all the extracts (NA, CO and MW) are close to each other, although NA extracts had much more 2-methylfuran and 2-pentylfuran than CO and MW (see table 3). This confirms that the addition of oil is the most important contributor of differences in this set of samples.
## Results

<table>
<thead>
<tr>
<th>Compound</th>
<th>Aldehydes</th>
<th>Ketones</th>
<th>Alcohols</th>
<th>Terpenes</th>
<th>Furan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NA flour</td>
<td>NA susp</td>
<td>NA extr</td>
<td>CO flour</td>
<td>CO susp</td>
</tr>
<tr>
<td>3-methyl-butanal</td>
<td>39 e</td>
<td>1673 a</td>
<td>119 de</td>
<td>455 bc</td>
<td>-</td>
</tr>
<tr>
<td>Hexanal</td>
<td>1533 d</td>
<td>2207 d</td>
<td>3743 cd</td>
<td>8782 a</td>
<td>5721 b</td>
</tr>
<tr>
<td>E-2-hexanal</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1451 a</td>
<td>1378 a</td>
</tr>
<tr>
<td>2,4-hexadienal</td>
<td>-</td>
<td>-</td>
<td>1819 a</td>
<td>2035 a</td>
<td>-</td>
</tr>
<tr>
<td>E-2-heptanal</td>
<td>22 d</td>
<td>-</td>
<td>166 d</td>
<td>5270 b</td>
<td>5966 a</td>
</tr>
<tr>
<td>2-octenal</td>
<td>54 f</td>
<td>297 ef</td>
<td>528 def</td>
<td>8172 a</td>
<td>6162 b</td>
</tr>
<tr>
<td>Nonanal</td>
<td>582 f</td>
<td>445 f</td>
<td>792 f</td>
<td>9751 a</td>
<td>8895 a</td>
</tr>
<tr>
<td>1-penten-3-ol</td>
<td>46 f</td>
<td>189 ef</td>
<td>315 def</td>
<td>7356 a</td>
<td>4786 b</td>
</tr>
<tr>
<td>3-methyl-butanol</td>
<td>308 t</td>
<td>1503 b</td>
<td>774 ef</td>
<td>1107 cd</td>
<td>881 de</td>
</tr>
<tr>
<td>Hexanol</td>
<td>260 b</td>
<td>11039 b</td>
<td>5990 b</td>
<td>10421 a</td>
<td>410 b</td>
</tr>
<tr>
<td>Hex-1-enol</td>
<td>29 c</td>
<td>-</td>
<td>220 c</td>
<td>4754 a</td>
<td>3035 b</td>
</tr>
<tr>
<td>Octen-3-ol</td>
<td>109 t</td>
<td>5481 c</td>
<td>2599 d</td>
<td>13864 c</td>
<td>6988 b</td>
</tr>
<tr>
<td>1-penten-3-one</td>
<td>50/ bco</td>
<td>-</td>
<td>290 t</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-pentanone</td>
<td>89 e</td>
<td>534 e</td>
<td>422 e</td>
<td>12784 a</td>
<td>4296 bc</td>
</tr>
<tr>
<td>2-heptanone</td>
<td>-</td>
<td>656 de</td>
<td>845 d</td>
<td>28/8 a</td>
<td>121/5 c</td>
</tr>
<tr>
<td>2-methyl-1,3-butadiene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22/5 a</td>
<td>215/5 a</td>
</tr>
<tr>
<td>3-ethyl-1,5-octadiene I</td>
<td>51 d</td>
<td>-</td>
<td>9761 a</td>
<td>7529 b</td>
<td>112 d</td>
</tr>
<tr>
<td>3-ethyl-1,5-octadiene II</td>
<td>-</td>
<td>-</td>
<td>13373 a</td>
<td>12318 a</td>
<td>-</td>
</tr>
<tr>
<td>3-ethyl-1,5-octadiene II</td>
<td>-</td>
<td>-</td>
<td>7196 b</td>
<td>11391 a</td>
<td>-</td>
</tr>
<tr>
<td>Linone</td>
<td>99 e</td>
<td>221 e</td>
<td>195 e</td>
<td>4148 a</td>
<td>2505 b</td>
</tr>
<tr>
<td>2-methyl-furan</td>
<td>-</td>
<td>3120 b</td>
<td>3706 a</td>
<td>1316 c</td>
<td>-</td>
</tr>
<tr>
<td>2-pentyl-furan</td>
<td>74 c</td>
<td>1938 ab</td>
<td>1952 ab</td>
<td>3049 a</td>
<td>2943 a</td>
</tr>
</tbody>
</table>

**Table 4.** Volatile compounds identified in the samples. Peak numbers correspond to volatiles elution order in chromatograms. Values are mean area units (divided by 10⁴) from three replicates. Different letters represent statistically significant differences.
Results

Subsequently, volatile compounds could be considered responsible for the aromatic profile of samples in this study are taken into account, together with their sensory perception.

Looking at the compounds (Table 4), aldehydes were the most represented class, with generally higher amounts in the emulsions, probably because of the rapeseed oil oxidation accelerated/driven by LOX activity. This clearly showed that addition of oil to faba bean flours in aqueous environments is a risk for off-flavour formation, if enzymes have not been inactivated. LOX activity has also positive effects on food flavor: volatile aldehydes and alcohols are important compounds which contribute to the characteristic flavour produced after physical disruption and subsequent processing of fruits, vegetables and plants (Stone et al., 1975; Kemp et al., 1973; Kazeniac et al., 1970; Buttery et al., 1969; Forss et al., 1962).

**Aldehydes.** Hexanal, typical lipid oxidation product, was the major aldehyde detected in all faba bean samples and it showed higher value after water addition (in suspensions) and even more after the oil addition (in emulsions), reaching the highest amounts in 10% oil emulsions. This could be clearly explained considering that hexanal, whose amounts depends on linoleate content in beans (Ho et al., 2005), is produced during lipid oxidation, mainly due to the LOX activity (Matoba et al. 1985). Additionally, hexanal can be formed by autoxidation (Sanches-Silva et al., 2004), which is enhanced by thermal treatments. This could be the reason why greater amounts of hexanal were found in MW and CO flours, than in NA flour. Hexanal has also been associated with different sensory notes according to different food matrices, like greeny and leafy notes in soymilk (Lozano et al., 2007).

**E-2-hexenal** has been associated to different sensory notes in linkage with food matrices as: green, fruity, almond notes in olive oil (Aparicio et al., 1998), beany-flavour in soybean meals (Tang et al., 2014), green-herbal in *Iulo* tomatoes (Forero et al., 2015). This compound was detected mainly in NA emulsions with 3% and 10% oil; this could be related to the LOX activity, as **E-2-hexenal** is a product of lipoxygenase pathway. The aldehyde **2,4-hexadienal**, mainly associated to green, fruity, citrus, waxy sensory notes and produced during lipid oxidation of rapeseed oil (Petersen et al., 2012); this could better explain why these volatiles were detected only in the NA emulsions where the LOX found more substrate.

Conversely, **E-2-heptenal** has been related to sensory notes that remind to fatty, soapy and oily and also this compound showed a similar trend with the highest values in all the
emulsions, with respect to extracts. The last two aldehydes detected, 2-octenal and nonanal were found in the flours and in the suspensions, albeit in lower amounts if compared to the emulsions. The fact that the concentration of these molecules was higher for CO and MW samples could be related to their origin, as 2-octenal and nonanal were considered to be produced during autoxidation processes (Tzschoppe et al., 2016), which probably occurred due to thermal treatments. The aldehydes have been associated to sensory notes of beany but also tallowy and fatty notes in raw peanut (Brown et al. 1973).

**Alcohols.** According to Oomah (2007) 1-penten-3-ol, produced from linoleate oxidation, could be associated with grassy ethereal odor. This compound was generally higher in the emulsions, with the highest peak areas in NA models. Hexanol was found in lower amounts in thermal treated samples: this is not surprising since this alcohol is produced by alcohol dehydrogenase, an enzyme of LOX pathway (Gargouri et al., 2004), and CO and MW models were produced from beans with a reduced LOX activity. Considering thermal treated samples, octen-3-ol showed different behavior: while in NA models the highest amounts were recorded in the emulsions, the same couldn’t be said for CO and MW models; in these cases, the suspensions showed the highest contents. Octen-3-ol is considered the main off-flavour compound in commercial oil-free soybean products (Samoto et al., 1998) because of its “mushroom-like” odor. The main enzymes involved in the biosynthesis of this alcohol are a lipoxygenase and a hydroperoxide dehydrogenase.

**Furans.** These compounds were earlier found to be relevant in explaining the variation of volatiles amounts in different genotypes of fabas as beans showed variability according to different genetic traits (Oomah et al., 2014). 2-methylfuran was detected in NA, CO and MW samples, and showed the highest peak areas in the extracts; in particular, this compounds was found in the highest amount in NA extract. 2-methylfuran had been detected in headspaces of many beans like faba bean, pea and beans. Moreover Mariotti et al. (2012) described 2-methylfuran as having a burnt aroma with a sweet odor, similar to that of coffee. Another furan compound, 2-pentylfuran, was found in all samples of this study; this furan is produced by free radical mechanism, from linoleic acid, when the vinyl hydroperoxide undergoes to cyclization via the alkoxy radical, to yield 2-pentylfuran (Ho et al., 1994). Moreover, formation of hydroperoxides by LOX may also enhance formation of
Results

this furan, justifying the high amounts of this compound in NA models, where the LOX activity wasn’t reduced by thermal treatments.

This molecule had been previously associated with beany odor (Lasekan et al., 2012; Hsieh et al., 1982; Wilkens et al., 1970; Krishnamurthy et al., 1967; Chang et al., 1966) and linked with musty/earthy notes (Vara-Ubol et al., 2004). According to what explained above, PCA highlights how 2-methylfuran and 2-pentylfuran are closer to native samples than to heat-treated samples; thus, they could be indicators of enzymatic activity in the samples, partially reduced in thermally treated faba beans as reported by LOX activity measurements.

**Ketones.** Among all ketones, 3-pentanone was found to be the most represented one for all the samples of this study, with the highest amounts in emulsions, in particular in the two produced with NA flour. This ketone seemed to behave as a LOX activity secondary product, and has been previously associated with caramel and sweet sensory notes (Olafsdottir et al., 2005). 2-heptanone is formed from alkanals, during lipid oxidation, (Labuza et al., 1971) and it is considered to be responsible for musty sensory notes.

**Terpenes.** Looking at the class of terpenes, many of these compounds were detected only in the emulsions, and could be considered as indicating the oxidative changes: this is the case of 2-methyl-1,3-butadiene, identified in NA 3% oil, NA 10% oil and MW 10% oil samples. Considering the stereoisomers of 3-ethyl-1,5-octadiene (I, II, III), the I was found in emulsions derived from NA beans and in all the samples coming from heat-treated beans, whereas the stereoisomers II and III were identified only in the NA emulsions. The large amount of these compounds in oil-added samples was probably related to oxidative processes caused both by LOX activity (in NA samples) and by thermal treatments, in MW and CO samples.
### Results

#### 3.3.4c Sensory analysis

While sensory characteristics and profiles of soymilk and other soy-derived products have been investigated (Ma et al., 2015; Granato et al., 2010; Torres-Pennarada et al., 2001), studies on the sensory characterization of products containing faba beans are not so numerous. Moreover, there are few studies reporting sensory evaluation of faba bean samples derived from different heat treatments (Ramos Diaz et al., 2016). The data obtained through 4 different evaluation sessions (7-8 samples per each one) are listed in Table 5.

![Table 5](image)

The evaluation of the 15 samples gave interesting results (Table 5) considering both the factors heat-treatment (NA, CO, MW) and sample type (flour, suspension, extract, emulsions). While the highest values of the attributes *pea* and *beany* for heat-treated (CO and MW) samples were recorded in suspensions and emulsions, the same couldn’t be said for the native samples, where the strongest intensity of these attributes was associated to the 3% and 10% oil emulsions followed by the suspension and by flour. In general, the mean intensities were higher for *beany* than for *pea*. The attributes *nutty* and *grain/seed* were associated with the heat-treated samples. Looking at the *nutty* intensities, the highest value

---

**Table 5.** Means and standard deviation (SD) values of the 8 sensory attributes (n= 15x3) selected for the sensory profiling of faba bean samples (flours, suspensions, extracts and emulsions) produced with native (NA), conventional thermal treated (CO) and microwave thermal treated (MW) beans.
Results

was for the CO samples, especially in flour, followed by extract and 3% oil emulsion. For MW samples, intensities seemed to be lower. The highest intensity was observed in 10% oil emulsion sample with mean value of 2 (1.94 ±0.34). The mean intensities of this attribute in NA samples were really low, with the only exception of the 10% oil emulsion. A similar perception regards grain/seed. In fact, the intensities of this attribute in NA samples were low, except for the 10% oil emulsion; anyhow, thermal treated samples showed higher intensities of this attribute. In particular, while for conventional thermal treated models the highest values of grain/seed were associated both with flour and extract (3.37 ±0.14 and 3.01±0.07 respectively), for the microwaved samples this descriptor had the highest intensities in extract but also in 3% and 10% oil emulsions. Grassy was always reported with very low intensities but seems to be mainly associated with emulsions, the 3% oil for MW and NA beans and the 10% oil for CO. The intensity of attributes considered as negative were generally low. Considering musty/earthy, the ranges of values were 0.81-1.31, 0.74-1.22 and 0.73-1.06 for NA, CO and MW models respectively; this descriptor seemed not to have statistical relevance in discriminating among different heat treated samples. A similar trend characterized fermented and rancid, that showed the highest intensities in NA and CO 10 % oil and in MW flour.

In order to describe the significant differences eventually existing among the samples, a multifactorial analysis of variance was carried out (Table 6). The analysis of variance was performed for 7 of the 8 attributes: pea, nutty, grassy, grain/seed, musty/earthy, rancid, fermented.
Results

<table>
<thead>
<tr>
<th></th>
<th>Pea</th>
<th>Nutty</th>
<th>Grassy</th>
<th>Grain/seed</th>
<th>Musty/earthy</th>
<th>Rancid</th>
<th>Fermented</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>df err</td>
<td>F²</td>
<td>p</td>
<td>F²</td>
<td>p</td>
<td>F²</td>
</tr>
<tr>
<td>Treatment</td>
<td>2</td>
<td>16</td>
<td>1.0</td>
<td></td>
<td>10.9 **</td>
<td></td>
<td>11.8 **</td>
</tr>
<tr>
<td>Kind of sample</td>
<td>4</td>
<td>32</td>
<td>0.6</td>
<td>*</td>
<td>2.9 *</td>
<td></td>
<td>3.2 *</td>
</tr>
<tr>
<td>Replicate</td>
<td>1</td>
<td>8</td>
<td>13</td>
<td>*</td>
<td>0.3</td>
<td></td>
<td>9.7 *</td>
</tr>
<tr>
<td>Sample x treat</td>
<td>8</td>
<td>64</td>
<td>1</td>
<td>**</td>
<td>6.2 ***</td>
<td></td>
<td>5.8 ***</td>
</tr>
<tr>
<td>Sample x rep</td>
<td>2</td>
<td>16</td>
<td>1.7</td>
<td>*</td>
<td>4.7 *</td>
<td></td>
<td>9.4 **</td>
</tr>
<tr>
<td>Treat X rep</td>
<td>4</td>
<td>32</td>
<td>7.4 ***</td>
<td></td>
<td>6.7 **</td>
<td></td>
<td>3.9 *</td>
</tr>
</tbody>
</table>

Table 6. Results of analysis of variance performed on the data; main effects and interactions.
Treatment: NA, CO, MW; kind of sample: flour, suspension, extract, emulsions.
1 df= degrees of freedom; df err= degrees of freedom for error.
2 F-ratio
3 ***p<0.001; **p<0.01; *p<0.1

The attributes nutty and grassy varied significantly between different thermal treatments, such that CO samples resulted in strong rating and NA samples resulted in weak values. The attributes nutty, grassy, grain/seed and fermented resulted significantly different among different models: nutty showed the lowest values in flours and 3 % oil emulsions, while grain/seed showed the highest intensities in MW and NA models.

The variability of the replicates was significant for four of the attributes: pea, grassy, musty/earthy and rancid, suggesting that training had not been sufficient to result in consistent ratings. The interaction between sample and treatment was significant for the majority of the attributes selected, indicating that different sample types differed in the way their sensory profiles changed in relation to thermal treatments.
3.3.4d Combining volatile compounds and sensory evaluation data

Data from sensory attribute intensities (pea, nutty, grain/seed, musty/earthy, fermented, rancid and grassy) and amounts of 22 volatile compounds detected were analysed by Multifactorial Analysis (MFA) 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 the 61.05 % of variance explained, 33.24 % and 27.81 % for F1 and F2, respectively.

![Figure 2](image-url)  
**Figure 2.** MFA biplot, obtained using part of sensory and volatile compounds data as variables, selected according to their contribution to the two main components.

Figure 2 summarises what described above: the variability among NA samples is bigger than that of thermal treated samples. Considering the extracts, while NA extract is described by 2-methylfuran and 3-methylbutanal but didn’t show high intensities of any sensory attributes. CO and MW extracts were much more similar one to each other, the both of them described by the attribute of musty/earthy and by a higher number of volatile compounds.
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The CO flour was close to the attributes of nutty and grain/seed, probably because of the effect of heat treatment, together with CO susp and emulsion CO 3% oil, while MW flour was characterized by the attributes of fermented, grassy and rancid. Additionally, the attributes grassy and fermented were positively correlated (r = 0.78) while the attribute nutty resulted to be positively correlated with 1-penten-3-one (r = 0.64). The most of the volatile compounds detected in samples in this study appeared to be close to the emulsions produced using native faba bean flour (emulsions NA 3% and 10% oil): this is not surprising as these compounds are generated during LOX activity, which was reported to be at its maximum in NA beans. For example, among aldehydes produced during oxidative processes, 2-octenal, associated with sensory notes of beany and tallow, resulted to be positively correlated with the attribute of musty/earthy.

3.3.5 Conclusions

At present, there are no scientific studies that have analysed both volatile compound amounts and sensory attribute intensities to characterize different faba bean samples. Starting from beans, different models were produce: in particular, beans were thermally treated with a conventional oven or with a microwave oven, then de-hulled; native untreated beans were analysed as control samples. After that, different models were prepared, in order to reproduce different uses of this bean in common food models: suspensions (with water addition) and emulsions, as centrifuged suspensions with 3 different levels of oil addition: 0%, 3% and 10%. As thermal treatments were proven to not completely stop the LOX activity in the samples, a compromise should be reached: where the enzyme activity is almost completely reduced, as in CO and MW samples, attributes related to rancid and nutty sensory notes appeared, maybe because of the autoxidation caused by the temperature rise. On the other hand, when beans were used without any treatment, it could be impossible to control the oxidation carried on by enzymes, and this could affect the shelf life of products in which faba beans are added.

