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Valorisation and protection of food quality

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"The bitterness of poor quality remains long after the sweetness of low price is forgotten."

Benjamin Franklin

Al sorriso delle persone che amo

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Introduction

Valuing and protecting a food product implies, first of all, identifying its quality and, therefore, the ability to satisfy consumers' explicit and implicit demands.

In the past, the term quality was equivalent to the concept of conformity, now this word has taken on many meanings, and it is influenced by multiple factors. Today, this term is in fact connected to several aspects: hygienic, healthy, organoleptic, nutritional, commodity, technological , of origin, environmental and ethical.

The food quality requirements fall into the category of primary needs and they are characterized by a strong social value. As such, they are protected, in the first instance, by national and international legislation. However, as other types of needs, the voluntary approaches to quality - regarding "system", "product", and "process", complementary and synergistic - are based on conscious and demanding choices of the operators involved and constitute instruments, that are not only preparatory to the respect of the laws, but pro-active and of improvement.

Unfortunately, these methods are not always sufficient enough for the enhancement and protection of products: there are gaps in the regulations, monitoring and sanctions are not always effective and reassuring, and the certifications are sometimes not confirmed by the choices of Italian consumers.

Italy and Europe have experienced situations in which producers have made a lot of investments in order to obtain higher quality products but there weren't enough tools to protect them on the market. The same operators in the food supply chain, often fail to perceive the benefits of these investments. For example, the quality connected to the origin responds to a series of specifications and is promoted across quality brands on the product. PDO and PGI products are subject to different laws, as set out in the EU regulation 1151/2012, which should protect them from counterfeiting, that is a debatable and punishable fraud. Unfortunately, though, this does not occur for the *Italian Sounding*, a phenomenon that involves the imitation of the Italian products, or rather another type of food fraud resulting in huge losses for the national turnover.

Despite this phenomenon, in 2017 Italy nevertheless registered an exceptional record with 814 PDO and PGI brands, for a production value of about 14 billion euro and a weight of 10% on the total turnover of the national agri-food industry.

In the first case study, we will try to define the importance of adopting analytical and chemometric tools in support of the certifications of origin of *made in Italy* products, in the fight against food frauds. In particular, the attention will be focused on the identification of quality markers, or the peculiarities of the PDO buffalo mozzarella that allow to differentiate it according to the cheese-making procedure used and, at the same time, to protect it on the European market.

However, the instrumental analysis are not enough to define in a univocal and objective way the sensorial profile of a food product, but it is necessary to conduct descriptive and discriminatory tests, in which a panel of experts evaluates the intensity of specific attributes related to the various aspects of the perceivable quality of the food in exam.

Descriptive tests are often accompanied by hedonistic tests, aimed at assessing sensory acceptability by consumers.

On the other hand, the consumers, often victims of informative asymmetry, are not always able to distinguish similar products, or products obtained by different production processes, or do not have the knowledge necessary to identify positive attributes and defects of the foods they consume every day.

Many people do not know the differences between the different brands of origin, others do not know what the biological production consists of. It is therefore necessary to conduct information campaigns aimed at educating and sensitizing the consumers.

In the second case study, we will investigate the perception of young Italian consumers concerning three different types of olive oil: extra virgin olive oil, PDO and organic extra virgin olive oils, taking into account that olive oil is a traditional product for our peninsula (Italy is in fact the second largest producer and importer of oil in the world).

By using consumer science tools, blind and non-blind panel tests, we will analyse if the products perception of panelists changes after showing them the labels and the brands of the olive oils.

If there is still confusion in the field of production specifications and of organic production, on the contrary, more and more consumers are attentive to the nutritional label.

The nutrition label contains information that helps consumers understand how different foods can contribute to a healthy and balanced diet.

Today, consumers prefer healthy and fat-free products, and it is very important to take into account their demands, experimenting with innovative products that are able to embrace the new food trends (e.g. the affirmation of vegan products and functional foods).

Precisely for this reason in the following studies, with the collaboration of Queen Margaret University, we will experiment with a new an egg-free mayonnaise, containing functional ingredients, and then we will analyse its use in a bakery product.

The rheological, analytical and consumer science tools allow us to understand if the new products have adequate chemical-physical characteristics and embrace the expectations and needs of consumers, in comparison with other products currently on the market.

The presence of allergens, GMOs, the percentage of saturated fats, the energy supply are certainly important factors, but the attention is too often focused on ancillary information, and the consumers do not give sufficient weight to the essential characteristic of the products they consume: the food security.

In addition to the stringent limits imposed by the Italian food safety authority, several factors as globalization, urbanization, lifestyle changes, industrialization, intensification of agricultural and livestock production continue to increase the responsibility of producers and operators in ensuring food safety. Food security is threatened by various kinds of risks.

In the final part of the research and in the last case studies, we will focus on the human health risks related to the intake of contaminated products.
In particular, reference will be made to environmental contaminants capable of bioaccumulation and biomagnification along the food chain.
It is important to control food along the supply chain, and to conduct continuously environmental monitoring especially to verify the safety of those products that are marketed without processing the raw material and/or sold wholesale or in local markets.

Chapter I

Quality

Defining the concept of quality is far from easy, since it has always been the protagonist of a profound evolutionary process that continuously transforms its meaning (Bergman & Klefsjö, 2010). In the past, talking about quality was equivalent to talking about conformity, today it would be absolutely reductive. Hence the disquisition between objective and subjective quality: objective quality puts the intrinsic characteristics of the good at the centre of interest, the subjective one is based on the considerations of the user of the good, the client, generally expressed in terms of satisfaction.

The relationship between the two is at the core of the economic importance of quality: only when producers can translate consumer wishes into physical product characteristics, and only when consumers can then infer desired qualities from the way the product has been built, will quality be a competitive parameter for food producers (Grunert, 2005).