This study represents a preliminary approach to the investigation on origin of off-flavour derived by mandatory heat-treatment applied to faba beans before their consumption or their addition to other ingredients for the preparation of food products.
3.3.6 References


Results


Petersen K.D., Kleeberg K.K., Jahreis G., Busch-Stockfisch M., Fritsche, J. 2012. Comparison of analytical and sensory lipid oxidation parameters in conventional and
Results


3.4 Characterization of typical Italian salami from Mora Romagnola pig breed: an integrated sensory and instrumental approach

Publication details:

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Authors: Federica Tesini\textsuperscript{a,*}, Enrico Valli\textsuperscript{a}, Federica Sgarzi\textsuperscript{a}, Francesca Soglia\textsuperscript{b}, Massimiliano Petracci\textsuperscript{b}, Alessandra Bendini\textsuperscript{a,b}, Claudio Cavani\textsuperscript{b}, Tullia Gallina Toschi\textsuperscript{a,b}

\textsuperscript{a} CIRI - Agrifood (Interdepartmental Centre of Industrial Agrifood Research), Alma Mater Studiorum – University of Bologna

\textsuperscript{b} Department of Agricultural and Food Sciences, Alma Mater Studiorum - University of Bologna

Submitted to: Journal of Food Quality

3.4.1 Abstract

A rapid sensory and instrumental analytical approach for characterizing a typical Italian salami, manufactured from Mora Romagnola, an autochthonous pig breed extensively farmed within a geographically confined area in Italy, was investigated. The aim was to highlight the importance of an integrated approach as a tool for supporting and ensuring the authenticity of traditional food products: in this case study, salami produced with Mora Romagnola were compared with salami produced using meat from conventional pig breeds. The sensory profiles of samples were defined through the attributes: seasoning, pepper, fermented, humidity, meat grain, and fat distribution were the major attributes to characterize dry fermented salami. Color differences between Mora Romagnola and conventional salami were clearly identified by an electronic eye; moreover, through the analysis of volatile compounds (SPME-GC-MS), 33 molecules were detected. According to instrumental texture analysis, a large variability among the Mora Romagnola samples was detected, likely related to the different types of salami (i.e. recipe, casing, dimension, ripening). This is not an unexpected result: salami produced with local breeds are traditional products, whose recipes and handcraft preparation are characterized by a certain variability,
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linkable to the concept of local food. The findings allowed to describe the characteristics of this salami, usable to define a single document to describe and protect the product.

**Keywords:** Mora Romagnola salami; sensory analysis; textural properties; typical product; volatile compounds; electronic eye, integrated approach.
3.4.2 Introduction

The concept of local food, traditionally related to its geographical origin, constitutes an important driver for consumer demand of typical products (Pieniak et al., 2009). Moreover, aspects like the history of a typical product preparation, together with specific ingredients, represent an important part of quality perception in food (Amli et al., 2001; Chambers et al., 2007). Traditional products are enriched by added cultural and identity values, as they contribute to maintenance and development of the rural areas they are linked to (Guerrero et al., 2009). Among these, fermented meats are unique products, often represented as elements of culinary heritage and identity, especially in Mediterranean Countries such as Italy and Spain (Frédéric et al., 2013). The particular category of sausage-shaped fermented meats traces back to the Romans, who seem to have learnt the craft from a tribe of southern Italy called the Lucanians. The word “salami” probably originates from the Medieval Latin word “salumen”, i.e., salted stuffs, or (less likely) from the Cypriot city of Salamis Spain (Frédéric et al., 2013). Nowadays, salami is used to define a specific type of cured sausage that is air dried, smoked, or salted and left to age; salami consumption, in Italy, is a perfect example for a product that was historically considered as a traditional national food, and tends to differ from one Italian region to another (Di Monaco et al., 2015; Monteleone et al., 2009; Conter et al., 2008). Among these products, in the past few years, an increasing interest has been given to the development of “mono-breed” labelled lines of meat products (Fontanesi, 2009). Until the 20th century, production of dried and fermented meat products throughout the Mediterranean Countries was essentially based on local breeds, developed over centuries to fit specific production targets and environmental constraints. The situation changed dramatically with the intensification of agriculture in the mid-20th century, when pig production moved to more intensive systems based on a reduced number of transboundary breeds (Gama et al., 2013). Native Italian pig breeds belong to the southern European native breeds that are characterized by dark-coloured skin, with a pointed snout, small litters, and by the presence of high amounts of subcutaneous and intramuscular fat. Among these, Mora Romagnola is an autochthonous pig breed that is farmed under extensive or semi-extensive management systems, mainly in the Emilia-Romagna region, and its meats are used mainly to produce traditional meat products such as dried hams and salami (Pugliese et al., 2012; Franci et al., 2007). Recently, a Mora Romagnola Consortium
has been founded to promote and increase the visibility of Mora Romagnola products and to protect them from potential fraud. However, there is a lack of detailed information concerning the quality properties and the sensory traits of traditional products made with Mora Romagnola. The inimitable sensory characteristics of dry-fermented salami are ascribed to a number of biochemical and physicochemical transformations occurring in the sausage batter during fermentation and maturation. A distinctive gel-like texture is created due to the bacterial acidification process, while specific color development results from interactions between the myoglobin of the meat and nitrogen monoxide, originating from the nitrate and/or nitrite in the curing salt (Gama et al., 2013). Finally, the complex flavor of fermented sausages is due to oxidative transformations, mostly of unsaturated fatty acids, and complex interactions between the sausage batter, meat enzymes, and microorganisms. As a result, flavor development does not depend only on the raw materials and processing conditions, but also on how these factors affect the composition, community dynamics, and metabolism of the sausage microbiota (Gama et al., 2013). As a consequence, characterization of the sensory profile of salami is very complex and requires the use of an advanced and wide range of sensory and analytical techniques. Therefore, the aim of this study was to highlight the relevance of the adoption of an integrated approach to describe salami produced with the Mora Romagnola breed by comparing this typical product to salami obtained using modern white-pig breeds available on the Italian market. For this purpose, the product was characterized by an advanced analytical approach using sensory descriptive analysis (DA) and instrumental techniques, such as image analysis by electronic eye, solid-phase microextraction coupled with gas-chromatography mass-spectrometry (SPME-GC-MS) for the determination of the volatile profile, and a TA-HDi Heavy Duty texture analyzer.

3.4.3 Materials and methods

3.4.3a Samples

The 13 samples selected for this study are listed in Table 1. In particular, 5 samples were produced using meat from the Mora Romagnola breed, while the other 8 were manufactured by meat from conventional pig breeds. The conventional samples “Contadino” (CON), “Felino” (FEL), and “Milano” (MIL) were selected as they are market leaders in Italy
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(Prakash et al., 2015; MIPAAF 2010; UNI 1993). Samples CO1, CO2, CO3, CO4 and CO5 were produced with meat obtained by conventional pig breeds and were selected according to their similarity (e.g. in terms of size, weight, meat consistency) to the Mora Romagnola samples MO1, MO2, MO3, MO4 and MO5, respectively. All salami samples were vacuum packed and stored protected from light at 4±1 °C; all instrumental determinations were carried out on at least three replicates, whereas for the sensory analysis the final score was the average of the ones assigned by each judge to the samples assessed in three different sessions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO1</td>
<td>Conventional pork salami with small size (50 g)</td>
</tr>
<tr>
<td>MO1</td>
<td>Mora Romagnola salami with the same size of CO1 (50 g)</td>
</tr>
<tr>
<td>CO2</td>
<td>Conventional pork salami &quot;Nostrano&quot; type (500 g)</td>
</tr>
<tr>
<td>MO2</td>
<td>Mora Romagnola salami similar to CO2 (500 g)</td>
</tr>
<tr>
<td>CO3</td>
<td>Conventional pork salami &quot;Cacciatore&quot; type (160 g)</td>
</tr>
<tr>
<td>MO3</td>
<td>Mora Romagnola salami similar to CO3 (260 g)</td>
</tr>
<tr>
<td>CO4</td>
<td>Conventional pork salami obtained directly by a local producer (300 g)</td>
</tr>
<tr>
<td>MO4</td>
<td>Mora Romagnola salami similar to CO4 (300 g)</td>
</tr>
<tr>
<td>CO5</td>
<td>Conventional pork salami obtained directly by a local producer (300 g)</td>
</tr>
<tr>
<td>MO5</td>
<td>Mora Romagnola salami similar to CO5 (300 g)</td>
</tr>
<tr>
<td>CON</td>
<td>Conventional pork Salami &quot;Contadino&quot; type (550 g) (Prakash et al., 2015)</td>
</tr>
<tr>
<td>FEL</td>
<td>Conventional pork Salami &quot;Felino&quot; (PGI) type (1,000 g) (Prakash et al., 2015; MIPAAF 2010)</td>
</tr>
<tr>
<td>MIL</td>
<td>Conventional pork Salami &quot;Milano&quot; type (1,000 g) (Prakash et al., 2015; UNI 1993)</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of salami samples selected for this study: Mora Romagnola samples are coded with MO capital letters, while conventional pig breed salami are labelled with CO capital letters or with the codes CON, FEL, or MIL.

3.4.3b Sensory analysis

The DA was performed by a panel of 12 trained judges recruited on the basis of their previous experience in sensory analysis (staff and PhD students at the Department of Agricultural and Food Sciences, University of Bologna, Cesena, Italy). The panel worked in a suitable panel room and each assessor performed the sensory evaluation in a single booth. During the training phase, each panelist received salami samples and found visual, aroma,
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taste and texture descriptors to describe them. The panel decided whether descriptors were redundant (so they had to be removed from the profile sheet) or if there were other attributes that should be added. A final list of 11 attributes was defined and descriptors were divided in olfactory (directly and indirectly perceived), taste, texture, and visual (Table 2). Panelists also used reference standards during the training phase, which were specifically formulated to help rating of the intensity of the selected attributes (Table 2).

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Definition</th>
<th>Reference</th>
<th>Anchor points</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Direct/indirect olfactory attributes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seasoning</td>
<td>Direct/indirect olfactory note that reminds seasoned meat</td>
<td>Minced pork meat (fresh) and sample CO1 (seasoned)</td>
<td>Fresh: 20; Seasoned: 80</td>
</tr>
<tr>
<td>Pepper</td>
<td>Direct/indirect olfactory note that reminds of pepper</td>
<td>4 g of pepper in 20 g of minced pork meat</td>
<td>Strong: 100</td>
</tr>
<tr>
<td>Garlic</td>
<td>Direct/indirect olfactory note that reminds of garlic</td>
<td>2 g of garlic powder in 20 g of minced pork meat</td>
<td>Strong: 100</td>
</tr>
<tr>
<td>Rancid</td>
<td>Direct/indirect olfactory note linkable to oxidized/old food</td>
<td>CO1 samples undergone to forced oxidation for 7 days</td>
<td>Strong: 100</td>
</tr>
<tr>
<td>Fermented</td>
<td>Pungent olfactory note reminiscent of acetic acid</td>
<td>&quot;Golfetta&quot; type pork salami</td>
<td>Strong: 100</td>
</tr>
<tr>
<td><strong>Taste attributes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spicy</td>
<td>Mouth inflammatory effect</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Salty</td>
<td>Salty elementary taste</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td><strong>Texture attributes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greasiness</td>
<td>Fat/greasy sensation perceived while biting (related to the fat part of the product)</td>
<td>&quot;Milano&quot; type pork salami</td>
<td>Strong: 100</td>
</tr>
<tr>
<td>Humidity</td>
<td>Juiciness released by sample while biting (related to the lean part of the product)</td>
<td>&quot;Golfetta&quot; type pork salami</td>
<td>Strong: 100</td>
</tr>
<tr>
<td><strong>Visual attributes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat grain</td>
<td>Lean part evaluation: lean meat distribution in the slice</td>
<td>/</td>
<td></td>
</tr>
<tr>
<td>Fat distribution</td>
<td>Fat part evaluation: fat part distribution in the slice</td>
<td>/</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Attributes used in the profile sheet of salami samples and their definitions; anchor points and references used during the training phase by panelists.
The attributes were presented to each panelist and specific training sessions on their use were carried out. Visual analysis of samples was performed at the end of each session in white light conditions, while the rest of sensory evaluation was conducted using a red light, in order to prevent the judge’s recognition of samples. The evaluation of red intensity of salami was too difficult, as declared by the judges; during training, they were not able to rate different intensities of red among the samples, and this attribute was thus removed from the final profile sheet. The panelists rated samples by indicating the intensities of attributes on an unstructured 100 mm scale with defined anchor points from 0 (not perceivable) on the left side, to 100 (perceivable at maximum level) on the right. Samples were presented to panelists in a randomized order; each session was performed on four different samples, all presented as whole slices cut with a fixed thickness of 3 mm. Salami were coded with alphanumerical codes and presented in white plastic dishes. Fresh water and breadsticks were provided to panelists between sample evaluation to clean their mouth. The software FIZZ (Biosystèmes, Dijon, France) was used to create the profile sheet adopted during sensory evaluation and for related data collection. Samples were analyzed in triplicate.

3.4.3c Volatile compounds analysis

An aliquot of 1 g of each sample, previously homogenized with a mixer, was weighed in a 20 mL amber glass vial. Each vial was closed with a silicone septum and conditioned at 40°C for 2 min. After this, 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 GC injector, with a split ratio of 1:10. 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 min, the temperature increased to 240°C at 10°C min⁻¹ and remained stable for 5 min. Helium was used as a carrier gas at a flow of 1 ml min⁻¹. Volatile compounds were analyzed by quadrupolar mass-selective spectrometry (in the 30–250 amu mass range), using a GCMS-QP2010 gas chromatograph (Shimadzu Co., Kyoto, Japan) coupled with an autosampler AOC-5000 Plus (Shimadzu Co., Kyoto, Japan). Peak identification was based on comparison of mass spectrum data with spectra present in the National Institute of Standards and Technology (NIST) library (2008 version) and only identification matching
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more than 90% were taking into account. Since it was not possible to use an internal standard (due to the difficulty to homogenously solubilize a standard molecule in a solid sample), a percentage normalization was applied: the presence of each compound was expressed as percentage with respect to the entire volatile fraction. Three replicates for each sample were performed.

3.4.3d 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 (resolution 2592 × 1944 p) combined with powerful data processing software. This instrument was equipped with an adjustable photo-camera (16 million colors) in a dedicated measurement small room with standardized, controlled and reproducible lighting conditions. The camera imaging was software-monitored, and equipped with several lenses of different focal length for accurately assessing from very small to large products. The instrument is furnished with 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) and 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 it is also possible to correlate these data with ones from sensory panels, if provided.

3.4.3e Textural properties

Warner Bratzler shear test was performed by shearing a 4 × 1 × 1cm parallelepiped samples by a TA-HDi Heavy Duty texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) (5 replications/sample) (Del Nobile et al., 2009). The instrument, equipped with a 25kg loading cell and a Warner-Bratzler triangular shear blade, was set to shear the sample at a
cross head speed of 2 mm/sec. Shear force was defined as the maximum force (expressed as kilograms) required to shear the sample.

Texture profile analysis (TPA) was performed on a cylindrical-shaped meat sample (3 cm diameter × 0.8 cm height) that was double compressed to 60% of its initial height (5 replications/sample). The test was run by using a TA-HDi Heavy Duty texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) equipped with a 25 kg loading cell and a 5 cm-diameter cylindrical aluminum probe. Next, the TPA parameters of Hardness, Cohesiveness, Springiness, Gumminess and Chewiness were obtained by elaborating the double compression diagram (force/deformation) of each sample (De Campos et al., 2008).

Tensile test was performed on a hexagonal sample (25 mm-long × 2 mm-thick; 5 replications/sample) according to the procedure described by Herrero et al. (Herrero et al., 2008). Briefly, tensile properties were evaluated by using a TA-HDi Heavy Duty texture analyzer (Stable Micro Systems Ltd., Godalming, Surrey, UK) equipped with a 5 kg loading cell and two tensile grips (one fixed to the base of the texture analyser and the second attached to the loading cell). After applying a grip separation of 25 mm at a cross-head speed of 1 mm/s, rupture force, defined as the maximum peak force required for breaking the sample (kg), and breaking strength, defined as the ratio between rupture force and cross-sectional area of the sample (kg/cm²), were calculated.

3.4.3f Statistical analysis

The software XLSTAT 7.5.2 version (Addinsoft, Belmont, MA) was used to elaborate data using the analysis of variance (ANOVA, with Fishers’ least square difference post-hoc test) and to elaborate data from chemical (volatile compounds), sensory, and textural property analyses through multifactorial analysis (MFA). The software Alphasoft version 14.0 (Alpha MOS, France) was used to explore data from image analysis with Principal Component Analysis (PCA).

3.4.4 Results and discussion

3.4.4a Sensory analysis

The reliability of the panel's performance and training efficiency was checked to ensure reproducibility and repeatability were good (data not shown). Additionally, panel analysis
and product characterization extensions of XLSTAT were used to monitor the judges’ performance during training and effective discrimination power of each selected attribute. The samples produced with conventional pig breeds were selected in relation to their resemblance to their Mora Romagnola counterpart; thus, the results of sensory evaluation will be discussed by pairing samples according to their similarities (e.g. MO vs. CO1, MO2 vs. CO2).

Sensory profiling results (Table 3) showed that MO5 and CO5 were among the samples with the highest intensity of seasoning attribute. The sample MIL showed one of the highest intensities of fermented and fat distribution: this could be related to its processing, as it was produced with the smallest size of fat particles (UNI, 1993). In general, attributes like seasoning, pepper, fermented, humidity, meat grain, and fat distribution could be potentially useful to discriminate among samples. Considering MO2 and CO2, the samples were statistically different for the attribute seasoning, with MO2 showing a higher intensity; on the other hand, among CO1 and MO1, the former had the highest intensity of this attribute, while taking into account MO3 and CO3 no such difference was found. Sample MIL had remarkably different characteristics with respect to the other samples as it was characterized by the lowest intensities of seasoning and meat grain and the highest value of fat distribution.

<table>
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<tr>
<th>Sample</th>
<th>Seasoning</th>
<th>Pepper</th>
<th>Garlic</th>
<th>Rancid</th>
<th>Fermented</th>
<th>Spicy</th>
<th>Salty</th>
<th>Greasiness</th>
<th>Humidity</th>
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<td>0 e</td>
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<td>11 e</td>
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Table 3. Sensory results of salami samples. Mean values followed by different letters significantly differ between the samples (p < 0.05).
Results

3.4.4b Image analysis

The characteristic color of salami is known to be produced by the interaction between the meat pigments and the products resulting from reduction of the nitrates and nitrites added. After nitrate reduction to nitrites, the latter are reduced to nitric oxide that reacts with myoglobin reducing it into chromogen (Ordònez et al., 1999). This process improves color stability; nonetheless, salami is a very complex product in which pigment oxidation is quite difficult to study due to the numerous factors affecting the redox potential within the products (Gøtterup et al., 2008). The image analysis of samples, conducted with an electronic eye, allowed us to obtain the color spectra of each sample in RGB coordinates (red, green, blue). Part of the colors that composed the spectra were selected and used as variables in order to perform a PCA, which was useful to distinguish samples according to red intensity. The projection of samples in the factorial plan, obtained as a result of the PCA, is reported in Figure 1: the different direction/location of vectors (PCA loadings) showed which variables (colors) were involved in the appearance variation among samples. The 16 colors selected permitted to distinguish between the two types of salami, highlighting that the Mora Romagnola meat yielded the darker samples, as they were described by darker shades of red.

![Figure 1. Projection of salami samples on a factorial plan (PCA, intensity and shades of red) built using data related to visual characteristics evaluated by an electronic eye. Conventional salami samples are labeled in green, while Mora Romagnola salami samples are labeled in pink.](image-url)
Results

In particular, variables like “colors-2424” and “colors-2440”, which describes the strongest red intensities, mainly described several Mora Romagnola samples. On the other hand, variables like “colors-2951” and “colors-2678” mainly characterized samples from conventional pig breeds.

This is an important result, as the judges declared that they were not able to evaluate the differences in red intensities among samples. Thus, the use of an electronic eye could support visual evaluation of red intensity in salami and could be useful for the characterization of salami produced with meat from Mora Romagnola. Moreover, this result could be used to define a specific range of color taints associated with Mora Romagnola salami. Starting from this, a non-targeted colorimetric method could be established to evaluate the conformity of different Mora Romagnola salami to their typical color.

3.4.4c Volatile compounds analysis

It is well known that during the processing of dry-cured meat products, many enzymatic and non-enzymatic reactions occur such as protein degradation, lipid degradation and oxidation, Maillard reactions, and Strecker degradation. These changes give rise to the formation of volatile compounds such as aldehydes, carboxylic acids, alcohols, ketones, esters, terpenes as well as sulfur, nitrogen, and other compounds (Jerkovic et al., 2010). In this investigation, a total of 33 volatile compounds were tentatively identified in the sample headspace; compounds are listed in Table 4 according to the chemical classes they belong to. Low percentages of ketones were detected in all samples, with the exception of 2-butanone that was present in a high amount in sample MO3 (12.76%): this molecule is already known as one of the contributors for an “apricot” note in sausages (Olivares et al., 2009). Considering alcohols, the compounds with highest amounts were ethanol and 2-buthanol. In particular, ethanol was detected in all samples produced with Mora Romagnola, with higher percentages compared to conventional samples CO1, CO2, and CO3. This compound can derive from the reduction of aldehydes formed by lipid metabolism (Marco et al., 2008) or from microbial fermentation (Procida et al., 1999); other alcohols such as pentanol, hexanol, and octen-3-ol are also produced in the same way; the latter is considered to be responsible for the mushroom-like odor (Montel et al., 1998) and is produced during
Results
degradation of lipid hydroperoxides (Frankel, 2014). The seasoning process of salami is known to have an important role in the formation of aldehydes; the main precursors of these compounds in meat products are unsaturated fatty acids (Moretti et al., 2004). Among aldehydes, hexanal was the most represented; this molecule showed the highest percentage compared to other volatile compounds detected in salami. Hexanal is already known to impart green, grassy, or floral notes (Meynier et al., 1999; Stanhke, 1995); the highest percentages of hexanal were recorded for samples FEL (72.58%), CON (46.86%), CO5 (44.78%), and MIL (31.56%). The second most abundant aldehyde is represented by phenylacetaldehyde, with the highest percentage (38.07%) in MO4; all the other samples showed lower abundances of this molecule, an aroma-active compound formed from phenylalanine through the Strecker degradation pathway (Hoffman et al., 2000). Allyl methyl sulfide is a volatile sulfur-containing compound, responsible for sensory garlic notes and naturally present in many plants from *Allium*, such as garlic, onion and leek (Lanzotti, 2006); this compound was detected in samples CO3, MO3, CO4, CO5, MO4, and MO5 (few amounts in MO1 and MO2). In accordance with previous studies (Moretti et al., 2004), the chemical class of terpenes was the most represented, whose presence is probably due to addition of spices during salami preparation. In fact, α-pinene, identified in all samples except for FEL, is a terpene already found in coniferous and rosemary essential oil (Simonsen et al., 1957). The same origin is attributed to β-pinene, which showed the highest percentage in CO1 (26.58%). The isomers of α-phellandrene and β-phellandrene are specific to certain plants: the first is typical of *Eucalyptus phellandra*, while the latter has been frequently found in fennel essential oil (Tholl et al., 2006). 3-carene, a molecule linked to pepper scents, was mainly found in samples CO4, MO4, CO5, and MO5, produced with the same recipe, while limonene was the most represented terpene and can be associated with pine/peppery-lemon sensory notes (El Zaeddi et al., 2017). Caryophyllene reminds of sensory notes of clover/turpentine, which could match the characteristic ‘green flavor’ (Burdock, 2016). As reported in previous studies, the presence of many terpenes may be associated with the use of spices, mainly pepper and garlic (Moretti et al., 2004; Meynier et al., 1999). It is interesting to highlight how sample FEL did not show the presence of terpenes; thus, it could be hypothesized that spices were not added to the meat during preparation. Finally, several acids were identified: butanoic acid is a carboxylic acid coming
Results

from hydrolysis of glycerides and could be responsible of rancid perception in food (Stahnke et al., 1995): this molecule was detected only in samples MO1, CO3, and MIL, while the most abundant acid was acetic acid, which showed the highest percentage in sample MIL (10.79%); the same behavior was also recorded for 3-methylbutanoic acid. The findings of this study on volatile compounds highlighted that, although several volatile compounds were significantly different among samples, there was no trend related to the different type (Mora Romagnola vs. conventional) of meat used. Conversely, it seemed that samples available on the market showed remarkably differences in terms of aromatic profile: this result could be associated with the product preparation and use of different spices. In this context, market leader products were characterized by a reduced number of volatiles: no terpenes were detected in samples FEL; in addition, ethanol was not detected in CON, FEL, or MIL. On the other hand, substantial variability was reported among the Mora Romagnola samples, which may be related to the different dimensions and weights, or to the curing period. This is not an unexpected result, since salami produced with this local breed are traditional products, whose recipes (specific ingredients) and handcraft preparation are characterized by a certain variability, linkable to the concept of local food (Amli et al., 2011; Chambers et al., 2007).
### Results

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<th>CO2</th>
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132
### Results

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**Table 4.** Volatile compounds (relative area and percentages%) detected in salami samples. Different letters indicate statistically significant differences between the mean values (LSD Fisher, p<0.05).
Results

3.4.4d Combining volatile compounds and sensory evaluation data

A multifactorial analysis (MFA) was performed with part of sensory and chemical (volatile compounds) data: in particular, 18 of the 33 molecules and 5 of the 11 sensory attributes were selected according to their discrimination power; the MFA biplot is shown in Figure 2. The first two components were responsible for 60.67% of total variance (35.24% for the first component (F1) and 25.43% for the second one (F2)).

Figure 2. Projection of salami samples on plan by multifactorial analysis (MFA biplot). Projection obtained by selecting some of the sensory attributes (blue labels) and volatile compounds (gray labels) data as variables, according to their contribution to the two main components. Mora Romagnola salami samples are labelled in pink, conventional pig breed salami samples are reported in green.
Results

The outcomes highlighted that samples MO4, MO5, CO4 and CO5 were similar and close to the attributes *seasoning*, *rancid*, and *garlic* and to volatile compounds related to oxidation (phenylacetaldehyde) and garlic (allyl methyl sulfide). Moreover, the attributes *seasoning* and *rancid* were positively correlated ($r = 0.75$); thus, it could be hypothesized that an increased intensity of *seasoning* attribute could be linked to a stronger perception of *rancid* descriptor. Samples MO1, MO2, CO1, and CO2 were characterized by the presence of many terpenes and by the sensory attribute of *pepper*. This latter descriptor was positively correlated with the presence of volatile compounds such as caryophyllene, $\beta$-phellandrene, and $\alpha$-pinene. This finding is in agreement with previous studies (Plessi et al., 2002; Meynier et al., 1999), where the aforementioned terpenes were reposted to be responsible for the perception of spicy notes, in particular with pepper. Samples MO3 and CO3 were characterized by the presence of acetic acid and the sensory perception of *fermented*, which were positively correlated ($r = 0.69$). This result is in accordance with Stankhe *et al.* (1995), since the acid perception in salami was associated with sensory notes of fermented or pungent.

3.4.4e Textural properties

The findings for textural properties assessed by Warner-Bratzler shear blade, TPA, and tensile test are reported in Table 5. If the entire set of samples is considered, as expected it can be clearly observed that, exhibiting the lowest values for all the parameters, MIL displayed remarkably different textural properties than the other samples. On the other hand, both FEL and CON exhibited a texture profile comparable to those of the 10 experimental groups. Within this context, since the conventional samples were selected according to the similarity with their Mora Romagnola counterpart, the results for textural properties will be discussed considering the samples as pairs. No significant differences were found in the texture profile of CO1 and MO1, which exhibited analogous values for almost all the parameters considered herein. Similarly, only negligible dissimilarities (not displaying any clear trends) were observed by comparing the textural properties of CO3 vs. MO3 and CO4 vs. MO4 samples. On the other hand, the main differences in the textural properties of conventional vs. Mora Romagnola samples were observed by comparing CO2 vs. MO2 and CO5 vs. MO5.
## Results

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<th>CO2</th>
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Table 5. Textural properties of salami samples. Different letters indicate statistically significant differences between the mean values (LSD Fisher, p<0.05).

sem = standard error of mean; *** = P<0.001
Results

In detail, if compared to its conventional counterpart (CO2), significantly higher hardness and hardness-related parameters (chewiness and gumminess) were found in MO2 (20.5 vs. 36.5 kg, P<0.001; 42.4 vs. 94.1 kg, P<0.001; 75.6 vs. 235.6 kg, P<0.001), which also exhibited increased cohesiveness (2.1 vs. 2.6, P<0.001), springiness (1.8 vs. 2.4, P<0.001), and rupture force (0.4 vs. 0.6 kg, P<0.001) values. Similarly, remarkably higher springiness and chewiness values were observed by comparing MO5 with its conventional counterpart (2.6 vs. 3.0, P<0.001 and 218.8 vs. 305.4 kg, P<0.001). In addition, MO5 exhibited the highest breaking strength. The findings of the present research, in agreement with previous studies performed by Dellaglio et al. (1996) and Saccani et al. (2013), shows that products exhibiting different textural properties can be found in the market. Among variables, the ripening period remarkably influences the textural properties of dry-fermented sausages (Saccani et al., 2013). Within this context, the highest values exhibited by the CO5 and MO5 samples for all parameters considered herein might be likely explained by a longer curing time. Indeed, according to previous studies, hardness, chewiness, and gumminess were found to increase over the ripening period as a consequence of protein coagulation at low pH and a decrease in the moisture content (Saccani et al., 2013; Lorenzo et al., 2012).

3.4.4f Combining data from texture analysis and sensory attributes

The results of textural properties and sensory evaluation were combined and analyzed by MFA (Figure 3). In particular, 5 sensory attributes and 5 textural parameters were selected according to their discrimination power. The first two components were responsible for 78.38% of total variance (59.00% for the first component (F1) and 19.38% for the second (F2)). The findings showed that MO5 was similar to its conventional counterpart (CO5) and both were close to the attributes of seasoning and meat grain and to the textural parameter of gumminess. This textural property, in its turn, was positively correlated to the attribute of seasoning (r = 0.80). This trend could be partly explained by considering the aforementioned increase in hardness and chewiness during curing (Saccani et al., 2013; Lorenzo et al., 2012). Thus, a longer curing period might account for the increased perception of the seasoning attribute by the panel. Accordingly, the Mora Romagnola MO2 sample was close to the vectors related to the textural parameters of WB shear force, hardness, and gumminess (Figure 3).
Results

Samples MO4 and CO4 were characterized by the attributes of *greasiness* and *humidity*, which were positively correlated ($r = 0.84$); consequently, as shown in Table 5, MO4 and CO4 exhibited the lowest hardness and WB-Shear Force values (MO4: 8.1 and 1.04 kg; CO4: 14.3 and 1.82 kg). Hence, the higher the intensities of *greasiness* and *humidity*, the lower the toughness (instrumentally assessed). Even pairs CO1/MO1 and CO2/MO2 appeared to be similar and were characterized by hardness and WB-Shear Force.

Samples CO3 and MO3 were characterized by high intensities of *fat distribution* and, consequently, by low values of cohesiveness and springiness. Indeed, a higher homogeneity of *fat distribution* perceived by the panel corresponded to the lower energy needed to irreversibly deform the sample structure.

![Figure 3](image)

*Figure 3.* Projection of salami samples in multifactorial analysis (MFA biplot). Projection obtained by selecting selected sensory attributes (blue labels) and textural parameters (orange labels) data as variables, according to their contribution to the two main components. Mora Romagnola salami samples are labelled in pink, conventional pig breed salami samples are reported in green.
3.4.5 Conclusions

In conclusion, on the basis of an integrated approach, specific sensory and instrumental parameters, useful to describe and discriminate Mora Romagnola salami, a typical Italian product, were detected. In particular, the sensory evaluation, conducted by a panel of trained judges, highlighted that the five Mora Romagnola salami here analysed were characterized by the attributes: seasoning, pepper, fermented, humidity, meat grain and fat distribution. Thirty-three volatile molecules, mainly belonging to the chemical classes of terpenes, aldehydes, alcohols, ketones, and acids, were separated and detected by SPME-GC-MS. In general, all the salami samples were characterized by the presence of aldehydes, as hexanal, mainly produced during autoxidation, and by many terpenes. In particular, higher amounts of terpenes as caryophyllene, β-phellandrene, and α-pinene, compounds due to the presence of pepper, were detected in Mora Romagnola samples that were also characterized by a higher intensity of the sensory attribute of pepper. Color differences among Mora Romagnola and conventional salami resulted to be unperceivable by human eyes; nevertheless, the electronic eye allowed to discriminate between samples produced with different pig breeds. Specifically, Mora Romagnola samples showed darker shades of red colour, if compared to their conventional counterpart. Salami produced with Mora Romagnola and their conventional counterpart were not discriminated by the instrumental texture analysis; on the other hand, the recipe, the dimension and the ripening are responsible for the large variability of textural properties among samples. This result puts on evidence that the traditional craftsmanship of this typical and local pig breed production determines a certain variability among Mora Romagnola salami, linkable to the concept of local food. The results of this rapid and integrated analytical approach can be useful to provide quality elements and attributes to be included in a single document devoted to describe and protect the peculiar characteristics of Mora Romagnola salami.

3.4.6 Acknowledgements

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3.4.7 References


Results


Results


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Results


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

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Authors: Barbieri S, Soglia F, Palagano R, Tesini F, Bendini A, Petracci M, Cavani C, Gallina Toschi T

a Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, Cesena (FC), Italy
b Interdepartmental Centre for Industrial Agrifood Research, Alma Mater Studiorum, University of Bologna, Cesena (FC), Italy

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3.5.1 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. In particular, high quality samples 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; standard cooked ham samples showed the lowest intensity of all visual attributes and the highest value of juiciness, whereas the intermediate category (selected cooked ham) was not discriminated from the other. Also physical-rheological parameters measured by electronic eye and texture analyzer were effective in classifying samples. In particular, the PLS model built with data
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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.

**Keywords:** Food science; cooked ham; sensory analysis; volatile compounds; combined analysis; flash profile.
3.5.2 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 et al., 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. Especially yield is associated with raw meat pH and raw material with extreme pH (i.e. pale-soft and exudative and dark-firm-dry meat) are avoided (Aaslyng, 2002). In addition, other involved factors 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).

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 allows to collect information about different physico-chemical properties (Iqbal et al., 2014; Iqbal et al., 2013; ElMasry et al., 2012). On the other hand, also the conventional image analysis represents an useful tool to the study of meat products’ appearance characteristics (Fongaro et al., 2015; Sánchez et al., 2008;), especially
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considering its cost effectiveness, consistency, speed and accuracy provided by its automated application (Brosnan et al., 2004).

Textural characteristics are also very important for the quality of cooked hams and depend on several factors that are related to biochemical constituents (water, fat, protein, connective tissue content etc.), raw meat pH, added non-meat ingredients, chemical reactions (entity of proteolysis and lipolysis prior to cooking) and processing variables such as the extent of heating (Toldrá et al., 2010; Aaslyng, 2002), 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 (Toldrá et al., 2010).

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 (Henrique et al., 2015; Tomović et al., 2013; Válková et al., 2007; Delahunty et al., 1997).

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 (Moretti et al., 2009; Casiraghi et al., 2007). However, the results from all these investigations are not always easily comparable because they take in account different raw materials and processing procedures (Tomović 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.
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3.5.3 Materials and methods

3.5.3a 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.

<table>
<thead>
<tr>
<th>Category</th>
<th>Raw materials</th>
<th>Ingredients/Additives</th>
<th>MDDP¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COOKED HAM</strong></td>
<td>Pork leg</td>
<td>Sodium chloride&lt;br&gt;Protein (milk and soy)&lt;br&gt;Starch (native or modified)&lt;br&gt;Phosphate&lt;br&gt;Sugar (dextrose, lactose, fructose, glucose syrup)&lt;br&gt;Ascorbic acid&lt;br&gt;Lactate&lt;br&gt;Glutamate&lt;br&gt;Nitrate and nitrite&lt;br&gt;Wine&lt;br&gt;Spices and aromas</td>
<td>≤81</td>
</tr>
<tr>
<td><strong>SELECTED</strong></td>
<td>Pork leg in which it is possible to identify at least 3 of the 4 major muscles</td>
<td>Sodium chloride&lt;br&gt;Protein (milk and soy)&lt;br&gt;Starch (native or modified)&lt;br&gt;Phosphate&lt;br&gt;Sugar (dextrose, lactose, fructose, glucose syrup)&lt;br&gt;Ascorbic acid&lt;br&gt;Lactate&lt;br&gt;Glutamate&lt;br&gt;Nitrate and nitrite&lt;br&gt;Wine&lt;br&gt;Spices and aromas</td>
<td>≤78.5</td>
</tr>
<tr>
<td><strong>HIGH QUALITY</strong></td>
<td>Pork leg in which it is possible to identify at least 3 of the 4 major muscles</td>
<td>Sodium chloride&lt;br&gt;Sugar (dextrose, lactose, fructose, glucose syrup)&lt;br&gt;Ascorbic acid&lt;br&gt;Lactate&lt;br&gt;Glutamate&lt;br&gt;Nitrate and nitrite&lt;br&gt;Wine&lt;br&gt;Spices and aromas</td>
<td>≤75.5</td>
</tr>
</tbody>
</table>

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. 1MDDP = moisture on defatted-deadditived product.
In particular, 15 samples (5 for each category) were selected from the Italian market in order to represent the variety of cooked pork hams available on the Italian market. The set of samples included: balanced number of samples belonging to the different commercial category (CH, SE and HQ); samples belonging to the most common Italian brand of cooked pork ham and presence of samples characterized by different intensities of sensory attribute (a larger set of samples was evaluated during the training as described in the paragraph 3.5.3b). A sensory description of each product was generated and sensory differences between products were described and quantified by a panel of highly trained assessors who have been preselected to have good sensory abilities and received general training as described in the following section. Moreover, textural and appearance properties (sensory profiling and instrumental) 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 physical analysis were carried out in several replicates, whereas for the sensory analysis, the final score was the average of the scores assigned by each judge to these samples in three different sessions.

3.5.3b Sensory analysis

Samples were tasted by a panel of 8 expert sensory assessors, balanced in terms of gender, varying in tasting experience, and previously trained in the assessment of cooked ham. All of them were regular consumers of cooked ham and interested in the study. Only assessors who demonstrated specific characteristics such as acuity, ability to communicate and/or to describe, knowledge of the involved product, interest, motivation and availability to attend both training and subsequent assessments, were selected. The recruitment, the preliminary screening and the training were done according to ISO 8586:2012 and ISO 13299:2010. The performance of selected assessors should be monitored regularly to ensure that the criteria by which they were initially selected continue to be met. During different sessions, the DA panel generated a list of appearance, aroma, taste and texture attributes using the consensus training (Heymann et al., 2014). 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. An agreement on the meaning of the attributes of the sensory lexicon, must be obtained. For this reason, it is important clearly define attribute name, written definition, method of assessment and standards reference able to help judges in the memorization of the different intensity levels for each of the selected descriptors. After attributes generation, the product assessment protocol must be determined in order to standardize the procedure and avoid bias. This step includes the way in which the product needs to be assessed and methods to reset the senses back to a neutral state between samples. Then, a wide range of samples of a product should be evaluated by rating the intensity of each attribute on a scale. This training improves the judges ability in using the sensory scale and promote the use of the ends of the scale. The performance check is generally carried out by applying statistical treatments to confirm that the panel works in a consistent and reliable way. The conventional profiling method was applied (Meilgaard et al., 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 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 (anchors) were selected taking into account the previously results of the training session and were used to illustrate the maximum, the
minimum or average intensity points on the scale 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.

3.5.3c 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 (resolution 2592 × 1944p) combined with powerful data processing software (see paragraph 3.4.3d).