To date, the commonly accepted definition of quality is that proposed by the standard EN ISO 8402:1995 (replaced by EN ISO 9000:2005), which defines it as the wholeness of the characteristics of an entity (product or service) that render it capable of satisfying stated and implicit needs - of a moral and material, social and economic nature, typical of the civil and productive life - translated in form of concrete and measurable requisites, through adequate regulatory and standardization processes.

In an increasingly globalized market, in continuous and rapid evolution, where conditions change with great dynamism and where the consumer becomes more and more attentive and aware of the choices he adopts (this also thanks to the widespread diffusion of mass media and Internet), the demand for quality is ever more insistent, and companies must be able to accept it and adapt to the situations that are gradually created (Grunert, 2005).

However, the satisfaction of the demand for quality is extremely complex and linked to a multifactorial set of components that are very difficult to define univocally and in any case extremely variable in time and space.

The requirements that quality is called to satisfy may be primary, connected to the protection of essential needs, such as safety, health, and in general, the fundamental rights of people, or of an ancillary nature, related to the satisfaction of material and spiritual needs, transcending essential aspects such as performance, reliability, durability, beauty, comfort. Furthermore, quality can have an essentially "economic" value (satisfying technical-economic needs within the framework of a specific contractual relationship) or a wider "social" value, not necessarily regulated by direct contractual relationships (e.g. environmental quality and other forms of socially responsible management of production and service processes).

In all cases, the quality must be "measurable" and the costs associated with its implementation and assurance (making products and services capable of meeting those consumer needs and giving demonstration of conformity) must be in proportion to the benefits actually achieved, as perceived, often subjectively, by users (Caswell, 1998).
Applying the Kano model (invented in 1984 to structure customer requirements and examine its influence on customer satisfaction) in the food field it is possible to identify: - pre-requisites, which correspond to safety and legitimacy expectations; - the essential requirements, related to the expectations of health-nutritional contents as well as perceptual and sensory; - the additional requirements respond to psychological gratification (for example, in the way a product is presented), to service expectations and convenience of use. Finally, the system requirements embrace the expectations of constancy of quality attributes, availability, and a fair relationship between quality and price (Xu, *et al.*, 2009).

1.1 THE APPROACHES TO THE QUALITY

Quality requirements in the food sector fall into the category of primary needs and are characterized by a strong social value; as such, they are protected, in the first instance, by specific national and/or international legislation.
As for other types of needs, however, the voluntary approaches to quality (both of "system", of "product", and finally of "process") based on conscious and demanding choices of the operators involved, are complementary and synergistic, and represent means, that aren't only preparatory to the respect of the laws (reactive action), but also pro-active and of improvement (Westhoek *et al.*, 2014).
The system approach is "indirect", as it does not refer to specific product requirements, but it ensures the organization's ability to structure itself and manage its resources and production processes in such a way as to identify and, in general, to satisfy the needs of the customers and stakeholders.
The product approach is "direct" as it aims to ascertain the conformity of the products to the requirements that "directly" characterize its ability to satisfy consumer needs (Meiselman, 2003). The "process" approach, typical of the agri-food sector, is based on the assessment of the capacity of the production processes to supply products that comply with the applicable requirements, and, as such, it represents a middle ground between the two previous approaches.
The agricultural enterprises and the agro-food industry, as any other organization that produces goods and services, are called to carry out and therefore to assure the quality to the market, as it has been defined above and in the various applicable forms, in proportion to the required needs and those they undertake to satisfy (Bryzhko & Kosheleva, 2012).
To this end, they must adequately identify these requirements, starting from those indicated by the applicable mandatory or voluntary regulatory references, and commit themselves to implementing the processes and the resources, that are necessary for their satisfaction.

1.2 THE FRONTIERS OF QUALITY

The quality of food products is the result of a combination of factors, including:

- hygiene and healthiness;
- organoleptic and nutritional characteristics;
- elements of use;
- cultural factors (tradition, local belonging, genuineness);
- environmental factors;
- ethical and social factors.

Regarding the first point, reference is made to the so-called sanitary quality that is directly related to food safety, and it is given by the compliance with minimum hygiene requirements, established by law, relating to content in chemical substances, microorganisms and their metabolites, which can cause damage to health. In this sense, the Codex Alimentarius and the EC Reg. 852/2004 underline the importance of the presence of securities that the food in question does not cause damage, after being prepared and/or consumed according to the use to which it is intended (Lairon, 2011).

The organoleptic quality of a product is defined according to the consumer's evaluation of some characteristics of the food itself, such as the appearance, the aroma and the consistency, perceived through the sense organs (sensorial quality) (Stone & Sidel, 2004). It is therefore a subjective assessment of the consumer, greatly influenced by psychological, social and cultural factors.

The nutritional quality is identified with the content of nutritive principles such as carbohydrates, lipids, proteins, vitamins, fibres and natural substances, and therefore with the nutritive capacity of the food itself. Many of these substances have a protective effect on preventing the onset of serious diseases and more and more often we hear about "functional foods" (Pravst, 2012). With "elements of use" we refer instead to all the purely commercial characteristics: such as packaging, shelf life, ease of use, the commercial category, the sales name, expiry dates, labelling that, together, fall within the concept of product quality.

The quality of origin is often associated with quality brands, or with product certifications that guarantee the place of origin of the product and/or that the production process takes place according to a typical territorial method, in compliance with the specification that regulates the quality brand (Fandos & Flavian, 2006).

In 1992 the European Community created some quality schemes known as PDO (Protected Designation of Origin), PGI (Protected Geographical Indication) and STG (Guaranteed Traditional Specialty) to promote and protect agri-food products (art. 10 Reg. CE 510/2006).