3.5.3d Texture analysis
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 et al. (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
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mm stainless steel spherical probe according to the procedure described by Petracci et al. (2009).

### 3.5.3e 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 analyses were performed by XLSTAT 7.5.2 version software (Addinsoft).
3.5.4 Results and discussion

3.5.4a Sensory analysis

Before sensory 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.

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). The importance of product appearance was also confirmed by a recent study in which the effect of different factors (visual appearance, price and pack label) in purchasing decision, were investigated (Resconi et al., 2016). Figure 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
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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.

![Figure 1](image.png)

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).

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 et al. (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. On the contrary, the highest intensity of juiciness mainly defined the standard quality of cooked hams; this result could be linked with the effect of the addition of phosphates as ingredient of brine, in increasing the amount of retained water and therefore on texture traits (Resconi et al., 2016; Toldrá et al., 2010).
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3.5.4b Image analysis

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 et al., 2010). 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 Figure 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, Valous, Mendoza, Sun, Allen (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.
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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 (Figure 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
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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.

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).

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.

3.5.4c texture analysis

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
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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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Categories</th>
<th>sem</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
<td>CH: 5</td>
<td>SE: 5</td>
<td>HQ: 5</td>
</tr>
<tr>
<td>Gel strength (N x cm)</td>
<td>12.68</td>
<td>12.45</td>
<td>13.01</td>
</tr>
<tr>
<td>Expressible moisture (%)</td>
<td>18.6^a</td>
<td>16.5^ab</td>
<td>12.9^b</td>
</tr>
<tr>
<td>Shear force (N/g)</td>
<td>18.23^b</td>
<td>19.72^b</td>
<td>28.15^a</td>
</tr>
</tbody>
</table>

Table 3. 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.

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 (Pancrazio et al., 2015; Moretti et al., 2009; Válková et al., 2007; Casiraghi et al., 2007). 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 et al. (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 et al. (2007) did not find any differences in product cohesiveness when hams with increasing brine injection level were compared.
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The results of PCA analysis of instrumental texture parameters are shown in Figure 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 and cohesiveness were mainly involved in product category discrimination. Otherwise, hardness, gumminess, chewiness, and gel strength seem to independently vary within each market category.

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).

3.5.4d. 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 sensory attribute of pink intensity was correlated with physical parameters (electronic eye and texture analyzer) 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) that, in this study, were related with products belonging to the high quality category. A negative correlation was found, instead,
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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). In contrast, Resconi et al., 2015, reported a positive correlation between juiciness and springiness, both enhanced with the increase in polyphosphates while, in the present work, only juiciness characterized the product category with the higher phosphate content (CH). Among texture instrumental parameters, positive correlations were found between: gumminess and hardness (0.95) as already observed by Válková et al. (2007), springness and cohesiveness (0.76), chewiness and hardness (0.75) and also between chewiness and gumminess (0.89) (p < 0.05), these two latter correlations were also confirmed by Resconi et al., 2015; which found a reduction in hardness and gumminess as a function of the increase of the phosphate content. 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) confirming that these attributes were significant in the evaluation of the sensory profile of cooked ham obtained from different raw materials and technological process parameters applied. 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.

The best results were obtained from models developed using electronic eye data that allowed an effective prediction of pink intensity (R2 = 0.95, RMSECV = 3.24) and presence of fat (R2 = 0.88, RMSECV = 5.84) as showed by Figure 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).
Results

<table>
<thead>
<tr>
<th>Sensory attribute (y)</th>
<th>$R^2$</th>
<th>RMSECV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture analyzer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.24</td>
<td>9.87</td>
</tr>
<tr>
<td>Juiciness</td>
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<td>37.99</td>
</tr>
<tr>
<td>Electronic eye</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pink intensity</td>
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<td>3.24</td>
</tr>
<tr>
<td>Presence of fat</td>
<td>0.88</td>
<td>5.84</td>
</tr>
</tbody>
</table>

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.

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.

3.5.5 Conclusions

In this investigation, the application of physical-rheological and sensory techniques was 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.
Results

(“high quality”) from each other. 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. Work in progress includes a consumer study on cooked pork hams to investigate the correspondence between attributes generated by the panel and consumer lexicon used in quality-related communications.

3.5.6 Acknowledgements

This study could not have been realized without the precious help of COOP ITALIA and NEGRINI SALUMI as the main suppliers of the samples used herein. This work was supported by the Italian program FARB – Financing of Alma Mater Studiorum – University of Bologna for basic research. Line of Action 2 – Project Meating “sensory and fast instrumental analyses of meat and meat products: an integrated approach for quality control and communication.”.
3.5.7 References


Ministerial Decree, G.U. n 231, 04/10/2005 Regulation of the production and selling of specific meat products.

Results


3.6 Children preferences of coloured cheese prepared during an educational laboratory

Publication details:

Title: Children preferences of coloured cheese prepared during an educational laboratory

Authors: Tesini F¹, Laureati M², Palagano R¹, Mandrioli M¹, Pagliarini E², Gallina Toschi T¹,³

¹DISTAL, Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, P.zza Goidanich 60, 47521 Cesena, FC, Italy
²Department of Food, Environmental and Nutritional Sciences (DeFENS), University of Milan, Via Celoria 2, 20133 Milan, Italy
³CIRI, Interdepartmental Centre for Industrial Grifood Research, Alma Mater Studiorum, University of Bologna, P.zza Goidanich 60, 47521 Cesena, FC, Italy

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3.6.1 Abstract

Choices among young consumers are mainly driven by food preferences; in particular, a connection between appearance and acceptance of food has been highlighted, together with a general lack of knowledge of food processing. For these reasons, educational activities are important to increase scientific knowledge and awareness. The cheese-making educational laboratory described herein involved children, adolescents, and their parents/teachers in the preparation of fresh and naturally-coloured cheeses. At the end of the activity, both the colour preference and possible relation between preference and colour of cheese prepared were investigated administering a short questionnaire.

Keywords: cheese preference; children food preference; colour preference; gender; neophobia; nutrition education.
3.6.2 Introduction

Food preference has a fundamental role in driving consumer’s choices and habits, especially in children (Laureati et al., 2015a; Cooke, 2007; Nicklaus et al., 2004). Therefore, the number of food products specifically developed for children is growing and this market is acquiring increasing importance (Issanchou, 2015). Consequently, the youngest consumers are frequently involved in research and development programs, since their food habits will influence choices as they grow older and because their preferences, even if partly driven by advertisements (Ustjanauskas et al., 2014), seem to be strictly related to the sensory aspects of food (Mustonen and Tuorila, 2010; Pagliarini et al., 2005). In 1994, Moskowitz (Moskowitz, 1994) highlighted how a visually pleasing product tends to be more appreciated by children, as visual attributes seem to be, among different sensory characteristics, those that mainly determine its success (Topcu, 2015; Kildegaard et al., 2011). Indeed, children tend to create an “ideal picture” of each food product that can be related to their own idea of “good”; this picture represent a sort of reference point that can be used to dislike products whose appearance is not close to their expectation (Mustonen et al., 2009). This phenomenon, called food neophobia, is defined as the fear of eating new or unfamiliar food (Pliner et al., 1992) and is related to both the quality and variety of diet (Laureati et al., 2015b). Nutritional education and food-related diseases prevention activities can be used for children and teenagers in order to: i) reduce the impact of poor food habits on health, and ii) avoid food neophobia, mainly responsible for refusing consumption of fruits and vegetables (Wardle et al., 2003). Moreover, a lack of awareness of procedural knowledge, both in terms of food processing and nutritional values, must be taken into account (Worsley, 2002), highlighting the link between traditional food preparation and sensory properties (Laureati et al., 2006). Nowadays, food education is mainly based on the social-cognitive theory (SCT) (Bandura et al., 1977), which incorporates the interaction of personal, environmental and behavioural factors. According to this theory, principles that are highly influential in establishing changing food behaviour in children are both models and repeated exposure. Among the models, those that have been found to be effective in children include cartoon characters, peers, mothers, unfamiliar adults and teachers; moreover, children seem to be influenced more by the behaviour of multiple rather than single models (Lowe et al., 2004). Furthermore, according to Zajonc’s “mere exposure”
Results

theory (Zajonc, 1968), repeated exposure to a specific food increases the liking and consumption of that food (Cooke et al., 2011; Wardle et al., 2003b) through a mechanism that is believed to be a “learned safety” behaviour (Kalat et al., 1973). According to this theory, repeated ingestion of an unfamiliar food without negative consequences leads to increased acceptance of that food. Several educational interventions for food have investigated all these factors, reporting promising results, especially for consumption of fruits and vegetables (Laureati et al., 2014; Evans et al., 2012; Reverdy et al., 2008). Both principles of imitation and repeated exposure characterise food laboratories aimed at involving children in food preparation, thus familiarising them with food ingredients and technologies. Furthermore, it has been reported that the involvement of children in food preparation can contribute to increasing their acceptance for that food, according to the principle of repeated exposure (Chu et al., 2012). For all these reasons, food educational interventions can play a relevant role in driving choice, interest and preference of children through better awareness of both sensory characteristics and technological process of traditional food products (Mustonen et al., 2010). The educational cheese-making activity reported herein, called Cheese making in one hour, was conceived by a committee of referees as part of the Festival della Scienza 2014 (which took place in Genoa, Italy, from October 24 to November 2, 2014) and is considered as a “teaching tool” in order to increase scientific information about the cheese-making process. This practical laboratory took the form of a family entertainment during which children and parents or teachers could share principles of traditional cheese making, spending some family or educational time away from daily work, but related to increased knowledge of food science. The activity involved children, teenagers and adults (age from 6 to 87 years) in the traditional preparation of a fresh “primo sale like” cheese. Cheeses were naturally coloured (using turmeric, rocket and beetroot juice), and in order to keep the attention of participants, each was actively involved in the process by preparing his/her personal small cheese. At the end of the activity, both the colour preference and possible relation between the colour of cheese prepared and preference were investigated by administration of a short and simple questionnaire.
3.6.3 Materials and methods

The cheese-making activity involved 738 participants (395 females and 343 males) with an age between 5 and 87 years and divided in groups of no more than 30 individuals. Both families and school classes could participate in the activity by asking the Festival staff to make a reservation; given the popularity of the Festival, mainly represented by children and partly by parents/teachers, a large number of participants were enrolled. Each participant had an active role in the laboratory, giving his/her contribution to one of the four phases of cheese making: 1) adding starter, rennet and natural flavour to milk; 2) curd cutting and synaeresis; 3) curd breaking and purge; 4) cheese shaping. During the Festival, the laboratory was repeated at least 3 times a day by 4 previously-trained scientific facilitators. Due to the fact that the cheese-making laboratory should last only one hour (related to the timing of the Festival), one pot (Bartscher GmbH, Germany) was dedicated to each of the 4 main phases; in this way, participants could move from one pot to the next instead of proceeding step-by-step using a single pot such that each of the phases could be observed in a short length of time.

3.6.3a Cheese-making phases

Starter, rennet and milk natural flavouring. Fresh whole cow milk was heated to 36° C and 17 g/L of natural whole yoghurt was added as a starter. In order to produce coloured cheeses, different natural dyes were added (rocket, turmeric and beetroot juice) as reported in Table 1. While turmeric and beetroot juice were added to the milk 30 minutes after the addition of starter, together with 0.5 mL/l milk of liquid rennet (80% chymosin, 20% pepsin, provided by Graco), rocket was added during the last step of cheese shaping.
Table 1. Natural dyes and their amounts are reported together with the final colour of each cheese and its visual aspect.

Curd cutting and synaeresis. After enzymatic coagulation, due to the addition of liquid curd, the gel obtained was cut into $4 \times 4$ cm squares, and 30 minutes later participants could observe the phenomena of curd synaeresis.

Curd breaking and purge. The squares of curd were broken into pieces of approximately 1.5 cm; during this phase, rocket was added (20 g/l milk) for preparation of green cheese.

Cheese shaping. Finally, the small pieces of curd were collected in dedicated shaper (60 g cheese shaper in polypropylene and polyethylene, Tecnolatte srl, Italy) by each participant as shown in Figure 1.

Questionnaire. At the end of the cheese-making laboratory, each participant was administered a dedicated and simplified questionnaire. The questionnaire contained a first part regarding personal information: age and gender. Next, each participant was asked to provide information on: i) which colour of cheese he/she preferred and ii) which one he/she prepared. The filled questionnaires were collected in three dedicated paper boxes, one for each cheese colour.
Results

Figure 1. Children at the cheesemaking educational laboratory, during collection of a yellow-curd into a dedicated shaper.
Results

3.6.3b Statistical analysis

Preference data were analysed according to chi square test (p>0.05). Statistical analysis of data was carried out using the SAS/STAT statistical software package version 9.3.1. (SAS Institute Inc., Cary, USA).

3.6.4 Results and discussion

The cheese-making laboratory was visited by 1900 participants. Many questionnaires were incomplete, while some participants preferred to not give their opinion. Data from 738 participants was collected considering the gender dimension and 5 age subgroups (1: 5-7 years old, 77 participants; 2: 8-10 years old, 142 participants; 3: 11-13 years old, 182 participants; 4: 14-18 years old, 203 participants; 5: >18 years old, 134 participants); subsequently, the visual preference for one of the three coloured cheeses was considered, as reported in Table 2. Data were divided in the 5 subgroups reported above, selected mainly according to different stages of cognitive development (Guinard, 2001).

<table>
<thead>
<tr>
<th>Age subgroup (years)</th>
<th>Preferred cheese</th>
<th>Total</th>
<th>Chi square</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>Yellow</td>
<td>27</td>
<td>77</td>
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<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Pink</td>
<td>35</td>
<td>142</td>
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<td>0.00</td>
</tr>
<tr>
<td></td>
<td>White and green</td>
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<td>79</td>
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<td>0.00</td>
</tr>
<tr>
<td>8-10</td>
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<td>89</td>
<td>142</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Pink</td>
<td>28</td>
<td>173</td>
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</tr>
<tr>
<td></td>
<td>White and green</td>
<td>45</td>
<td>79</td>
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<td>0.00</td>
</tr>
<tr>
<td>11-13</td>
<td>Yellow</td>
<td>59</td>
<td>182</td>
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<td>182</td>
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<td>0.00</td>
</tr>
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<tr>
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<td>Pink</td>
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<td>27.4</td>
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</tr>
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<td>65</td>
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</tr>
<tr>
<td>&gt;18</td>
<td>Yellow</td>
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</tr>
<tr>
<td></td>
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<tr>
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<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td>All</td>
<td>Yellow</td>
<td>243</td>
<td>738</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Pink</td>
<td>162</td>
<td>59.5</td>
<td>2</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>White and green</td>
<td>333</td>
<td>738</td>
<td>2</td>
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</tbody>
</table>

Table 2. Non-parametric statistical analysis of data to identify significant differences in preference of cheese colour by age. Data were analysed using a Chi square test. Interviewees were subgrouped by age: 5-7, 8-10, 11-13, 14-18, >18 years.

In addition, different food preferences and consumption behaviour observed in these age groups confirmed that this type of age subdivision is relevant (Laureati et al., 2015). Considering colour preference, regardless of age, the majority of participants preferred the white and green cheese (333 vs. 243 yellow and 162 pink; chi square=59.49; p<0.0001), which could be considered as the most “traditional” because it is present on the Italian market, rather than pink or yellow cheeses, and probably seen for the first time during the laboratory activity by the most of the respondents. As reported in Table 2, sorting by age, it
Results

could be seen that the youngest participants (between 5 and 7 years old) chose the pink cheese as their favourite, while next oldest age subgroup (8 to 10 years) preferred yellow. Subjects from 11 years old and older chose the white and green cheese as their favourite, as did the overall population. This result may be associated with both the attractive effect of colours on the children and to the tendency of the youngest, as observed by Moskowitz (Moskowitz, 1994), to prefer food products with a pleasing appearance. Starting from the age of 11, children started to prefer, in accordance with adults, the white cheese mixed with rocket. Moreover, the youngest children’s preference can be explained by food neophobia, which is particularly high for fruits and vegetables, reaching its highest level between 2 and 6 years (Laureati et al., 2015c; Pliner et al., 1997; Pelchat et al., 1995; Pliner, 1994). It then tends to decrease when children move towards adolescence (Addessi et al., 2005), finally becoming relatively stable in adulthood, probably because of an increased number of food experiences (Cooke et al., 2005). On the other hand, the preference of older respondents for a white cheese enriched with a vegetable (rocket), may be linked to a low acceptance of adults for an innovative food, while its dislike among the younger interviewees could be related to the visual presence of rocket inside cheese (Laureati et al., 2015; Russel et al., 2008). It has already been demonstrated that traditional food products have been always recognised by consumers as linked to a specific geographical origin and highest sensory quality (Guerrero et al., 2009); as a consequence, this cheese was probably considered much more similar to product already on the Italian market compared to the pink and yellow ones. Sorting by gender (Table 3), no differences in preferences were seen regarding the white and green cheese, except for the oldest group (>18 years). A clear gender-related difference was, however, found for the pink and the yellow cheeses: the former seemed to be much more appreciated by females (all age classes except 14–18 years, but only if p<0.10 is considered as marginally significant), while the latter was favoured among males (especially from 8-10, 14-18).
Results

<table>
<thead>
<tr>
<th>Preferred cheese</th>
<th>Gender</th>
<th>5-7</th>
<th>8-10</th>
<th>11-13</th>
<th>14-18</th>
<th>&gt;18</th>
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<tr>
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<td>57</td>
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<td>1</td>
<td>1</td>
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</tr>
<tr>
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</tr>
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<td>23</td>
<td>22</td>
<td>23</td>
<td>18</td>
<td>115</td>
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<td>12</td>
<td>21</td>
<td>3</td>
<td>47</td>
</tr>
<tr>
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<td>Total</td>
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<td>34</td>
<td>44</td>
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<td>162</td>
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<td>10.7</td>
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<tr>
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<td>0.5</td>
<td>0.6</td>
<td>0.4</td>
<td>0.0</td>
<td>0.5</td>
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</tbody>
</table>

Table 3. Non-parametric statistical analysis of data to identify significant differences in preference of cheese colour by gender and age. Data were analysed using a Chi square test. F= female; M=male; df=degrees of freedom. Interviewees were subgrouped by age: 5-7, 8-10, 11-13, 14-18, >18 years.

In order to determine if this could influence the visual preference of participants, they were also questioned about the colour of the cheese they prepared; however, a significant association between cheese preferred and colour preference for one of the three coloured cheeses was not seen, even if some significant differences were seen among the youngest consumers (Table 4). In particular, when sorting by age, the subgroup from 8 to 10 years who prepared the yellow cheese more frequently preferred this cheese over the others (26 of 52, chi square=7.5 p=0.02). Likewise, the subgroup from 5 to 7 years who prepared the pink cheese preferred it more frequently than others (12 of 21 choices, chi square=7.1 p=0.03). However, the older participants did not show a consistent trend of preferred cheese over than prepared. Data from literature suggest that 7–8 exposures are needed to produce a learning effect that can influence consumer appreciation regarding a product (Maier et al., 2007). Thus, it might well be that the single exposure during the cheese-making educational activity was not enough to influence children choice.
Results

<table>
<thead>
<tr>
<th>Prepared cheese</th>
<th>Age subgroup (years)</th>
<th>Preferred cheese</th>
<th>Total</th>
<th>Chi square</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
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<td>Yellow</td>
<td>5-7</td>
<td>Yellow</td>
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<td>37</td>
<td>2</td>
<td>0.31</td>
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<tr>
<td></td>
<td></td>
<td>Pink</td>
<td>14</td>
<td>52</td>
<td>2</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White and green</td>
<td>8</td>
<td>27</td>
<td>2</td>
<td>0.00</td>
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Table 4. Non-parametric statistical analysis of data to assess if the colour of sample prepared could affect the overall colour preference. Data were analysed using Chi square test. F= female; M=male; df=degrees of freedom. Interviewees were subgrouped by age: 5-7, 8-10, 11-13, 14-18, >18 years.

3.6.5 Conclusions

The results of this study highlight how visual preference for a food, in terms of colour, changes during different stages of life. Indeed, the data demonstrates how children can be influenced by food appearance and how the aspect of a product can be related to its acceptance, especially among younger individuals; in fact, the youngest participants tended to prefer intensively coloured cheese vs. the white and green version, probably because of the presence of rocket. Moreover, the cheese-making laboratory was considered to be a useful tool in order to catch and keep participants’ attention, involving them in a practical activity while sharing educational and scientific information related both to food processing (fresh cheese production) and sensory (visual) aspects.

3.6.6 Acknowledgements

The activity described in this work was made possible by the kind permission of Festival della Scienza. A special thank goes to Emanuele Bargelli, events Creator & Coordinator for Festival della Scienza.
3.6.7 References


Results


Results


3.7 Exploring influences on food choice in a large population sample: The Italian Taste project

Publication details:

Title: Exploring influences on food choice in a large population sample: The Italian Taste project

Authors: Monteleone E\textsuperscript{a}, Spinelli S\textsuperscript{b}, Dinnella C\textsuperscript{a}, Endrizzi I\textsuperscript{b}, Laureati M\textsuperscript{c}, Pagliarini E\textsuperscript{c}, Sinesio F\textsuperscript{d}, Gasperi F\textsuperscript{e}, Torri L\textsuperscript{e}, Aprea E\textsuperscript{b}, Baleetti L I\textsuperscript{f}, Bendini A\textsuperscript{g}, Braghieri A\textsuperscript{b}, Cattaneo C\textsuperscript{c}, Clicer D\textsuperscript{d}, Condelli N\textsuperscript{h}, Cravero MC\textsuperscript{i}, Del Caro A\textsuperscript{j}, Di Monaco R\textsuperscript{k}, Drago S\textsuperscript{l}, Favotto S\textsuperscript{m}, Fusi R\textsuperscript{a}, Galassi L\textsuperscript{n}, Gallina Toschi T\textsuperscript{o}, Garavaldi A\textsuperscript{p}, Gasparini P\textsuperscript{q}, Gatti E\textsuperscript{a}, Masi C\textsuperscript{a}, Mazzaglia A\textsuperscript{a}, Moneta E\textsuperscript{d}, Piasentier E\textsuperscript{m}, Piochi M\textsuperscript{a,e}, Pirastu N\textsuperscript{f}, Predieri S\textsuperscript{q}, Robino A\textsuperscript{l}, Russo F\textsuperscript{u}, Tesini F\textsuperscript{s}

\textsuperscript{a} University of Florence, Italy
\textsuperscript{b} Edmund Mach Foundation, San Michele all’Adige, Italy
\textsuperscript{c} University of Milan, Italy
\textsuperscript{d} CREA – Research Centre on Food and Nutrition, Rome, Italy
\textsuperscript{e} University of Gastronomic Science, Bra, Italy
\textsuperscript{f} Italian Center of Sensory Analysis & Innovation, Matelica, Italy
\textsuperscript{g} University of Bologna, Italy
\textsuperscript{h} University of Basilicata, Potenza, Italy
\textsuperscript{i} CREA – Research Center for Enology, Asti, Italy
\textsuperscript{j} University of Sassari, Italy
\textsuperscript{k} University of Naples, Italy
\textsuperscript{l} Mérieux NutriSciences, Prato, Italy
\textsuperscript{m} University of Udine, Italy
\textsuperscript{n} ERSAF – Regional Agency for Services to Agriculture and Forestry, Italy
\textsuperscript{o} C.R.P.A. S.p.A., Reggio Emilia, Italy
\textsuperscript{p} Medical Genetics, UNITS0 – IRCCS Burlo Garofalo, Trieste, Italy
\textsuperscript{q} IBIMET-CNR, Bologna, Italy
\textsuperscript{r} Centre for Global Health Research, Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, United Kingdom
\textsuperscript{s} University of Catania, Italy
\textsuperscript{t} Institute for Maternal and Child Health – IRCCS “Burlo Garofolo”, Trieste, Italy
\textsuperscript{u} Adacta S.p.A., Naples, Italy

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3.7.1 Abstract

Food choice is influenced by many interacting factors in humans. Its multidimensional and complex nature is well recognized, particularly within the sensory and consumer food science field. However, the vast majority of the studies aimed at understanding determinants of food choices, preferences, and eating behaviours are affected by important limitations: the limited number of factors that are considered at once and the sample size. Furthermore, sensory and hedonic responses to actual food stimuli are often not included in such studies. The Italian Taste project is a large-scale study (three thousand respondents in three years) launched by the Italian Sensory Science Society aimed at addressing these limitations by exploring the associations among a variety of measures – biological, genetic, physiological, psychological and personality-related, socio-cultural – describing the dimensions of food liking, preference, behaviour and choice, and their relevance in determining individual differences within a given food culture framework. In addition, the study includes also the collection of sensory and hedonic responses to actual food stimuli commonly consumed in Italy and prepared to elicit a variation in the strength (from weak to strong) of bitterness, sweetness, saltiness, sourness, pungency, umami and astringency. The aims of the present paper are twofold. Firstly, the paper is aimed to illustrate the variables selected to explore the different dimensions of food choice and to report the experimental procedure adopted for data collection. Secondly, the paper is aimed at showing the potential of the Italian Taste dataset on the basis of the data collected in the first year of the project. For the purpose, we selected a small number of variables known to influence food choices from data collected in the first year of the project on 1225 individuals.

**Keywords:** Food preference; sensory; PROP; neophobia; sensitivity to reward; sensitivity to punishment; food attitudes.
3.7.2 Introduction

Food choice is influenced by many interacting factors in humans. The selection of a given food depends on the interplay of its intrinsic and extrinsic characteristics with person-related dimensions that are biological, physiological, psychological, and socio-cultural (see Köster, 2009; Mela, 2006; Rozin, 2006; Sobal et al., 2006; Sobal et al., 2014). Food choice is also subject to changes over the lifetime. Its dynamic nature is evident, varying from person to person and from situation to situation (Köster et al., 2007; Sobal et al., 2014). Cultural traditions, social organizations and conditions, shared values and beliefs tend to determine common experiences, while still allowing for individual differences in food choice (see Köster, 2003). The simplest expression of food choice is relative intake, calculated per capita in a population (Rozin, 2006). In the absence of economic and availability constraints, the major role played by food preferences and liking in determining food choice and intake has been emphasised (Eertmans et al., 2001; Rozin, 1979, 1990; Tuorila, 2007). The development of food likes and dislikes reflects the operation of multiple influences, from our genetic inheritance, to maternal diet, child-raising practices, learning, cognition and culture, each of which is expressed through hedonic responses to sensory qualities (Prescott, 2012). Preferences are generally defined as choices among available and generally acceptable (i.e. edible) foods in the context in which eating is the issue at hand (Rozin, 2007). However, when faced by a choice, one may prefer one food rather than another for specific reasons such as health, convenience, price, and so on, but actually like better the food not chosen. Thus, preference and liking can be seen as necessary but not sufficient to explain food choice. The multidimensional and complex nature of food choice is well recognized, particularly within the sensory and consumer food science field. However, the majority of studies examine only a few variables related to specific aspects of one or two dimensions regulating choices, preferences or behaviours. Although these studies have the merit of clarifying specific effects and interactions on a response variable of interest, a lack of research aimed at identifying the associations among the numerous relevant variables in food choice is evident. To address this, more multidisciplinary and multidimensional approaches are needed (Köster, 2009). In recent years, such multidisciplinary approaches have been increasingly used. Many studies that investigate food behaviours are taking into account health, sociodemographic, psychological and lifestyle factors, thanks also to the
Results

support to multidisciplinary networks offered by the European Union (e.g. HabEat project: Caton et al., 2014). Törnwall et al. (2014) reported one of the few recent and most interesting examples of a multidisciplinary approach in exploring the inter-relationships between the different dimensions of food perception and preference. The study was aimed at obtaining a coherent picture of flavour preferences among young adults in relations to different factors, including genotype, gender, age, education, sensory and hedonic responses to varied flavours, taste sensitivity index (PROP), food neophobia, attitudes and food and smoke habits. Food neophobia, pleasantness of pungency, liking of fruits and vegetables and genetic variability were found to be the main factors discriminating two subgroups in a young twin population differing in their liking of sour and pungent foods. However, studies such as this are in the minority. In addition, many studies tend to generalize findings from small samples to whole populations (Meiselman, 2013). Moreover, academic research is often conducted on convenience samples, e.g., students, that do not necessarily represent larger populations (Golder et al., 2011). The uncertainty about relationships between the responsiveness to PROP and the density of fungiform papillae is an example of this limitation. The association between fungiform papillae density and responsiveness to PROP bitterness found in small size studies (Essick et al., 2003; Yackinous et al., 2002) has not been confirmed in the more recent, larger studies (Fischer et al., 2013; Garneau et al., 2014). Understanding the associations among factors involved in food choices requires large-scale studies aimed at making statements about population as a whole, as well as about significant subgroups within the population. A successful model of such an approach can be found in research on the causes of diseases that has benefited from epidemiological studies of genuine population samples (Willett, 2012). In the same way, food choice and behaviour studies can gain predictive power by enlarging the sample size and collecting data on multiple variables in order to identify key explanatory factors and to estimate their actual weight in determining food behaviours. In line with studies indicating that food hedonics may be better predictors of health outcomes than food intake (Duffy et al., 2009), recent epidemiological studies have included food liking and preference in addition to dietary intake, physical activity, anthropometry, lifestyle, socioeconomic conditions and health status (NutriNet Santé: Hercberg et al., 2010; Lampuré et al., 2014, 2015; Méjean et al., 2014). In addition, large-scale studies (e.g. with three or four thousand respondents) aimed
Results

at studying the associations among several factors such as genetics, demographics, taste sensitivity, lifestyles, anthropometrical measures and stated liking for several food categories have been recently published (Pirastu et al., 2012, 2016). Although these studies show the potential of explorative large scale studies on some determinants of food choice and behaviour, they do not include the data collection relative to important dimensions, such as sensory and hedonic responses to actual food stimuli, or psychographics, in particular food-related attitudes. To our knowledge, there are no examples in the literature of genetic studies aimed at understanding food choice and preferences that include hedonic and intensity responses to tastants and odorants presented at different concentration in food product and not in solution, with the exception of Törnwall et al. (2014) on tastes and Jaeger et al. (2013) on odours. Investigating how sensory perception and hedonics vary in relation to an increase of the concentration of a tastant could give us important information for a better understanding of liking. Food-related motivations and attitudes have been associated with different patterns in food preferences and diet. Hence, general health interest was associated with a lower intake of fat, a lower consumption of high-fat savoury snacks and high-fat oils and fats, and an increased consumption of vegetables and fruit (Zandstra et al., 2001). Restrained, emotional and external eating behaviours have been linked to food choice. Thus, consumption of sugar-sweetened soft drinks was associated with less restrained and more external eating in adults (Elfhag et al., 2007), and Oliver, Wardle, and Gibson (2000) reported that emotional eaters consume more sweet, high-fat foods in response to emotional stress than did non-emotional eaters. However, the relationship of these eating behaviours with liking and sensitivity is much less explored. In addition, the investigation of food-related lifestyles, including information about attitudes and behaviour relating to purchase, preparation and consumption of food products, has been revealed to be useful in identifying consumer segments and in better understanding the attitudes behind food choice (Brunsø et al., 2007).

In addition to genetic, biological, physiological and sociocultural variables, it has been proposed that personality may play a large role in determining food preferences and food behaviours. This was shown not only for food-related personality traits such as neophobia (Eertmans et al., 2005; Knaapila et al., 2011), but also in the case of more general personality traits not explicitly related to food, such as sensitivity to reward (SR) and to
Results

punishment (SP). The investigation of the relationships between SR, SP and food preferences and choices is new and still limited but recent studies presented interesting findings. SR was found to be positively associated with the frequency of chilli consumption and weakly, though significantly, correlated with the liking of spicy foods (Byrnes et al., 2013, 2015). Recent studies have also highlighted an association between sensitivity to reward and unhealthier behaviours (higher fat intake, higher alcohol consumption, smoking frequency) (Morris et al., 2016; Tapper et al., 2015). Other relevant associations include those between taste perceptions and preferences and personality dimensions such as private body consciousness, the awareness of bodily sensations (Stevens, 1990; Stevens et al., 1998) but the results are controversial (Byrnes et al., 2013; Jaeger et al., 1998). Sensitivity to visceral disgust (Herz, 2011, 2014) and alexithymia (inability of individuals to identify and name their emotional states) (Robino et al., 2016) have both been linked to variations in PROP bitter taste responsiveness, with high alexithymia linked in addition to liking of alcohol, sweets and fats/meats, and lower alexithymia related to liking of vegetables, condiments and strong cheeses. The objectives of the present paper are twofold. Firstly, the paper aims to describe the Italian Taste project, to illustrate the variables selected to explore the different dimensions of food choice and to report the experimental procedure adopted for data collection. The Italian Taste project is a large-scale study (three thousand respondents in three years) aimed at exploring the associations among a variety of measures – biological, genetic, physiological, psychological and personality-related, sociocultural – describing the dimensions of food liking, preference, behaviour and choice, and their relevance in determining individual differences within a given food culture framework. It includes also the collection of sensory and hedonic responses to actual food stimuli commonly consumed in Italy and prepared to elicit a variation in the strength of bitterness, sweetness, saltiness, sourness, pungency, umami and astringency from weak to strong. Secondly, the paper aims to show the potential of the Italian Taste dataset to explain food choice. For these objectives, we selected a small number of variables known to influence food choices from data collected on 1225 individuals in the first year of the project.
3.7.3 The Italian Taste Project

3.7.3.1 Objectives
The aims of Italian Taste (IT) are twofold. Firstly, at a strategic level the targets are:

- to show that large scale and multidisciplinary studies are the necessary condition to increase the understanding of food choice mechanisms.
- to show that large and complex studies can be managed considering several aspects which are economic, cultural and social as we describe here:
  - Economic: IT is a cost-sharing project among several partners in which the contribution of each partner reflects their available human and financial resources.
  - Cultural: IT is a multidisciplinary study with a knowledge-sharing approach in which researchers with different scientific backgrounds not only give their own contribution, but learn more about the complex and multidisciplinary factors affecting food preference and choice.
  - Social: IT is close to the type of epidemiological studies that have been so successful in determining causes of disease and health-related states. The IT dataset has the potential of generating valuable information for human health and wellbeing.

Secondly, the target of the project is to contribute to the uncovering of associations among variables along multiple dimensions that are presumed to be important in determining individual differences in food preference and choice.

3.7.3b Organization and management of the study
The Italian Taste project was initiated in 2014 by the Italian Sensory Science Society (SISS). It involves, on a voluntary base, 58 SISS members working in 19 sensory laboratories of public and private organizations, across the country (see Appendix 1). The study is conducted in agreement with the Italian ethical requirements on research activities and personal data protection (D.L. 30.6.03 n. 196). The study protocol was approved by the Ethics Committee of Trieste University where the genetic unit of the project is based. The
respondents gave their written informed consent at the beginning of the test according to the principles of the Declaration of Helsinki.

3.7.3c General project methods

Respondents: Recruitment and inclusion. The recruitment procedure aims to reach a balance between genders, three age classes (18–30; 31–45; 46–60 years) and main geographical areas of the country. Exclusion criteria are pregnancy and not being born in Italy or having lived at least 20 years in Italy. Participants are recruited on a national basis by means of announcements published on the Italian Taste project website (www.it-taste.it), the SISS website (www.scienzesensoriali.it) and social networks (Facebook), articles published on national newspapers, and in food and wine magazines. Furthermore, each research unit recruits subjects locally by means of social networks and emails, pamphlet distribution and word of mouth.

Overview of data collection. At the time of recruitment, respondents are given general information about the study aims. They are asked to complete an online questionnaire (OQ; Tables 1 and 2) at home in the days preceding the data collection and invited to attend two sessions, in two days, in a sensory lab. The data collection scheme is presented in Figure 1. On day 1, participants sign the informed consent and are introduced to the general organization of the day which includes a liking and an odour session, followed by the measurement of PROP responsiveness. Designated breaks (10–15 min) between tests are carefully observed. During these breaks, participants are seated together in a comfortable room where water and unsalted crackers are available. Participants are encouraged to comment on, and ask questions about, the procedures with the purpose of giving them the feeling of being part of an important research project, thus increasing their attention and motivation and avoiding fatigue and boredom. During the breaks, participants are given instructions on scaling methods and asked to fill in questionnaires. Before starting the hedonic evaluation of food samples participants are introduced to the use of the Labeled Affective Magnitude scale (LAM; Schutz & Cardello, 2001). They are seated in individual booths and introduced to the use of the PC for data collection. They are asked to rate their appetite and are presented with four series of products (pear juice, chocolate pudding, bean purée and tomato juice) for liking evaluations. Each series includes four samples with varied intensities of target sensations (Table 3). Food product series are presented in independent
sets, each consisting of four samples of the same product. The presentation order of food series is fixed and is designed to avoid perceptive interferences across samples due to the long-lasting sensations of chocolate pudding and tomato juice spiked with capsaicin. Pear juice is presented as first set followed, after a 10 min break, by chocolate pudding.

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<td>Monthly food spending (euro)</td>
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<tr>
<td></td>
<td>Practice of restricted diets (type and reasons)</td>
<td>Up to 20/€20 to 40/€40 to 90/€90 to 100/€100/More than 100</td>
</tr>
<tr>
<td></td>
<td>Practice of smoking habits</td>
<td>No:Yes, low calorie diet/Yes, for medical reasons (if yes, which one?)</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>1. Never tried/2. Not smoking (have tried or quit) Yes: No</td>
</tr>
<tr>
<td></td>
<td>No:Yes, for some colours/Yes, in general</td>
<td>- E:3:</td>
</tr>
<tr>
<td></td>
<td>Food intake</td>
<td>- T:1:</td>
</tr>
<tr>
<td></td>
<td>Tendances to binge/monetary behaviours</td>
<td>Behaviours in the last months such as miss meals, use of medicines, want to control weight: No:Yes</td>
</tr>
<tr>
<td></td>
<td>Use of medicines</td>
<td>Regular use for: Blood pressure/Cholesterol/Arthritis/Neurological diseases/</td>
</tr>
<tr>
<td></td>
<td>Heart and chronic diseases</td>
<td>No:Yes</td>
</tr>
<tr>
<td></td>
<td>Ear infections/otitis</td>
<td>Never/1 time/2 times/3 times/From 3 to 5 times/6 or more times</td>
</tr>
<tr>
<td></td>
<td>Problems in taste perception</td>
<td>Yes/No</td>
</tr>
<tr>
<td></td>
<td>Problems in odour perception (except cold)</td>
<td>Yes/No (if yes, details asked)</td>
</tr>
<tr>
<td></td>
<td>Self-rated smell</td>
<td>Above the normal/below the normal/Normal</td>
</tr>
<tr>
<td></td>
<td>Heart and chronic diseases in relatives (first degree)</td>
<td>Type 1 diabetes/Type 2 diabetes/Obesity (if yes, which one?)</td>
</tr>
<tr>
<td></td>
<td>Childbirth (natural/cesarean section)</td>
<td>Yes/No/Do not know</td>
</tr>
<tr>
<td></td>
<td>Breast feeding</td>
<td>Yes/No (if yes, specify if for medical reasons: age,</td>
</tr>
<tr>
<td></td>
<td>Pregnancy: Age of first menstr.; Memopausal status</td>
<td>Yes/No (if yes, specify if for medical reasons: age,</td>
</tr>
<tr>
<td></td>
<td>Self-rated health (5R)</td>
<td>Very good/Good/Fair/Bad/Very bad (Shrier et al, 2008)</td>
</tr>
</tbody>
</table>

Table 1. Socio-demographic and socio-economic, anthropometric and physical health variables: questionnaires and their relative acronym and code. The options were presented as check the one/s that apply, if not differently specified. The symbol “-” Indicates that the options include every unit in the range indicated.