Environmental quality is linked to the use and certification of eco-compatible production methods. In today's society, consumers are paying greater attention to food produced with systems that safeguard the environment and ensure the welfare of farm animals, which use integrated pest management systems or biological pest control and no longer pesticides (Vighi *et al.*, 2006).

Specifically, in integrated control systems, farms use only the plant protection products that are easily denatured and non toxic, in a selective and targeted way, as well as more resistant crop varieties and sustainable practice as the crop rotation (Oskarsson, 2012).

On the other hand, the main important biological pest control strategy (the importation) involves the introduction of a pest's natural predators in the same habitat, without damaging the crops.

The ethical quality underlies all that has to do with the protection of the ecosystem and of the human being. There are special guarantees/certifications of production aimed at highlighting the absence of exploitation of labour, with particular reference to minors (SA 8000).

Each of these needs must receive a response by identifying the requirements that guarantee its satisfaction (mandatory regulatory or voluntary regulatory references), as well as through the verification and certification of compliance with these requirements (conformity certification). Needless to say, while the consumer generally pays more attention to the organoleptic qualities, nutritional qualities and freshness of a product, from the point of view of public health, the securities on the quality of hygiene, the nutritional value and compliance with the law are absolutely essential.

Furthermore, it should not be underestimated that a foodstuff can’t be fully evaluated from the qualitative point of view, if not only after consumption and, in part, not even after that. This makes us understand the importance, especially in these cases, of how the control, security and communication systems, including the brands, are designed to build a reputation and a relationship of trust that is central to the appreciation and enhancement of quality.

This context drives farms to introduce a series of activities and stringent controls, that often involve substantial investments for which operators do not always perceive the benefits.

1.3 FOOD QUALITY POLICIES

Quality is guaranteed by relevant legislation and appropriate controls on the market (Jahn *et al.,* 2005). The tools for the protection and enhancement of food quality are regulated by EU / national policies and by international organizations (Fig 1).

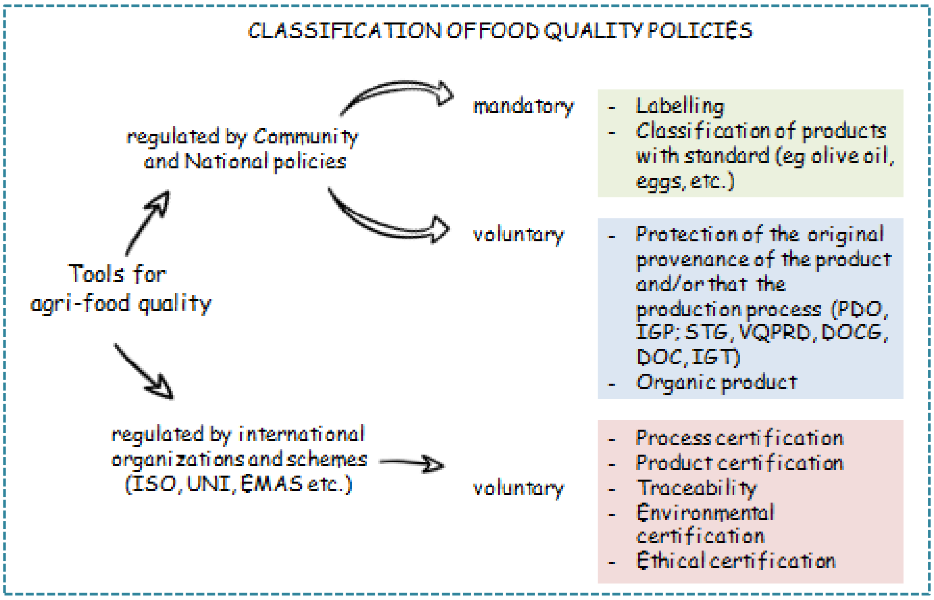


Fig. 1 Classification of food quality policies

In the first case, three different areas of action must be distinguished:

<u>*Cogent scope:*</u> It is represented by all the national and community legislation that establishes the general principles to be respected by the Organizations, in order to guarantee to consumers a safe product regarding health and hygiene in terms of nutritional characteristics (Commission Regulation (EC) No. 607/2009; EU Hygiene Package)

The reform of the European regulations on food hygiene, which came into force on 1 January 2006, simplified and harmonised the legislation applicable in the European Union. This set of regulations, known as the “Hygiene Package”, concerns the entire food chain from primary production to the consumer, including the food industry, catering trades, transport and distribution ("from farm to fork"), with traceability as a basic concept. Its aim is to harmonise the level of food safety by involving all stakeholders in the food chain, subjecting them to the same requirements, by formalising the responsibility of professionals, and by optimising controls by the health authorities.

The “hygiene package” is a body of EU law laying down hygiene rules for foodstuffs produced in the EU and non-EU countries exporting to the EU, and includes the following acts (O’Rourke, 2005):

- Regulation (EC) No. 852/2004 on the hygiene of foodstuffs
- Regulation (EC) No. 853/2004 laying down specific hygiene rules for food of animal origin in order to guarantee a high level of food safety and public health
- Regulation (EC) No. 854/2004 putting in place a Community framework of official controls on products of animal origin intended for human consumption

The “hygiene package‟ is supplemented by other EU legislations on food hygiene, taking particular account of the following principles (Stilo, *et al.* 2009):

- Primary responsibility for food safety borne by the food business operator
- Food safety ensured throughout the food chain, starting with primary production

- General implementation of procedures based on the Hazard Analysis and Critical Control Points principles (HACCP)
- Application of basic common hygiene requirements, possibly further specified for certain categories of food
- Registration or approval for certain food establishments
- Development of guides to good practice for hygiene or for the application of HACCP principles as a valuable instrument to aid food business operators at all levels of the food chain to comply with the new rules
- Flexibility provided for food produced in remote areas

Regulatory area is represented by the community regulations that protect the productions of specific geographic areas, or those obtained in compliance with traditional production techniques and / or using traditional raw materials.