1 OQ = online questionnaire.

* Indicates that the question was open-ended.
Results

Table 2. Food preferences, choice, familiarity and frequency of consumption measurements: Questionnaire, their relative code, items and categories and rating scale.

* OQ = online questionnaire.

Figure 1. Overview of data collection
Results

Table 3. Hedonic and sensory responses to food products, solutions and odours: aims, samples and rating scales.

<table>
<thead>
<tr>
<th>Stimuli</th>
<th>Response</th>
<th>Aim</th>
<th>Samples</th>
<th>Rating scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food products</td>
<td>Liking</td>
<td>To measure variations in liking for real food products due to the variation of the intensity of specific basic tastes or other oral sensations (astringency and pungency)</td>
<td>4 series of 4 samples (spiked with a relevant tastant): - pear juice (citric acid 0.5%; 2.0; 4.0; 8.0 g/kg) - chocolate pudding (sucrose 38; 83; 119; 233 g/kg) - bean puree (sodium chloride 2.0; 6.1; 10.7; 18.8 g/kg) - tomato juice (capsaicin 0.3; 0.68; 1.01; 1.52 mg/kg)</td>
<td>Labeled Affective Magnitude Scale (0–100) (Schutz &amp; Cardello, 2001)</td>
</tr>
<tr>
<td>Sensory</td>
<td></td>
<td>To measure individual differences in responsiveness to overall flavour, specific basic tastes or other oral sensations (astringency and pungency) in real food products</td>
<td>4 series of 4 samples (spiked with a relevant tastant): - pear juice (citric acid 0.5; 2.0; 4.0; 8.0 g/kg); target sensations: sourness and sweetness - chocolate pudding (sucrose 38; 83; 119; 233 g/kg); target sensations: bitterness, sweetness and astringency - bean puree (sodium chloride 2.0; 6.1; 10.7; 18.8 g/kg); target sensations: saltiness and umami - tomato juice (capsaicin 0.3; 0.68; 1.01; 1.52 mg/kg); target sensations: pungency</td>
<td>Generalized Labeled Magnitude Scale (0–100), gLMS (Bartoshuk et al., 2004)</td>
</tr>
<tr>
<td>Water solutions</td>
<td>Sensory</td>
<td>To measure individual differences in responsiveness to basic tastes, astringency and pungency in water solutions</td>
<td>7 samples: - citric acid 4 g/l (sourness) - caffeine 3 g/kg (bitterness) - sucrose 200 g/kg (sweetness) - sodium chloride 15 g/kg (saltiness) - monosodium glutamate 40 g/kg (umami) - K aluminum sulfate 0.8 g/kg (astringency) - capsaicin 1.5 mg/kg (pungency)</td>
<td>Generalized Labeled Magnitude Scale (0–100), gLMS (Bartoshuk et al., 2004)</td>
</tr>
<tr>
<td>Odours</td>
<td>Liking</td>
<td>To measure individual differences in liking for odours</td>
<td>4 samples: - mint - anise - pine - banana</td>
<td>9-point hedonic scale (1 = extremely disliked; 9 = extremely liked); (Peyton &amp; Pilgrim, 1957)</td>
</tr>
<tr>
<td>Sensory</td>
<td></td>
<td>To measure individual differences in odour responsiveness</td>
<td>4 samples: - mint - anise - pine - banana</td>
<td>Identification: multiple choice Intensity: 9-point scale (extremely week/very strong) Irritation: 9-point scale (not at all irritant/extremely irritant)</td>
</tr>
</tbody>
</table>

Subjects have a 15 min break and are then presented with the bean cream set followed, after 10 min break, by tomato juice. The presentation order of food samples within each set is randomized across subjects.

After the liking session, participants are presented with the Food Liking Questionnaire (Q1; Table 2). Then, participants are instructed about the odour test (Table 3) and receive general information about Food Related Life Style (Q2), Food Neophobia Scale (Q3) and Private Body Consciousness (Q4) questionnaires (Tables 4 and 5). They complete Q2 and the odour test, followed by a break during which they complete Q3 and Q4. Participants are then trained to the use of gLMS (0: no sensation-100: the strongest imaginable sensation of any kind) following published standard procedure (Bartoshuk, 2000; Green et al., 1993; Green et al., 1996). Subjects are instructed to treat the “strongest imaginable sensation” as the most intense sensation they can imagine that involves remembered/imagined sensations in any sensory modality. They are informed about Sensitivity to Punishment and Reward (Q5) and Alexithymia (Q6) questionnaires (Table 5). Then they rate the intensity of PROP solutions and fill in Q5 and Q6. At the end of day 1, respondents are instructed on fasting conditions preceding the collection of a saliva
Results

Table 4. Eating behaviours, food-related lifestyles and attitude measurements: questionnaires and their relative acronym, code, items and domains, rating scale and references.

Day 2 starts with a general introduction to tests, instructions on saliva collection and introduction to the Choice Questionnaire (Q7). Then, participants are seated in individual booths where they rate their appetite and, before completing the saliva collection procedure, complete questionnaire Q7. After that, the gLMS is briefly introduced again and the Health and Taste (Q8) and the Dutch Eating Behaviour (Q9) questionnaires are illustrated. Then, the first part of intensity data collection starts. Participants are first asked to rate the intensity of basic tastes, astringency and burn in a series of seven samples (Table 3). The presentation order of stimuli is randomised for the basic tastes and astringency while the burning solution is always evaluated as the last sample to avoid perceptive interferences across samples due to the long lasting sensation of capsaicin. They have a break and are asked to fill in Q8. Finally, taste and oral sensation intensities are collected from four series of the same food products presented in day 1. During breaks between sample series, participants are asked to fill in Qs 9, 10 and 11. The picture of the tongue for papillae counting is taken at the end of day 1 or 2, according to individual availability. Session 2 lasts around 120 min. At the end of the session participants receive a certificate of attendance to the project and are compensated for their time with a gift. From 1 to 7 days were left between the two sessions, according to subject availability.
Results

Table 5. Psychological and personality trait measurements: questionnaires with their relative acronym, code, items and domains, rating scale, and references.

Questionnaires. Using questionnaires, information is collected concerning socio-demographic and socio-economic, anthropometric and physical health (Table 1); food preferences, choice, familiarity and frequency of consumption (Table 2); eating behaviours, food-related lifestyles and attitudes (Table 4); psychological and personality traits (Table 5). For those questionnaires not originally developed in Italian, or when an Italian validated version was not available, the questionnaires were translated to Italian by two different bilingual Italian native-speakers and then back translated into the source language. Back translations were reviewed by an expert in semantics and adjustments were made when necessary to select the most appropriate translation.

Socio-demographic, socio-economic, anthropometric and physical health questionnaires. Information is collected on socio-demographic and socio-economic indices, and anthropometric and physical health measures through an online questionnaire (Table 1). The questionnaire includes multiple choice questions (select one or select multiple) and open-ended questions to collect further details. Information is collected concerning marital status, number of children, number of family members, current job, education level, tobacco smoking habit, past medical history, current use of medication, dietary supplements, familial medical history, causes of death of first-degree relatives (when appropriate) and, for women, obstetric history, pregnancies, and menopausal status. Self-rated health is measured
Results

using the European World Health Organization (WHO) version (Jürges et al., 2008). Anthropometric includes questions on height and weight and dietary restraint. To determine eating habits, respondents are asked to indicate which diet they follow out of list of nine eating diets descriptions adapted from De Backer and Hudders (2015). Information about physical and sedentary activity is collected using the Italian version of the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003; Mannocci et al., 2012). Physical activity is described according to 3 levels of exercise intensity (walking, moderate or vigorous), frequency of exercising (days/week) and daily duration of each performed activity.

Food Familiarity, liking and choice questionnaires. Information about frequency of consumption is collected for alcoholic beverages (beer; wine; spirits; aperitif/cocktail), coffee, sugar addition in coffee and chilli pepper and spicy food (Table 2). The Food Familiarity and Food Liking questionnaires were developed to measure, respectively, familiarity with, and liking for, a selection of 184 foods appropriate in different eating situations (Table 2). Eating situations were identified considering either the traditional Italian meal pattern (breakfast, lunch and dinner), as well as new habits, such as snack/light meals and aperitif, that tend to substitute lunch or dinner, thus breaking the traditional meal timing. The item selection reflected variations in familiarity (more/less familiar foods), flavour (strong/mild) and energy content (high-energy/low-energy dense) as well. Items are grouped in product categories based on their chemical composition. The presentation order of the items within each product category as well as the product category order are randomized across participants. The Food Choice Questionnaire was developed in order to evaluate preferences within a pair of items. For each pair, respondents are asked to indicate which food they would choose in that specific eating situation. In this questionnaire, food items (selected among the 184 items of the Food Familiarity and Liking questionnaires) were grouped in 79 pairs and distributed in specific eating situations as follows: breakfast (13 pairs), snack/light-meal (13 pairs), main meal (either lunch or dinner, 43 pairs) and aperitif (10 pairs). Items in each pair represent variations in terms of familiarity, taste (e.g. bitter vs sweet) and energy content (e.g. low-fat vs full-fat). In some cases, pairs consist of different foods or food categories (e.g. fruit vs cake) both suitable for a specific eating situation (e.g. breakfast). The presentation order of the food items within each pair, and of
the pairs within each eating context, is randomized across participants, while the presentation order of the eating situations is the same for all participants (breakfast, snack/light-meal, main meal, aperitif).

**Eating behaviours, food-related lifestyles and attitudes.** Questionnaires are completed during the course of the testing days to assess eating behaviours and attitudes towards foods. Food-related lifestyles are determined using the Food Related Lifestyle (FRL) questionnaire, while consumers’ orientations towards health and hedonic characteristics of foods is determined through the Health and Taste Attitudes Scale (HTAS). The Dutch Eating Behaviour Questionnaire (DEBQ) is used to assess restrained, emotional and external eating behaviours (Table 4).

**Psychological and personality traits.** Questionnaires are completed during the course of the testing days to assess seven psychological or personality related traits: food neophobia (FNS); private body consciousness (PBC), that is, awareness of internal sensations; sensitivity to punishment and reward (SPSRQ); sensitivity to core disgust (DS-SF); alexithymia (TAS-20); and orientation to value (PVQ); (Table 5).

**Sensory stimuli. Water solutions.** Seven water solutions, corresponding to five basic tastes, astringent and burning sensations are rated for intensity (Table 3). The concentration of the tastants were decided based on published psychophysical data (Feeney et al., 2014; Hayes et al., 2010; Masi et al., 2015) and previous preliminary trials conducted with one-hundred untrained subjects recruited in five Italian sensory laboratories (unpublished data) in order to select solutions equivalent to moderate/strong on a gLMS. The results of the preliminary trials were confirmed in a pilot study performed in 10 sensory laboratories with an average number of 5 subjects per lab.

**Food products.** The criteria followed for the selection of foods for the study were: i) being food or drink products widely consumed and distributed in Italy; ii) being simple and reproducible to prepare (e.g. preferable ready-made products), to handle (e.g. to be consumed at room temperature) and homogeneous in composition and aspect to be easily portioned (e.g. liquids or semi solid). A pear juice (PJ), a chocolate pudding (CP), a bean purée (BP) and a tomato juice (TJ) were selected as the most appropriate food matrices for testing the responses to target tastes. For each food product, four levels of tastant concentration were
Results

selected to elicit a variation in the strength of target sensations (the five basic tastes and two chemestetic sensations- astringency and pungency from weak to strong, Table 3). As with the water solutions, the choice of concentration of tastants for each product was based on published psychophysical data, preliminary tests (unpublished data) and the pilot study.

Odours. The odours were selected from the ones included in the European Test of Olfactory Capabilities (Joussain et al., 2016) and presented using cardstocks designed for the project “La Prévalence des troubles Olfactifs en France” (Projet DEFISENS – PREVAL– OLF) coordinated by Moustafa Bensafi (CRNL, Lyon, France) who kindly provided the material. Odorant molecules were trapped in tight microcapsules (aminoplast type, diameter: 4–8 micro). The microcapsule-based ink was printed on a cardstock (SILK-250 g; Dimension: 11 cm x 21 cm). Each odorant was printed on a delimited area (2 cm² disc). The release of the odour is done simply by rubbing the printed microcapsule reserve. Liking, intensity, identification and irritation are measured for each odour: mint, anise, pine, banana. First, the odorant is presented and the respondent is asked to identify the name of the odour among four possibilities. Then, the respondent is asked to evaluate the odour’s intensity, its degree of irritation, and how much they like it. The odorants are presented in a randomized order and a break of one minute is observed between each evaluation.

Taste function indices. *Fungiform papillae number.* The anterior portion of the dorsal surface of the tongue is swabbed with household blue food coloring, using a cotton-tipped applicator. This made the FP easily visible as red structures against the blue background of the stained tongue. Digital pictures of the tongue are recorded (Shahbake et al., 2005) using a digital microscope (MicroCapture, version 2.0 for 20×-400×) (Masi et al., 2015). For each participant, the clearest image is selected, and the number of FP is counted in two 0.6 cm diameter circles, one on right side and one on left side of tongue, 0.5 cm from the tip and 0.5 cm from the tongue midline. The number of FP is manually counted by two researchers independently according to Denver Papillae Protocol (Nuessle et al., 2015). The average of these values is used for each subject.

*PROP taster status.* A 3.2 mM PROP solution is prepared by dissolving 0.5447 g/L of 6-n-propyl-2-thiouracil (European Pharmacopoeia Reference Standard, Sigma Aldrich, Milano, IT) into deionized water (Prescott et al., 2004). Subjects are presented with 2
Results

identical samples (10 ml) coded with a three-digit code. Subjects are instructed to hold each sample (10 ml) in their mouth for 10 s, then expectorate, wait 20s and evaluate the intensity of bitterness using the gLMS (Bartoshuk et al., 2004). Subjects have a 90s break in order to control for carry-over effect after the first sample evaluation. During the break, subjects rinse their mouths with distilled water for 30 s, have some plain crackers for 30 s, and finally rinse their mouths with water for a further 30s. The average bitterness score is used for each subject.

Genotyping. Saliva samples are collected from all participants using the Norgen Saliva DNA collection and preservation devices. DNA extraction is then performed using the Saliva DNA Isolation kit, according to the manufacturer’s instructions (Norgen Biotek Corp; Ontario, Canada). Genotyping of these samples is carried out using Illumina MEGAEX high-density SNP chip array (Illumina, Inc., San Diego, CA, USA), which contains > 2 millions of selected markers. After quality control, samples will be imputed using the 1000G Project phase 3 reference (Auton et al., 2015) plus an INGI (Italian Network of Genetic Isolates) reference panel, for a total of about 88.000.000 markers.

3.7.4 Preliminary project dataset and analysis of selected variables

One of the aims of the present paper is to show the potential of the IT dataset on the basis of the results from the first year of the study, based on data from 1225 individuals. For this purpose, we selected a limited number of variables from the complete set in the project. The aim of reporting this particular set of data is to show how measurement of multiple variables provides an advantage in understanding food preferences. The variables reported here are: demographics (age and gender), biological (PROP status), psychological (food neophobia, sensitivity to reward and punishment), socio-cultural (health and taste attitudes) and behavioural (familiarity for specific vegetables). For these variables, we described the distribution of the data and studied both gender and age effects. In addition, we investigated the role of these variables in determining preferences (stated liking) for specific vegetables: rocket and radish salads. We selected these items for the following reasons: 1) understanding consumer liking for vegetables is relevant in itself because of the general interest in promoting health eating in many countries (Appleton et al., 2016); 2) the sensory properties of radish and rocket (bitterness and pungency) may represent a potential barrier
to consumption (Dinnella et al., 2016). Liking for Brassica vegetables has been reported to be affected by PROP status (Shen et al., 2016) and psychological traits (i.e. the level of neophobia in adult subjects has been found to be a barrier to the development of preference for vegetables in relation to their sensory properties; Törnwall et al., 2014). Thus, they are appropriate to set up a multidimensional model to show the potential of the Italian Taste dataset in studying the association among several different variables affecting food choice.

3.7.4a Materials and methods

Participants. The data from 1225 participants were collected during 2015. Their demographic and social characteristics are reported in Table 6. The sample was 61% female with a mean age of 36.9 years (SD 12.8; 18–60 years old range). The age distributions of the male and female groups were not significantly different. Regarding the region of residence of the respondents, the Northern of Italy was the most represented (46%), followed by the Southern and Islands (34%) and by the central area of Italy (20%) in line with ISTAT data (ISTAT, 2011). As expected, more females were in the normal range and underweight than males, whereas more males were overweight or obese ($\chi^2 = 15.8; p < 0.01$). 14% percent of the respondents smoked regularly and 11% occasionally. The vast majority of respondents (more than 90%) reported no history of food allergy and/or intolerance. Vegetarians were the 2.2% of the total. Almost all enrolled subjects attended the first laboratory session (more than 99%). Around 3% of subjects dropped out after the first session, generally due to time constraints.

Measuring sensitivity to PROP. PROP status was assessed according to the procedure described above.

Personality and attitude measures. Food neophobia scale (FNS). The trait of food neophobia, defined as the reluctance to try and eat unfamiliar foods, was quantified using the 10-item instrument developed by Pliner and Hobden (1992). The individual FNS scores were computed as the sum of ratings given to the ten statements, after the neophilic items had been reversed; the scores thus ranged from 10 to 70, with higher scores reflecting higher food neophobia levels.

Sensitivity to punishment and sensitivity to reward questionnaire (SPSRQ). According to Gray’s neuropsychological theory of personality, two basic brain systems control behaviour and emotions: the Behavioural Inhibition System (BIS) and the Behavioural Activation
Results

System (BAS). The responsiveness to these systems was measured using the SPSRQ (Torrubia et al., 2001). The SP scale is formed by a set of items reflecting situations which describe individual differences in reactivity and responsivity to BIS. The SR scale was conceived as a single measure of the functioning of the BAS dealing with specific rewards (i.e. money, sex, social power and approval, and praising). The SP and SR scales were scored with a yes/no format. For each subject, scores for each scale were obtained by adding all the “yes” answers.