The European Commission has responded to the requirements of typicality, tradition and consumer habits with the enactment of the Community Regulations on products with a protected designation of origin (PDO), protected geographical indication (PGI), and traditional specialty guaranteed (TSG) (Council Regulation (EEC) No. 2081/92, 2082/02 of 14 July 1992, Council Regulation (EC) No. 510/2006, Regulation of the European Parliament (EU) No 1151/2012) (Elortondo, *et al.*, 2007).

The demands of genuineness, protection of the environment and sustainable development coming from the market, have been meet through the introduction of the organic production system (Dimara & Skuras, 2005), also defined by specific Community Regulations (Council Regulation (EC) No. 834 / 2007, 889/2008, 1235/2008.

With the introduction of PDO and PGI products and organic farming production, "regulated quality brands" have been created; producers access these brands by choosing voluntarily but respecting the reference regulatory criteria and the conformity assessment/certification procedures, that are defined by binding rules.

These regulated certifications are issued by political bodies and institutions specifically authorized by the competent Authority (Jahn *et al.*, 2005).

The products covered by regulated certification represent, however, a relatively small fraction of the agro-food market and do not necessarily cover all the consumer's needs in the terms previously highlighted.

Therefore, additional elements are required to better guide the consumers in their choices of quality. These elements are represented by the voluntary certification of unregulated products (voluntary brands of food quality).

Voluntary scope: it is represented by references standards elaborated by Standards Bodies with the consent of all the interested parties, or regulations elaborated by Organizations that determine the definition of food product qualitative standards (Technical Regulations) (Segerson, 1999).

The voluntary product brands are issued by the competent third party Certification Bodies, in the context of specific certification schemes based on reference standards (voluntary but not regulatory production technical specifications) elaborated with the consent of the interested parties and on evaluation procedures adequate to the characteristics of the object (of the certification) and to the expectations of the market.

Among the tools for agri-food quality, regulated by international organizations, we include voluntary certifications of: process, - product, - traceability, - environment and ethics.

To complement and integrate the more or less direct forms of quality assurance of the agri-food products (certification), mentioned above, indirect forms of insurance have also been introduced. They are of considerable importance for agricultural production and the agro-food industry and are represented by the certification of management systems on a voluntary basis. Among them, we find the certification of quality management system (QMS) (regulated by the ISO 9001: 2015 standard), the certification of environmental management systems (EMS) (regulated by ISO 14001: 2015), and finally, the certification of food safety (FSM) (regulated by the ISO 22000: 2005 standard) based on the HACCP principles defined by the Codex Alimentarius.

Certified Management Systems provide standards in setting up and operating the organization's processes, or activities, so that its products or services meet the objectives of: satisfying the customer's quality requirements, complying with regulations, or meeting environmental objectives.

ISO 9001 is an international standard that gives requirements for an organization's quality management system, for providing assurance about the quality of goods and services in supplier-customer relations (Sampaio *et al.*, 2009). ISO 14000 confirms its global relevance for organizations wishing to operate in an environmentally sustainable manner in order to minimize harmful effects on the environment caused by its activities, and to achieve continual improvement of its environmental performance (Massoud *et al.*, 2010; Heras & Boiral, 2013). ISO 22000 specifies requirements for a food safety management system where an organization in the food chain needs to demonstrate its ability to control food safety hazards in order to ensure that food is safe at the time of human consumption (Escanciano & Santos-Vijande, 2014).

Chapter II

The quality of origin and enhancement tools

Products of origin-linked quality have a reputation, quality or characteristics that are essentially a result of their geographical origin and are related to resources special to the production or processing area. This differentiation can be attributed to the unique local features of the product, its history or its distinctive character linked to natural or human factors such as soil, climate, local know-how and traditions (all covered by the term "terroir") (Allaire *et al.*, 2011).

Quality linked to geographical origin represents both a heritage to preserve and a potential to differentiate the product on market. This specific quality linked to geographical origin can be defined through a code of practice (or specifications) and be promoted with a designation or “label” referring to the origin. This strategy is based on voluntary action by local producers to define the associated characteristics collectively and produce the product in accordance with these specifications (Bureau & Valceschini, 2003).

The European Union protects the typicality of some food products through the recognition of PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication). These designations, recognised throughout Europe, are awarded only to those high quality products whose production takes place in a defined geographical area, and for which there is a causal link between the geographical area and the quality or characteristics of the product and the characterising aspects of the production process (Vecchio & Annunziata, 2011).

In both cases, the product should show a strong link to the territory, to whose name must be traced certain characteristics of the product itself. It is to be noted that there is an important difference among the two designations.

The PDO mark identifies a product originating in a specific place, region or country, whose quality or characteristics are essentially or exclusively due to a particular geographical environment with its inherent natural (raw materials, environmental characteristics, location) and human (traditional and artisanal production) factors the production, processing and preparation steps of which all take place in the defined geographical area and in line with the strict production regulations established (De la Guardia & Illueca, 2013).

The PGI mark introduces a new qualitative protection level that takes into account the industrial development of the agri-food sector, giving greater priority to the production techniques rather than the territorial constraint.

Thus, the PGI mark designates a product originating in a specific place, region or country whose given quality, reputation or other characteristic is essentially attributable to its geographical origin and at least one of the production steps of which takes place in the defined geographical area (De la Guardia & Illueca, 2013).

All PDO and IGP products are subject to a set of traditional codified rules within the framework of a detailed Production Specification provided by the European Commission (Council Regulation (EEC) No. 2081/92 of 14 July 1992, Council Regulation (EC) No. 510/2006, Regulation of the European Parliament (EU) No.1151/2012).
The function of these trademarks is threefold: to protect quality products from misuse and imitation; give consumers reliable information about the products they purchase; contribute to the protection of rural areas, whose socio-economic system often depends on the development of typical agricultural food production and quality. According to *ISTAT*, Italy holds the European record for the number of DOP and IGP awards, with more than 261 quality products recognised. Due to the international importance of these designations, the awarded products are subject to strict and specific controls, in addition to the routine checks laid down by European and national legislation.

2.1 COUNTERFEITING AND ITALIAN SOUNDING

In the food sector, the counterfeiting is a fraud that consists in giving a deceptive appearance of genuineness to food products, that are made from substances which differ in quality or quantity from those than are usually found in that type of food (substitutes), or it is related to illegal practices of substituting, removing conventional ingredients or adding new ones (Spink & Moyer, 2011).
A very common type of counterfeiting in the Europe is the falsification of a brand, protected geographical indication (PGI) or denomination of origin (PDO), logo, design . This relates to a false data, either on food or on its packaging, to the violation of the registered trademark, or to an illegal reproduction of the patent according to which the food is made, sometimes with very serious sanitary consequences.
Among the main causes there are: false indications of Made in Italy (products made abroad), the abuse of quality brands indications (Temperini *et al.*, 2016); the use of harmful ingredients for health or the practice of unsuitable production and/or storage procedures (e.g. the absence of traceability). If counterfeiting can be legally contestable and punishable, it isn't the same for the so-called Italian Sounding products, an expression referring to the imitation of Italian product/brand name through a fake reference to the alleged nationality of the product (Nicoletti *et al.*, 2007).
The term Italian Sounding refers to an evocative phenomenon that is more widespread in the United States, Canada, Australia, Latin America and several other countries, including European markets, with reference to the products made there, which through the use of words, colors, images and geographical references on labels and packaging, induce the consumer to mistakenly associate the local product with the authentic Italian one.
The most imitated Italian products in the stores are those produced locally that aren't imported, or also the foodstuff that, although imported as in the case of DPO and IGP products, exploit the non-recognition of some exclusive features of the product (which

constitute its value components), contributing to its value decrease on the market (Temperini *et al.*, 2016).

The most affected categories are: cheeses, in particular the typical ones, pasta, pasta sauces, peeled tomatoes and tomato preserves, olive oil, vinegars, cold cuts and cold cuts, wine, balsamic vinegar, frozen pizzas, etc., until “polenta”.

The Italian Sounding often makes use of the experience and productive knowledge of Italian emigrants - it is more widespread in the countries that have represented the traditional historical destinations of emigration and where Italian communities are more deeply rooted - and it is one of the main causes of reduced impact of Italian exports on turnover (just under 20% for Italy, against the European average of 22% and against the 26% in France and the 28% in Germany), because it allows some local companies to have a competitive advantage that they do not deserve, producing at lower prices and selling the product at a higher price, thanks to appeal to the *Italianness*.

A fundamental point in the fight against counterfeiting was obtained in the European Union when, in December 2014, the new EU Regulation 1169/2011 of the European Parliament on the provision of food information to consumers became applicable, thanks to which it is possible to implement a more effective contrast action (Spink & Moyer, 2013).

The regulation, in confirming the general voluntary nature of the indication of the country of origin, provides that "The indication of the country of origin or place of provenance is mandatory: (a) if where failure to give such particulars might mislead the consumer as to the country of origin or place of provenance of the food, in particular if the information accompanying the food or the label as a whole would otherwise imply that the food has a different country of origin or place of provenance "(Article 26 Country of origin or place of provenance, paragraph 2).

However, despite the efforts made to date, the fight against counterfeiting remains an open challenge.

2.2 QUALITY MARKERS

Since certifications of origin are not often sufficient to counteract the counterfeiting and protect the local products from food fraud, alternative tools should be used.

Some of these include:

- the strengthening of the regulatory instruments and the establishment of a law firms network, in charge of the Public Administration, to support companies in legal disputes in order to protect their products and claim the damages inflicted by counterfeiting, also with the aim of reinforce the application of the rules and the sanctions.
- the inclusion of clause to protect food products (trademarks, Designations of Origin and Geographical Indications) within the bilateral free trade agreements, as well as clauses that prohibit the evocation of known brands.

- strengthening the relations with the main stakeholders of the ho.re.ca. channel and of the organised large-scale distribution system to encourage the entry and the permanence of authentic Italian products on foreign markets.
- educational information and communication campaigns on the true value of the "really" Italian product in order to inform and protect the final consumers.

With regard to this last point, first of all it is necessary carry out studies that compare PDO products with those counterfeited, highlighting peculiarities and differences, through sensory evaluations and experimental analytical methods.

To ensure the food quality and safety a correct process management is certainly necessary but. at the same time, also the knowledge and the application of chemico-physical, microbiological and sensorial instrumental techniques are equally important, whose fields of application and the effectiveness in representing the food alteration phenomena must be known (Belletti & Marescotti, 2004).

It is precisely through the physico-chemical characterization that it is possible to identify objective and analytically measurable parameters ("quality markers").

These markers are very useful for optimizing the initial quality of food and for describing the processes and the reaction mechanisms that occur during technological treatments, fermentation processes or the shelf life of the product. Using these descriptors it's possible to define: the quality classes, evaluate the product genuineness and ascertain the correct denomination of many foods.

In Italy there are 47 PDO and 1 PGI (Protected Geographical Indication) cheeses and dairy products, which makes it the second world producer of PDO cheese (Fig. 2). The PDO cheeses have specific characteristics and enhanced quality, and are defined according to their geographical area of production, as well as in terms of the material and technology used in their manufacturing. Certainly, the determination of origin is a key component for PDO.