<table>
<thead>
<tr>
<th></th>
<th>Males (n = 474) %</th>
<th>Females (n = 751) %</th>
<th>Total (n = 1225) %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>38.7</td>
<td>61.3</td>
<td>100</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>18–30</td>
<td>41.6</td>
<td>40.9</td>
<td>41.1</td>
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<td>31–45</td>
<td>25.3</td>
<td>28.5</td>
<td>27.3</td>
</tr>
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<td>46–60</td>
<td>33.1</td>
<td>30.6</td>
<td>31.6</td>
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<td><strong>Region of residence</strong></td>
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<td>18.5</td>
<td>18.0</td>
</tr>
<tr>
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<td>28.7</td>
<td>26.9</td>
<td>27.6</td>
</tr>
<tr>
<td>Centre</td>
<td>18.1</td>
<td>19.4</td>
<td>18.9</td>
</tr>
<tr>
<td>South</td>
<td>16.0</td>
<td>17.0</td>
<td>16.7</td>
</tr>
<tr>
<td>Islands</td>
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<td>8.1</td>
</tr>
<tr>
<td><strong>Education level</strong></td>
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</tr>
<tr>
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<td>0.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Lower secondary school</td>
<td>7.6</td>
<td>6.0</td>
<td>6.6</td>
</tr>
<tr>
<td>Upper secondary school</td>
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<td>42.1</td>
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<td>34.7</td>
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<td>15.2</td>
<td>14.5</td>
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<tr>
<td><strong>Occupation</strong></td>
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<tr>
<td>Employees</td>
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<td>51.8</td>
<td>54.8</td>
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<tr>
<td>Unemployed</td>
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<td>10.8</td>
<td>8.6</td>
</tr>
<tr>
<td>Retired</td>
<td>2.5</td>
<td>1.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Students</td>
<td>32.5</td>
<td>35.3</td>
<td>34.2</td>
</tr>
<tr>
<td><strong>Body mass index (kg/m²)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Underweight (&lt;18.50)</td>
<td>1.1</td>
<td>5.6</td>
<td>3.8</td>
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<tr>
<td>Normal range (18.50–24.99)</td>
<td>53.6</td>
<td>72.0</td>
<td>64.9</td>
</tr>
<tr>
<td>Overweight (25.00–29.99)</td>
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<td>15.8</td>
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<td>Obese (≥30.00)</td>
<td>9.5</td>
<td>6.5</td>
<td>7.7</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never tried</td>
<td>53.2</td>
<td>61.3</td>
<td>58.1</td>
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<tr>
<td>Not or smoking (have tried or quit)</td>
<td>17.1</td>
<td>15.3</td>
<td>16.0</td>
</tr>
<tr>
<td>Occasionally</td>
<td>12.2</td>
<td>10.5</td>
<td>11.2</td>
</tr>
<tr>
<td>Regularly</td>
<td>17.1</td>
<td>12.4</td>
<td>14.2</td>
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<tr>
<td><strong>Monthly expense for food (euro)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Up to 200</td>
<td>16.9</td>
<td>20.6</td>
<td>19.2</td>
</tr>
<tr>
<td>From 201 to 400</td>
<td>46.2</td>
<td>45.0</td>
<td>45.5</td>
</tr>
<tr>
<td>From 401 to 600</td>
<td>29.3</td>
<td>26.4</td>
<td>27.5</td>
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<tr>
<td>More than 600</td>
<td>7.4</td>
<td>8.0</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Table 6. Socio-demographic characteristics of respondents recruited in the first year of the Italian Taste study.

Health and taste attitude scale (HTAS). The HTAS questionnaire was developed to assess orientations toward the health and hedonic characteristics of foods (Roininen et al., 1999).
Results

The HTAS items were scored on a seven-point category scale with the scales labeled from “disagree strongly” to “agree strongly”. For each participant and each subscale, after recodification of negatively worded items, a mean score was computed from the individual scores.

Measuring food liking and familiarity. We selected from the Food Liking and Familiarity questionnaires stated liking for and familiarity with rocket and radish salads (for details on the rating scales see Table 2: Q1; OQ).

Data analysis. For the variables PROP, FNS, SR, SP and HTAS we analysed the distributions of data (by means of descriptive statistical tools) and both gender and age effects (by means of a Two-Way ANOVA model with interactions). A Partial Least Square (PLS) regression model was computed assuming the sum of liking data for rocket and radish for each subject as response variable (Y) and 23 explanatory variables (X). The selection of the regression model was made considering the multi-block nature of the X matrix (several food choice dimensions) and the expected co-variation between the different X variables (interplay among factors affecting food choice). In fact, as reported by Martens, Tenenhaus, and Esposito Vinzi (2007), PLS can model many types of data simultaneously and treats natural co-variation between variables as a stabilizing advantage. In particular, we considered the following X variable blocks: two demographic variables (gender and age); three psychological traits (FNS, SR and SP); five domains of the Health and Taste Attitude Scale (GHI, LPI, NPI, CSF, FR); PROP status and familiarity. PROP ratings were first categorized using the characteristic values of the percentile distribution (first and third quartiles); then, three dichotomic variables were considered: Non Taster (NT), Medium Taster (MT) and Super Taster (ST). Familiarity scores with rocket and radish were included in the model as ten dichotomic variables (from category 1 to category 5 of the familiarity scale for each of the vegetables). PROP status and familiarity with rocket salad and radish were introduced in the model as dummy variables (Martens et al., 2001). The PLS model was computed on standardized variables in order to have unit variance. Cross-validation was used to estimate the number of statistically reliable principal components while jack-knifing was used for stability assessment (significance) of estimated regression coefficients (Martens et al., 2000).
Results

3.7.4b Results

PROP status. Distributions of PROP ratings were compared among research units. Distributions of two units differed from the others showing higher frequency of ratings close to the maximum of the scale, due to the lack of compliance with the procedure for training subjects to the gLMS use. Thus, data from these units were excluded (79 subjects) and analysis were performed on 1149 participants. Distribution of PROP bitterness ratings of the whole sample is described in Figure 2. Based on the theoretical distribution of haplotypes, the percentile distribution of ratings was computed. The upper limit of the first quartile and lower limit of the third quartile were 17 and 58 on gLMS, respectively. These values are in good agreement with the arbitrary cut-offs used in previous studies to categorize subjects in Non Taster (arbitrary cut-off gLMS < moderate, 17) and Super Taster (arbitrary cut-off gLMS > very strong, 53) (Fischer et al., 2013; Hayes et al., 2010). The distribution of PROP bitterness ratings in males and females is reported in Figure 3. Based on an a priori cut-off, 27.7% of males and 23.6% of females were classified as NT; 21.1% of males and 34.6% of females were classified as ST. Females and males significantly differed in PROP group distribution ($\chi^2 = 5.99; p < 0.0001$). MT males and ST females were significantly larger groups than expected. The male distribution in PROP taster groups roughly reflected the haplotype frequencies of 25, 50 and 25% for NT, MT and ST, respectively, while the female distribution did not. The Two-Way ANOVA model (gender and age) shows that the PROP bitterness mean value was significantly higher in females (mean = 40.74) than in males (mean = 34.94) ($F = 16.77; p < 0.001$) (Table 7). Age effects on PROP ratings are also significant ($F = 4.19; p = 0.015$), while the gender * age effect is not significant ($p = 0.501$). In order to better analyse the age effect on PROP bitterness ratings, data from males and females were independently submitted to a Two-Way ANOVA model with interactions, considering age (three levels: 18–30; 31–45; > 46 years) and PROP group (three levels: NT, MT, ST) as effects. Age significantly affects PROP bitterness ratings of the three PROP taster groups in females (age effect: $F = 5.46; p = 0.004$; age * PROP group: $F = 2.82, p = 0.04$). PROP intensity ratings decrease significantly in MT and ST groups over 45 years old. No significant effect of age was observed in males.
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Figure 2. Distribution of PROP bitterness ratings (n = 1149).

Figure 3. Gender differences in PROP bitterness ratings. Median (line) and mean (cross) values.

Food neophobia scale (FNS). The internal consistency of the FNS score, as measured by Cronbach’s $\alpha$, was satisfactory ($\alpha = 0.87$). Overall, the mean was 27.4 ($n = 1225$, $SD = 11.7$, range = 10–69). Correlation among items was always highly significant ($p < 0.0001$) with Pearson correlation coefficients ranging from $r = 0.19$ and $r = 0.72$. The score distribution (Figure 4) had a skewness of 0.60 and a kurtosis of -0.20. Gender- and age-related
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differences in FNS scores were tested through Two-way ANOVA with interaction (Table 7), which showed a significant main effect of gender ($F = 4.24$, $p < 0.043$) and age ($F = 7.26$, $p < 0.001$). Males (mean = 28.3) were significantly more neophobic than females (mean = 26.9) and the youngest participants (18–30 years: mean = 25.9) were significantly less neophobic than the older group (>46 years: mean = 28.9). FNS scores of the middle-aged group (31–45 years: $M = 27.9$) lay in between. The age * gender interaction was not significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gender (G)</th>
<th></th>
<th></th>
<th>Age (A)</th>
<th></th>
<th></th>
<th></th>
<th>GA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-value</td>
<td>Male</td>
<td>Female</td>
<td>P-value</td>
<td>18-20</td>
<td>31-45</td>
<td>46-65</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>PROP Fatking</td>
<td>&lt;0.001</td>
<td>34.9</td>
<td>41.7</td>
<td>0.015</td>
<td>40.5</td>
<td>28.1</td>
<td>35.2</td>
<td>0.501</td>
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</tr>
<tr>
<td>Food neophobia scale</td>
<td>0.043</td>
<td>28.3</td>
<td>26.9</td>
<td>&lt;0.001</td>
<td>25.9</td>
<td>27.9</td>
<td>28.9</td>
<td>0.822</td>
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<td>&lt;0.001</td>
<td>10.1</td>
<td>8.2</td>
<td>&lt;0.001</td>
<td>11.4</td>
<td>8.9</td>
<td>9.1</td>
<td>0.951</td>
<td></td>
</tr>
<tr>
<td>Sensitivity to reward</td>
<td>&lt;0.001</td>
<td>10.1</td>
<td>8.2</td>
<td>&lt;0.001</td>
<td>11.4</td>
<td>8.9</td>
<td>9.1</td>
<td>0.951</td>
<td></td>
</tr>
<tr>
<td>General Health Interest</td>
<td>&lt;0.001</td>
<td>36.5</td>
<td>38.5</td>
<td>&lt;0.001</td>
<td>38.1</td>
<td>37.4</td>
<td>40.4</td>
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</tr>
<tr>
<td>Light Product interest</td>
<td>0.311</td>
<td>20.8</td>
<td>20.4</td>
<td>0.081</td>
<td>21.2</td>
<td>21.0</td>
<td>20.2</td>
<td>0.149</td>
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</tr>
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<td>Natural Product interest</td>
<td>&lt;0.001</td>
<td>25.8</td>
<td>27.3</td>
<td>&lt;0.001</td>
<td>25.1</td>
<td>24.7</td>
<td>28.5</td>
<td>0.066</td>
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<tr>
<td>Cravings for Sweet Foods</td>
<td>&lt;0.001</td>
<td>26.2</td>
<td>30.3</td>
<td>0.064</td>
<td>29.8</td>
<td>28.5</td>
<td>27.9</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Using food as Reward</td>
<td>0.096</td>
<td>26.1</td>
<td>27.1</td>
<td>&lt;0.001</td>
<td>28.0</td>
<td>27.2</td>
<td>24.2</td>
<td>0.090</td>
<td></td>
</tr>
<tr>
<td>Pleasure</td>
<td>&lt;0.001</td>
<td>30.2</td>
<td>31.2</td>
<td>0.171</td>
<td>30.8</td>
<td>31.2</td>
<td>30.5</td>
<td>0.034</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Two-Way ANOVA. Gender, age and their interaction effect on selected variables: mean scores and p-values.

In bold significant values. Letters indicate significantly different mean scores (Tukey’s Honest Significant Difference, HSD).

* The total degree of freedom (d.f.) for each of the computed ANOVA models was 1219 with exception of the variable PROP (d.f. = 1143).

** HTAS domains.

Sensitivity to punishment (SP) and sensitivity to reward (SR). The Cronbach’s a for each of the scales was good, this being slightly higher for the SP (0.84) than for SR (0.75) scale. The two scales were poorly correlated with each other ($r = 0.061$, $p = 0.035$). We also observed sufficient variation in scores: out of a possible range of 0–24, SP scores ranged from 0 to 24 (mean = 10.01; SD = 5.26) while SR ranged from 0 to 22 (mean = 8.92; SD = 3.96). The Two-Way ANOVA model with interaction (Table 7) computed on the SP and SR scores showed a significant effect of both gender and age, while the interaction effect was not significant. Females obtained higher scores than males on the SP scale, while males clearly score higher than females on the SR scales ($p < 0.001$). Both SP and SR scores in participants aged 18–30 were higher than in participants > 31 years old. In addition, on the SR scale, participants 31–45 obtained higher scores than subjects 46–60 ($p < 0.001$).
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Health and taste attitudes scale (HTAS). Concerning the internal consistency of each Health and Taste domain, only Pleasure revealed a low internal validity (Cronbach’s α = 0.42). The differences in α-values across countries seem to indicate that the internal consistency of this domain changes in relation to cultural aspects (Table 8). The effect of gender and age and their interaction was tested by a Two-Way ANOVA model (Table 7). Significant gender differences were found for General Health Interest (F = 24.64; p < 0.001), Natural Product Interest (F = 16.16; p < 0.001), Craving for Sweet Food (F = 66.16; p < 0.001), Pleasure (F = 12.19; p < 0.001), with females having more positive attitudes towards both the Health and Taste domains. The gender effect is stronger for the domain Craving for Sweet Foods than for General Health Interest, Natural Product Interest, and Pleasure. We did not find a gender effect for Light Product Interest (F = 1.026; p = 0.311), that had also the lowest mean score among the HTAS domains. No gender effect was found for the domain Food as a Reward. A significant association with age was found for General Health Interest (F = 34.89; p < 0.001) and Natural Product Interest, (F = 37.72; p < 0.001), which were rated gradually higher with the increasing age of the groups. In addition, older respondents (>45 years old) rated lower Using Food as a Reward compared to the other two age groups (F = 19.31; p < 0.001). A Gender * age interaction was found in the case of Craving for Sweet Foods (F = 6.87; p = 0.001) and Pleasure (F = 3.39; p = 0.034). Females aged 18–30 years old and 31–45 years old rated higher than males on Craving for Sweet Foods, and females aged 18–30 years rated higher than males on Pleasure.
Results

Figure 4. Distribution of Food Neophobia Scores (n = 1225).

<table>
<thead>
<tr>
<th>HTAS Domain</th>
<th>Theoretical range</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>α</th>
<th>α a</th>
<th>α b</th>
<th>α c</th>
<th>α d</th>
<th>α e</th>
<th>α f</th>
<th>α g</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Health Interest</td>
<td>8-56</td>
<td>11</td>
<td>56</td>
<td>37.94</td>
<td>8.08</td>
<td>0.79</td>
<td>0.80</td>
<td>0.89</td>
<td>0.87</td>
<td>0.84</td>
<td>0.80</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Light Product Interest</td>
<td>6-42</td>
<td>6</td>
<td>42</td>
<td>20.56</td>
<td>6.98</td>
<td>0.81</td>
<td>0.78</td>
<td>0.82</td>
<td>0.78</td>
<td>0.66</td>
<td>0.70</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>Natural Product Interest</td>
<td>6-42</td>
<td>6</td>
<td>42</td>
<td>26.73</td>
<td>6.84</td>
<td>0.74</td>
<td>0.70</td>
<td>0.76</td>
<td>0.76</td>
<td>0.65</td>
<td>0.69</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Craving for Sweet Foods</td>
<td>6-42</td>
<td>6</td>
<td>42</td>
<td>28.84</td>
<td>8.75</td>
<td>0.87</td>
<td>0.80</td>
<td>0.86</td>
<td>0.77</td>
<td>0.74</td>
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</tr>
<tr>
<td>Eating Food as a Reward</td>
<td>6-42</td>
<td>6</td>
<td>42</td>
<td>26.73</td>
<td>7.57</td>
<td>0.81</td>
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<td>0.67</td>
<td>0.67</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Pleasure</td>
<td>6-42</td>
<td>9</td>
<td>42</td>
<td>30.82</td>
<td>4.60</td>
<td>0.42</td>
<td>0.33</td>
<td>0.67</td>
<td>0.63</td>
<td>0.39</td>
<td>0.54</td>
<td>0.53</td>
<td></td>
</tr>
</tbody>
</table>

* SD = Standard Deviation.

a Values from Endrizzi et al. (2015) (Italian data).
b Values from Roininen et al. (1999) (Finnish data).
c Values from Roininen et al. (2001) (1Finnish data, 2English data, 3Dutch data).
d Values from Zandstra et al. (2001) (Dutch data).

Stated liking for rocket and radish. Four PLS components were estimated and retained as significant with a total explained variance of 45%. The PLS loading plot for the first two components (Figure 5) allows the observer to explore the associations among variables. Liking increases with age, when the familiarity with the products is high and when GHI and NPI scores increase. In contrast, liking decreases when food neophobic scores, sensitivity to reward and sensitivity to punishment increase. Gender does not seem to influence liking. The PLS regression coefficients and their significance are shown in Figure 6. It is

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interesting to note that being a PROP ST is, as expected, negatively associated with liking and positively associated with a low familiarity with the two vegetables.

3.7.4c Discussion

We applied a PLS model to study the associations among a selected number of variables in affecting stated liking for two vegetables. The purpose of the analysis was to give an example of how to explore and understand the complex picture determined by the interplay of biological, physiological, psychological and sociocultural factors determining individual differences in food preferences and choice, very well depicted by several authors already cited in the introduction of this paper. Our relatively simple example clearly showed that individual differences in stated liking for two specific vegetables characterized by sensory properties such as bitterness and pungency are driven by experience and exposure. However, some psychological traits, such as being neophobic or sensible to reward and to punishment may act as barriers to this process. The importance of food neophobia among a variety of variables in modulating flavour preferences in young adult subjects (21–25 y.o.) has been highlighted by Törnwall et al. (2014). In addition, our findings indicate that psychological traits potentially involved in explaining individual food choices are not limited to food neophobia. Our results suggest that sensitivity to reward and punishment could also play a relevant role as barriers to exposure and familiarization with specific foods. In fact, both higher SP and SR were associated with a lower liking for radish and rocket salad, thus representing a possible barrier to vegetable consumption. Recent studies have highlighted an association between these traits and unhealthier behaviours; higher sensitivity to reward predicted higher fat intake, higher alcohol consumption, greater likelihood of binge drinking, greater likelihood of being a smoker and, amongst smokers, smoking frequency. Higher sensitivity to punishment predicted lower alcohol consumption but higher sugar intake (Tapper et al., 2015). Higher SR scores were significantly related with a more frequent drinking and heavier consumption per occasion of alcohol. In addition, drinkers more sensitive to reward reported feeling more stimulated shortly after drinking and exhibited an attenuated rate of decline in stimulation over the blood alcohol curve, relative to drinkers with less strong reward sensitivity (Morris et al., 2016). The Italian Taste dataset represents an opportunity to study more in depth the contribution of these
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traits to unhealthy food behaviours investigating their association to preferences for specific food categories such as vegetables. This may be particularly worth of investigation in the case of younger adults, that we found more sensitive both to reward and punishment: for this age group these traits can play a role in creating a barrier to consumption of healthier products or encouraging unhealthier food behaviours. Finally, the model suggests that being a ST phenotype may also mediate familiarity with and thus liking for specific food, as reported by Prescott and co-workers (Lee et al., 2008; Yeomans et al., 2009). In this example, there is good evidence for the interplay between factors affecting liking: some psychological traits like food neophobia, sensitivity to reward and punishment and phenotype characteristics (PROP taste group) represent possible barriers to consumption of the considered vegetables because of their negative effect on liking. In contrast, age and experience, interpreted as familiarity with the products and acquired attitudes (GHI, NPI), facilitated liking and thus consumption. Considering the PLS model as an example and interpreting its results in a broader view, we suggest that coupling the measurement of many variables related to food preferences with appropriate multidimensional statistical analysis allows the researcher to obtain relevant information to answer to either applied or more fundamental research questions. In fact, it is possible to identify variables that are relevant for consumer segmentation in relation to the acceptance of specific products. At same time, the obtained information is relevant even when the research question is how to overcome barriers to the consumption of specific healthy foods in respect to segments clearly characterized for their physiological, psychological, and socio-cultural traits. Overall, the project sample to date has been quite well balanced in terms of gender, age (within the range 18–60 years) and geographic areas. The proportion between the two sexes among respondents is in line with other large scale studies (e.g. Pirastu et al., 2016) and can be judged acceptable, considering that males tend to be less inclined to volunteer for research than females, as clearly shown also in the NutriNet Santé study (Hercberg et al., 2010; Méjean et al., 2014). The analysis of the structure and distribution of the data for each of the selected variables allowed us to draw several conclusions regarding the variables presented here.
Figure. 5. PLS regression loading plot (n = 1149). Variance accounted for X and Y for PC 1 and PC2 are reported in brackets. Health and Taste Attitudes Scale variables: Natural Products Interest (NPI), General Health Interest (GHI), Light Products Interest (LPI), Food as a Reward (FR). PROP Status: Non Taster (NT), Medium Taster (MT), Super Taster (ST). Psychological traits: Food Neophobia Scale (FNS), Sensitivity to Reward (SR), Sensitivity to Punishment (SP). Demographics: Age, Gender. Familiarity with rocket: Fam Ro 1–5. Familiarity with radish: Fam Ra 1–5.
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Figure 6. PLS regression coefficients displayed with 95% Jack-knife confidence interval (n = 1149). Variables with interval overlapping 0 (white bars) are not significant. Health and Taste Attitudes Scale variables: Natural Products Interest (NPI), General Health Interest (GHI), Light Products Interest (LPI), Food as a Reward (FR). PROP Status: Non Taster (NT), Medium Taster (MT), Super Taster (ST). Psychological traits: Food Neophobia Scale (FNS), Sensitivity to Reward (SR), Sensitivity to Punishment (SP). Demographics: Age, Gender. Familiarity with rocket: Fam Ro 1–5. Familiarity with radish: Fam Ra 1–5.