Fig. 2 Italian cheeses and dairy products with Denomination of Origin and Geographical Indication (http://www.afidop.it/)

New tools and strategies need to be developed to assess the authenticity of these high-quality products, protecting consumers against cheaper industrial imitations. Besides, regulatory agencies are concerned with the prevention of economic fraud, while food processor needs confirmation of added-value claims showed on the product label (i.e. food ingredient authenticity, geographic origin, processing history etc.) in order to protect a brand (Fadzilillah *et al.*, 2013; Aparicio *et al.*, 2000).

2.3 CASE STUDY I

AUTHENTICATION ANALYSIS OF PDO BUFFALO MOZZARELLA USING FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY COUPLED WITH CHEMOMETRIC TOOLS

One of the world's most popular Italian products with Protected Designation of Origin is the buffalo mozzarella, a stretched curd cheese exclusively made with buffalo milk from Campania and produced using a process that meets the PDO specification (Fig. 3).

Fig. 3 The logos that distinguish the PDO buffalo mozzarella made in Campania.

Buffalo milk's composition is different from cow's or sheep's milk, in that it is rich in protein, fat and calcium. The peculiarity of this cheese is entirely due to the technology used in its traditional preparation. Furthermore, since it does not contain carotenoids, the mozzarella is very bright, almost a porcelain white colour.

Many factors such as composition of the milk, moisture, starter cultures, manufacturing process, and biochemical events occurring during maturation affect the quality of mozzarella. There are sensible differences between traditional and industrial buffalo cheese-making processes, and the market prices of the two final products reflect such differences.

Thus, a considerable interest is increasing in the development of instrumental techniques to enable faster, more objective and less expensive assessment to define and control the qualitative characteristic of typical cheeses. The methods used for the verification of food authenticity and traceability have to be suitable for the prevention of deliberate or accidental mislabeling. They are important for commercial reasons and play a considerable role in the assurance of public health.

Rapid, inexpensive and reliable analytical methods are required to measure food characteristics for authenticity and traceability purposes, and to link a finished product to its ingredients. These analytical techniques have to be applied to the food manufacturing industry for process performance evaluation, detection of faults, and achievement of persistently high-quality food product (Fadzilillah *et al.*, 2013).

Infrared spectroscopy is an attractive technology for rapid, inexpensive, sensitive, and high-throughput analysis of food components which does not require special skills by the users.

In these last years, Infrared spectroscopy has been applied for the authentication of products by commodity, variety and geographic origin. The potential of IR spectroscopy has been shown in monitoring the geographic origin of cheeses (Karoui *et al.*, 2005(a), 2005(b)) and for discriminating them according to the nature of the milk used in the cheese-making process (Picque *et al.*, 2002, Subramanian *et al* 2009). Specifically, mid-infrared spectroscopy (4000 - 700 cm^{-1}) has been used not only to monitor the geographic origin, but also to determine the major components in cheese, such as protein, fat, moisture, lactic acid, sugars, casein, cholesterol, etc. (Rodriguez-Saona *et al.*, 2007; Subramanian *et al* 2008), each with a distinct and reproducible biochemical fingerprint.

In this study, the potential of mid-infrared spectroscopy coupled with chemometric tools was evaluated for the authentication and discrimination of PDO buffalo mozzarella produced by traditional and industrial cheese-making processes.

Samples of mozzarella provided by local producers and supermarkets were analysed through both official destructive methods and Attenuated Total Reflectance-Fourier transform infrared spectroscopy (FTIR/ATR). In particular, destructive methods allowed to determine the content of fatty substances, proteins, moisture and total nitrogen.

The objectives of the present research were the following:

- developing a simple and rapid screening tool for the definition of the qualitative characteristics of Italian buffalo-cheese by means of Fourier transform infrared spectroscopy (FTIR);
- discriminating between traditional and industrial cheese-making processes by means of a statistical model working on measurements of the absorbance at different wavelengths.

EXPERIMENTAL PART

Fig. 4 PDO buffalo mozzarella

We purchased twenty samples of PDO Italian buffalo mozzarella (Fig. 4) from authorized manufacturers and supermarkets, and according to their cheese-making process and we categorized them into two groups. Specifically, ten samples of traditional cheese-making mozzarella and ten samples of industrial cheese-making mozzarella were collected in different production dates and analysed on the same day of purchase.

First of all we determined the fat content via Gerber reference method using Soxtec system, the moisture content via vacuum-oven method, and the protein content applying the Kjeldahl method (Bradley, 1992; AOAC, 1995), testing all the samples in duplicate.

Subsequently, the PDO buffalo mozzarella samples were analysed using Fourier Transform Infrared spectrometer (Spectrum 1000 PerkinElmer), mounted with an ATR accessory equipped with a grip (Fig. 5).

Fig. 5 Fourier Transform Infrared spectrometer (Spectrum1000 PerkinElmr), mounted with an ATR accessory equipped with a grip.

Thin sections of samples were taken from the centre of the mozzarella and pressed through the high-pressure clamp to ensure good contact between the sample and the crystal. Infrared spectra were recorded in the 3000-900 cm^{-1} region, and 64 scans were coadded for each spectrum at 4cm^{-1} resolution in order to improve the signal to noise ratio.

The data analysed concerned the values of absorbance registered at different wavelengths for the two categories of PDO buffalo mozzarella. The data were intended to reflect the spectral range of 3000–1000, which is the typical range of measurements suggested by literature (Rodriguez-Saona *et al.*, 2007; Van de Voort *et al.*, 1994 a; Bertrand and Dufour, 2006; Cevoli *et al.* 2013).

FTIR technique was applied to identify the main types of structure present in the analysed samples, through the correct association between absorption bands and fundamental vibrations of functional groups of molecule. The intensities of the different bands are correlated with the concentration of the chemical functional groups. Figure 6 shows the mid infrared spectra of traditional and industrial PDO buffalo mozzarella between 3000 cm^{-1} and 900 cm^{-1}.

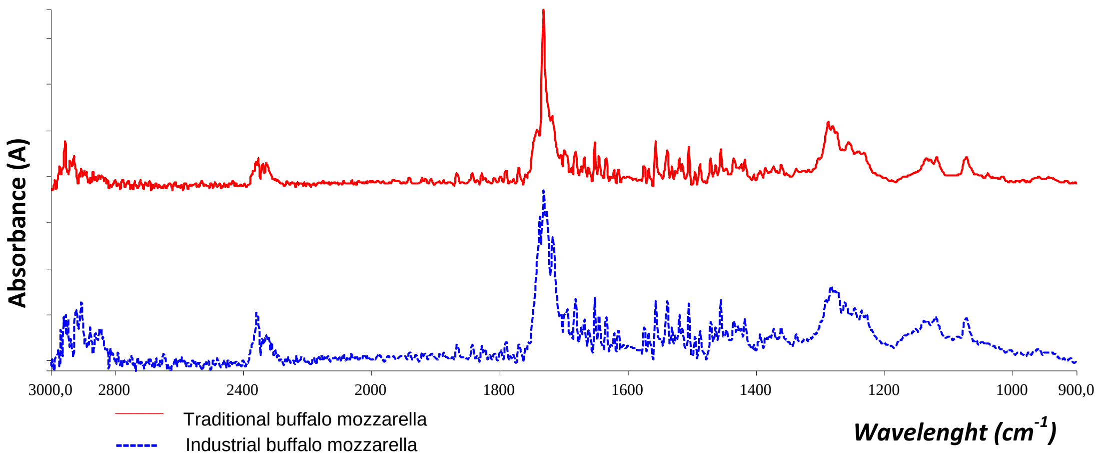


Fig. 6 Mid Infrared spectra of traditional (red solid line), and industrial (blue dotted line) PDP buffalo mozzarella between 3000 cm^{-1} and 900 cm^{-1}.

According to Boubellouta *et al.,* (2010), three spectral regions were identified to be the most important to assess the quality attributes of cheese:

- 3000-2800 cm^{-1} region corresponding to lipids,
- 1700-1500 cm^{-1} corresponding to amide I and II bands,
- 1500-900 cm^{-1} corresponding to carbohydrates region.

It is worth noting that the spectral values of 1700-700 cm^{-1} are considered to be "fingerprint" values, specific fingerprint of each compound (Cevoli *et al.* 2013; Nurrulhidayah *et al.* 2013; Reid *et al.*, 2006).

From literature, it is known that the area has a coverage between 800-1200 and 1000-1500 cm^{-1} (Van de Voort *et al.*, 1994 (a), 1994 (b)) but, this study has extended it to 1700 cm^{-1}. The region between 3000-2800 cm^{-1} consists of absorbance from C-H stretching vibrations of –CH_3 and >CH_2 functional groups of fatty acids (Bertrand and Dufour, 2006). Specifically, the intervals 2900-2827 cm^{-1} and 1782 to 1705 cm^{-1} correspond to fat content in cheese. Figure 7 shows two major bands corresponding to methylene/methyl groups, that were observed at ~ 2963 and at ~ 2955 cm^{-1}. The observed changes in methyl and methylene bands were attributed to difference in nature concentration and physical state of fatty acids (Boubelouta *et al.*, 2010).

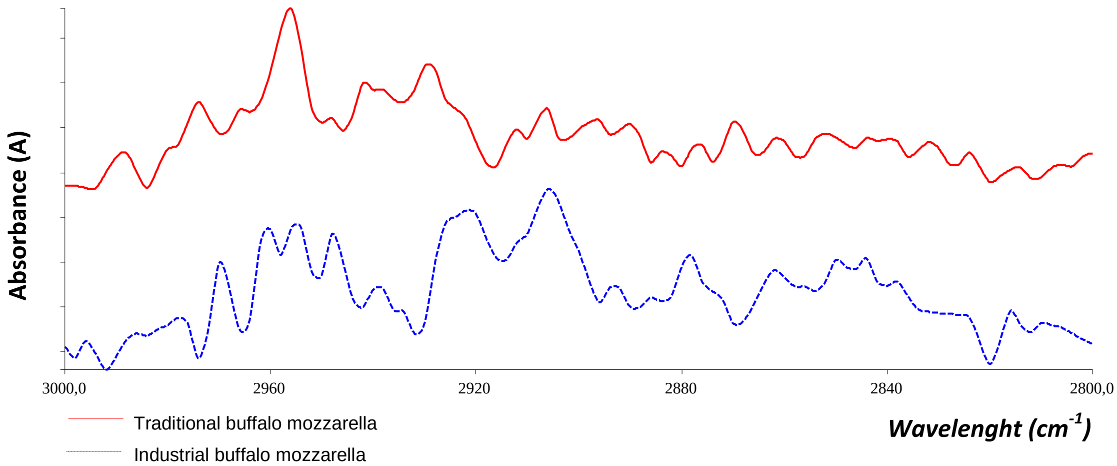


Fig. 7 Spectra of traditional buffalo mozzarella (red solid line), and industrial buffalo mozzarella

The spectral region between 1701-1507 cm^{-1} corresponds to protein content. Figure 8 shows spectra of traditional buffalo cheese (solid line), and industrial buffalo cheese (dotted line) in the region between 1850 and 1500 cm^{-1}. Two well-defined peaks were observed at 1715 and 1730 cm^{-1} associated with esters and organic acids. Besides, the solid line in figure 8 shows many other picks, two of which are associated with the Amide I at ~ 1651 cm^{-1} , and Amide II at ~ 1556 cm^{-1} (Rodriguez-Saona *et al.*, 2007, Mendenhall & Brown 1991). According to Bertrand and Dufour, 2006, these two peaks are probably associated with hydrolysed proteins. Changes in intensity and position of these bands in the 1700-1500 cm^{-1} range were associated with changes in casein secondary structure, protein aggregation and protein-water interaction (Othman *et al.,* 2012; Bertrand and Dufour, 2006; Karoui *et al.*, 2006; Mazerolles *et al.,* 2001).