PROP status. The distribution of PROP ratings and the relative values of the first and third quartile supported the validity of previously proposed arbitrary cut-offs to classify subjects as NTs, MTs and STs (Fischer et al., 2013; Hayes et al., 2010). In line with the present results, studies on large population samples identified gender as significant predictor of PROP bitterness intensity, with male mean ratings lower than those of females and a higher frequency of ST among females (Fischer et al., 2013; Garneau et al., 2014). Our data revealed an age effect on PROP ratings in females. In supra-threshold studies, age has been reported as a negative predictor of PROP bitterness (Garneau et al., 2014). A decrease in PROP bitterness sensitivity over the life span has been reported only in PROP taster subjects in a large size threshold study (Mennella et al., 2010). The general decoupling of threshold and supra-threshold PROP sensitivity has been often reported (Bartoshuk, 2000; Hayes et al., 2011; Webb et al., 2015); thus, the age effect on PROP bitterness sensitivity
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deserves further investigation. PROP status classification based on phenotype might also reflect the oral responsiveness due to other factors, such as fungiform papillae density, which in turn are affected by age. The interplay between responsiveness to PROP bitterness and fungiform papillae density has been reported in taster subjects depending on their genotype (Hayes et al., 2008). The relationships between genotype and phenotype, as well as responsiveness to PROP and fungiform papillae density, deserve further investigation and will be explored as part of the Italian Taste project as soon as population genotyping is completed.

Food neophobia. Since research on food neophobia suffers from a lack of standardization in the age groups being compared, and in the number of participants involved (Meiselman et al., 2010), the present results will be discussed only considering previous nationally representative samples of consumers with a similar age range as the one considered in our study. The analysis conducted on Food Neophobia scores showed that the internal validity (a) of data was similar to that reported in other large studies, confirming that FNS is a robust and efficient tool even when translated in other languages (Ritchey et al., 2003). In fact, internal consistency of the FNS scores in the present study (a = 0.87, n = 1225, age range = 18–66 years) was similar to those reported in previous research involving large population samples of Finns (a = 0.88, n = 2191, age range = 18–57 years, Knaapila et al., 2015; a = 0.85, n = 1083, age range = 16–80 years, Tuorila et al., 2001) and Swiss (a = 0.80, n = 4436, age range: 21–99 years, Siegrist et al., 2013). The mean FNS score observed here (27.4, SD = 11.7) was considerably lower than the one reported in a study performed in a sample of Italian subjects of similar age (mean = 34.0, SD = 15.5, n = 167, age range = 20–59 years, Demattè et al., 2013) and moderately lower than the mean FNS score found for Finns (mean = 28.5, SD = 11.0, N = 2191, age range = 18–57 years, Knaapila et al., 2015). Cultural origins may explain the difference between our results and those by Knaapila et al. (2015) but not the difference with the outcome of Demattè et al. (2013). In this latter case, it might be hypothesized that the sample was small, local and not representative of the general Italian population. However, considering that in Italy strong regional differences in food culture exist, the Italian Taste dataset has the potential to explore the differences among geographic macro-areas of the country (North, Central and South) that also reflect socio-economical differences. Significant effects of age and gender on FNS were found. We
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found a significant, though somewhat modest, effect of gender on FNS score, with males being more neophobic than females. Analysis of nationally representative studies involving consumers of comparable age to the one considered in the present study showed no gender effect in one study (Knaapila et al., 2015) or a slight effect in three other studies (Hursti et al., 1997; Siegrist et al., 2013; Tuorila et al., 2001). When gender-related differences were found, all studies agreed that males were more neophobic than females. This has been explained by the greater involvement of women rather than men in food purchase and preparation (Hursti et al., 1997). However, it should be pointed out that the effect of gender on FNS scores was always very small (from 1.5 to 2.9 points on a scale ranged from 10 to 70), leading to the conclusion that such effects are likely to be less important than many other variables related to food rejection (Nordin et al., 2004). Similarly, the effect of age, although significant, was somewhat weak. However, FNS scores tend to increase with age. Age-related differences in the level of food neophobia are often reported in large population studies, with FNS scores increasing with age (Meiselman et al., 2010; Siegrist et al., 2013; Tuorila et al., 2001). Further analysis of the current dataset may reveal age and gender effects on specific FNS items. At the same time, the Italian Taste dataset will facilitate the study of the associations between this trait and other psychological and biological measurements, as well as with attitudes relevant to food choice.

Sensitivity to reward and sensitivity to punishment. In line with previous results (O’Connor et al., 2004; Torrubia et al., 2001), the internal validity (α) of both scales was good, being slightly higher for the SP than for SR scale. Our results confirm that the two personality traits seem to be uncorrelated. The gender effect was in line with previous results (Caseras et al., 2003; Torrubia et al., 2001), with females more sensitive to punishment than males, and males more sensitive to reward than females. To our knowledge, the age effect on sensitivity to reward and sensitivity to punishment scores in adult populations (e.g. from 18 to 60 years old) has not been studied in depth yet. In a study that used the BIS/BAS scale developed by Carver and White (1994), Pagliaccio et al. (2016) observed that both sensitivity to reward and punishment scores tended to be higher in young adulthood (18–22 years old) than in later adulthood (30–45 years old) and in childhood. Our data clearly show that both sensitivity to reward and sensitivity to punishment are higher in the younger adults.
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aged 18–30, and that individuals aged 31–45 tend to be more sensitive to reward than older individuals.

Health and Taste attitudes. It has been shown that the HTAS predicts choices between products varying in health and hedonic aspects and it has been consequently used to segment consumers (Tuorila, 2015). In the present study, the internal validity (a) of the subscales is generally in line with other studies for five out of six domains. The Cronbach’s a value is not satisfactory for the Pleasure domain only. It seems that when this domain is used in countries different from the one in which the questionnaire was developed, the scores for each of the statements tend to be not strongly related each other. The interpretation of the meaning of the statements describing the link of food with pleasure could vary from culture to culture (Rozin et al., 1999), thus a translation-back translation could not always be sufficient to guarantee the adherence with the original meaning. Further studies on the adaptation of this domain taking into account the relevant socio-cultural aspects of the country in which the study is conducted are needed. Roininen et al. (1999, 2001) registered comparable mean scores in the three domains of the Health subscale, although with some differences between countries (2001). We noticed a low interest of the Italian sample for light products which reflects a general tendency in the country to consider the Mediterranean diet healthy and tasty at same time (Monteleone et al., 2009), with a consequent low interest in light foods. Early studies from the HTAS questionnaire creators pointed out a noticeable variability in values among gender, age and countries and their interactions (Roininen et al., 1999, 2001). Our results partially confirm previous findings, with females having more positive attitudes towards both the Health and Taste domains (Endrizzi et al., 2015; Roininen et al., 1999, 2001). However, we found a stronger gender effect for the domain Craving for Sweet Foods than for General Health Interest, Natural Product Interest, and Pleasure, while in the previous studies reported above, a strongest effect of gender for the General Health Interest domain was reported. The variability induced by the gender by age interaction on HTAS scores deserves further investigations as well as the effectiveness of this set of scales of predicting choices, even in association with other variables.
3.7.5 Conclusions

Studies on influences on food choice are subjected to two main limitations: the sample size and an approach based on a limited perspective that does not take into account at the same time genetics, taste sensitivity, psychographics and sensory and hedonic responses to foods based on evaluations of samples and not only of names or tastant in solution. The Italian Taste project plans to overcome the above-mentioned limitations and may be seen as a model to explore the complex interplay of factors contributing to food choices. The design of the study we presented here may in fact easily be reproduced in other countries, with the precaution of adapting the Food Liking and Choice questionnaires taking into account the specificities of the food culture considered and selecting appropriately the products used for the sensory and hedonic tests among commonly consumed products. The exploration of cross-cultural differences will contribute to a further deeper insight into the understanding of food choices. In recent years, multicenter research has become increasingly common in situations in which single research centres have the tools and skills to investigate a question, but the power of data would suffer due to slow data collection or too few available respondents. The present report describes a project in which Italian researchers have rallied their resources to investigate human food choice behaviour and preferences using current knowledge of possible predictors from genetic, physiological and psycho-social domains. When planning a multicentre research, it is particularly important to ensure the alignment in the procedure of data collection of the different labs involved in the study. Specific attention should be paid to the procedure of sample preparation, papilla count and to the instructions on the use of the gLMS. Training periods of all the researchers involved in data collection are recommended to guarantee the reduction of differences due to the operators. Great attention is also required to align the instructions to give to the respondents before and during the test, in order to avoid an effect of different type of information given.

3.7.6 Acknowledgements

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Italian Sensory Science Society and was partly supported in 2015 by the Louis Bonduelle Foundation.

3.7.7 Appendix 1

A scientific committee of thirteen members of the Italian Sensory Science Society (SISS), all experienced and internationally recognized researchers, designed the study. Each member of the scientific committee coordinated one of the following activities: ethics, bibliography, recruitment; liking/choice/familiarity questionnaires; attitudes and psychological traits; liking and sensory tests; genetic tests; data analysis; database implementation and management; communication; fund raising. The corresponding author of the present paper served as project coordinator. Working groups open to all SISS members were organized to define a procedure for each activity under the responsibility of a coordinator. All the procedures related to data collection and data analysis were reviewed by the members of an international advisory board composed by experienced sensory and consumer researchers. Procedures were revised according to their advice and tested in pilot studies before the approval of the scientific committee. Similarly, a general procedure for data acquisition was designed, reviewed, and tested in a pilot study run in April-June 2015 with 95 respondents (5 in each of the 19 laboratories involved in the project, (Table 9). During the pilot study a checklist was provided to each unit to report deviations from the procedures. The checklist included the control of the following critical points: sample preparation (time between preparation of food sample and testing; time between the two sessions; temperature of conservation of samples); papilla count (two-way ANOVA to investigate the effect of the operators, if any), critical points for each step of the test; missing data; data control. After a final revision, the data acquisition procedure was approved by the scientific committee and data collection started in July 2015 with the objective of recruiting three thousand respondents in three years across the laboratories and the country. Sixteen labs out of nineteen utilized the same computerised system for data collection in the lab (Fizz, Biosystèmes, France). Methods were centrally designed at Florence University. Four units collected data on paper forms prepared on the basis of the Fizz sessions. Before the pilot studies, two days of training were organised at the Sensory Lab of Florence University in order to uniform the PROP and papillae procedures. Special attention was paid to training researchers from all the labs in the use of the gLMS.
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Similarly, researchers were trained in the preparation of food samples participating in the preparation of a session.

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Table 9. SISS Sensory Laboratory Network: Institutions and their geographic distribution.

To assist all the units involved in the project during data collection five help-desks were activated on: sample preparation (Trento Unit); PROP and Papillae (Florence unit) and Data acquisition (Florence, Bra, Bologna). In each research unit, after the collection of the data the researcher responsible for the data entry uploaded the collected data on a default spreadsheet in order to obtain the unit dataset. Then, the researcher responsible for the whole database of the study merged the 19 unit datasets to obtain a complete dataset. In order to check the reliability of the data entry process, a data control procedure was applied to the both unit datasets and complete dataset. At the unit level, each responsible completing the data entry, firstly applied a filter function to each variable of the unit dataset to verify the absence of anomalous data. Secondly, the correspondence between the data reported in the unit dataset and the original data reported on the result files of the software used for data acquisition (or on the paper forms filled in by the participants) was checked. In particular, all the responses provided by at least the 20% of the subjects who took part in the study in the sensory laboratory of each research unit were controlled. Similarly, at a global level, the responsible controlled the merged datasets, firstly, applying a filter function to each variable of the complete dataset to verify the absence of anomalous data, and secondly, checking the
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correspondence between the data reported in the complete dataset and the original data reported on the unit dataset for the 20% of the subjects who performed the testing in each sensory laboratory. Additionally, a second researcher controlled the correspondence between the data reported in the complete dataset and the original data reported on the unit dataset for an additional 10% of the subjects who performed the testing in each sensory laboratory.
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4. Conclusions

The results of this PhD project dealt with the application of a combined approach, in which the aromatic profile of different products was studied: the sensory evaluation of different food samples was paired with the volatile compounds analysis, in order to define if any correlation exists among what human being can perceive (either is judges are more or less trained) and detectable volatile molecules of a product. Additionally, this approach was supported by the application of other instrumental techniques, like texture measurement or electronic eye evaluation.

In particular, this thesis took into account the characterization of various types of food matrices in order to support them as added-value and consumer appealing foods for modern consumers. Even if characterization was done using several methodologies, sensory studies were always included as the most important means to evaluate the products, and other methods were to support and/or relate to them.

In the first study, a new method using PCA and discriminant analysis applied to the volatile profile of the headspace as a fingerprint was shown suitable to verify the geographical traceability of EVOO. In particular, the headspace fingerprint was obtained using an FGC-E-nose and subsequently verified by SPME-GC-MS. 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, highlighting that when a good, reliable training set coming from a certain production year is available, it is possible to verify 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. 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.

The second study investigated the possibility to use the industrial tomato by-product (skin and seeds) for co-milling with olives to obtain a vegetable oil that is naturally rich in antioxidants, especially in carotenoids. Specifically, the presence of lycopene in this came from the natural passage of this lipophilic molecule into the lipid matrix only through a mechanical process and avoiding the use of solvents or chemicals. In both the experiments, the co-milling showed significant enrichment in carotenoids, especially in lycopene (mean values of 5.4 and 7.2 mg/kg oil from defrosted and freeze-dried by-products, respectively). This new product might be marketed as a “condiment produced using olives and tomato by-products” or “OO dressing enriched in lycopene”.

The third study, entirely carried out in Helsinki University, took into account faba beans and problems related to the development of off-flavours during the processing. As thermal treatments were proven to not completely stop the LOX activity in samples object of this study, a compromise should be reached: where the enzyme activity is almost completely reduced, as in CO and MW samples, attributes related to rancid and nutty sensory notes appeared, maybe because of the autoxidation caused by the temperature rise. On the other hand, when beans were used without any treatment, it could be impossible to control the oxidation carried on by enzymes, and this could affect the shelf life of products in which faba beans are added. This study represents a preliminary approach to the investigation on origin of off-flavour derived by mandatory heat-treatment applied to faba beans before their consumption or their addition to other ingredients for the preparation of food products.

The fourth work dealt with a sensory and instrumental analytical approach for characterizing a typical and high-quality Italian salami, manufactured from Mora Romagnola pig breed, an autochthonous breed, extensively farmed within a geographically confined area in Italy, located in the east part of Emilia Romagna region. The aim of this work was to highlight any difference, detectable by sensory profile and volatile compounds analysis, between conventional and Mora Romagnola salami, thus proposing an integrated approach for quality control. The aim of this study was to characterize salami produced with
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Mora Romagnola breed as compared with salami obtained using modern white-pig breeds in the Italian market. The characterization was carried out by means of both sensory and instrumental measurement (volatile compounds, texture analysis and image analysis).

The sensory evaluation carried out by a panel allowed to define that the major attributes to characterize dry fermented salami; however, color differences between Mora Romagnola and conventional salami, not detectable by panelists, were clearly identified by an electronic eye. Moreover, through the analysis of volatile compounds, 33 molecules, mainly belonging to the chemical classes of terpenes, aldehydes, alcohols, ketones, and acids, were detected. According to instrumental texture analysis outcomes, large variability was reported among Mora Romagnola samples, highlighting that salami produced with this local breed are traditional products, whose recipes and handcraft preparation are characterized by a certain variability, linkable to the concept of local food. Finally, the importance of the integrated use of advanced analytical and sensory techniques to describe specific characteristics of this typical product, which can eventually be used to define a specific regulation to enforce quality control, was confirmed.

The fifth work dealt with the characterization of different commercial categories of Italian cooked pork hams, by means of 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 “electronic eye” and texture analyser. In this investigation, sensory analysis resulted effective in defining the profile and the quality of the product. Also physical-rheological parameters measured by electronic eye and texture analyzer were effective in classifying samples. This study can be considered a first application of this combined approach that will be integrated with the volatile compounds analysis.

The sixth work is represented by a cheese-making educational laboratory, mainly dedicated to children, adolescents, and their parents/teachers. The participants were involved in the preparation of fresh and naturally-coloured cheeses. At the end of the activity, both the colour preference and possible relation between preference and colour of cheese prepared were investigated administering a short questionnaire. The results of this study highlighted how visual preference for a food, in terms of colour, changes during different stages of life.
and in relation to gender. Indeed, the data demonstrated how children can be influenced by food appearance and how the aspect of a product could be related to its acceptance, especially among younger individuals; in fact, the youngest participants tended to prefer intensively coloured cheese vs. the white and green version, probably because of the presence of rocket. This first preliminary investigation was useful to collect data about visual preference for differently coloured cheeses among interviewed and will be integrated by the volatile profile investigation for the three samples and the sensory evaluation of olfactory and taste attributes, linked with volatiles molecules.

In the last study, 1225 individuals were involved in a large-scale study, aimed at exploring the associations among biological, genetic, physiological and personality-related socio-cultural dimensions. The aims were to describe the Italian Taste project, describing the variables selected to explore the different dimensions of food choice, and to show the potential of Italian Taste dataset to explain food choice. The results of this work put on evidence the importance of The Italian Taste project as it represents a model to explore the complex interplay of factors contributing to food choices. In fact, the design of the project may be easily reproduced in other countries, with the precaution of adapting the Food Liking and Choice questionnaires taking into account the specificities of the product culture considered and selecting appropriately the products used for the sensory and hedonic tests among commonly consumed products.

This PhD project results highlighted how relevant could be a combined approach: the use of sensory and chemical (volatile compounds) data for a combined analysis represents an important tool for the valorization and communication of quality characteristics of products. This research topic was studied from many points of view and applying different sensory and analytical methodologies.

The food products selected to be included in this thesis were either traditional food products from Italy or new products with added value. Thus, the thesis put on evidence how relevant the development of quality indicators using modern research methods could be. The new knowledge could be used to promote these products to Italian and/or European consumers, as well as to better understand the characters of food matrices, which helps in developing new products and creating innovations.
5. I want to thank...Grazie a...

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