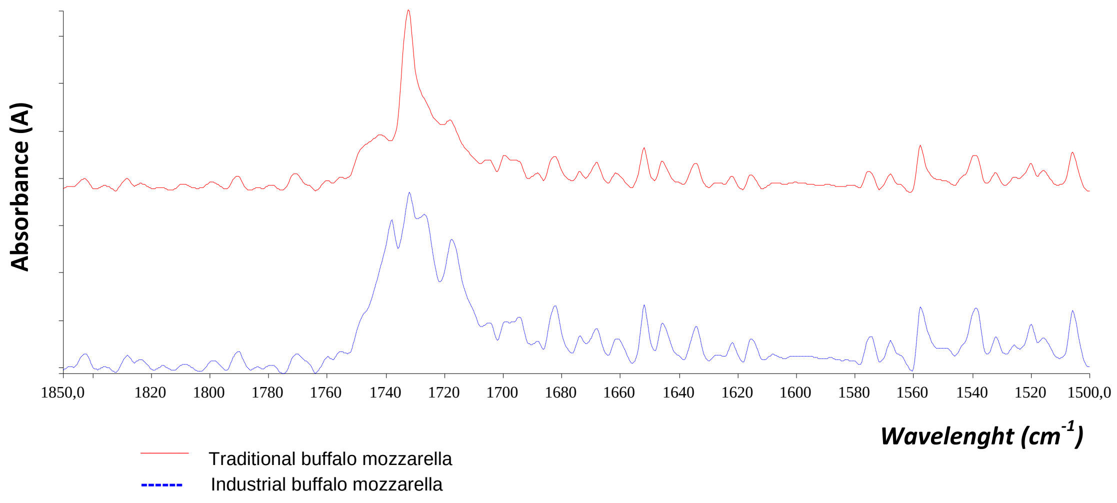


Fig. 8 Spectra of traditional buffalo mozzarella (red solid line), and industrial buffalo mozzarella (blue dotted line) in the region between 1850 and 1500 cm^{-1}.

In the third region of FTIR spectra (1500-900 cm^{-1}), wavelengths for specific chemical groups were recognised: 1477-1400 and 1195-1129 cm^{-1} for ester carbonyl C-H and C-O group (Belton *et al.*,1988). The solid line in figure 9 depicts more peaks in the range between 1477 and 1400 cm^{-1}, but we can find in both spectra (solid line and dotted line) peaks at ~ 1420, 1435, 1470 cm^{-1}, in spite of a different absorbance. The highest peak was observed at ~ 1289 cm^{-1}. In both spectra were observed two peaks: at ~ 1073 cm^{-1} and at ~ 1120 cm^{-1}, probably related to sum of lactose, monosaccharide (Karoui *et al* 2006, Petibois *et al.*, 2000), and the ester linkage of lipids (Martin del Campo *et al.*, 2007-2009). No well-defined peaks were attributed to glucose and galactose - which may be located at ~ 1377 cm^{-1}, according to Othman *et al.*, 2012.

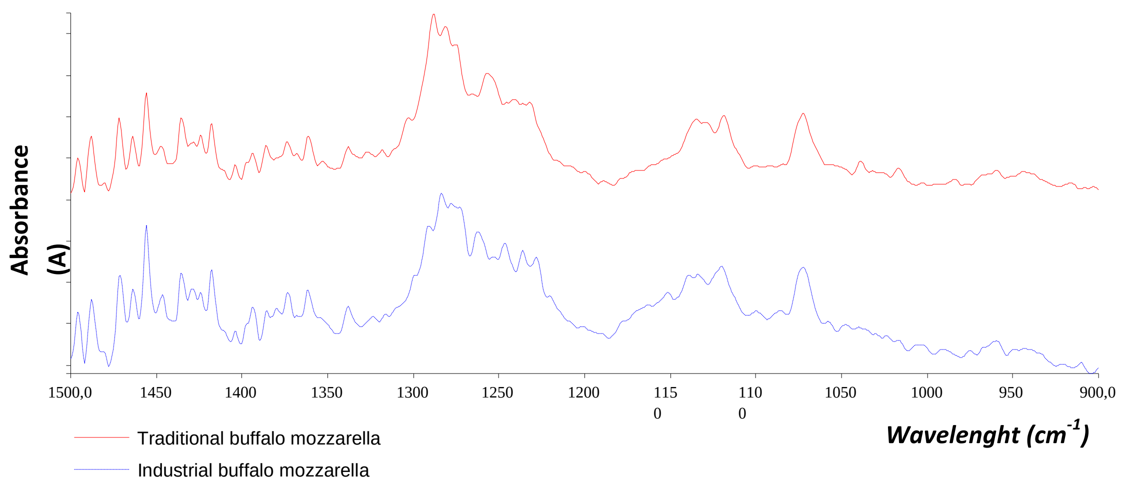


Fig. 9 Spectra of traditional buffalo cheese (red solid line), and industrial buffalo cheese (blue dotted line) in the region between 1500 and 900 cm^{-1}.

Throughout the MIR region (3000-900 cm^{-1}), and specifically in the intervals 2900-2827 and 1782-1705 cm^{-1} (corresponding to fat content in cheese), in the range between 1701 and 1507 cm^{-1} (corresponding to protein content), and finally in that from 1200 to 967 cm^{-1} (carbohydrates), the “traditional” buffalo cheese samples showed higher absorbance, corroborating the chemical and physical results according to which these samples possess a higher content of protein and fat respect those obtained through industrial buffalo cheese-making processes. Considering that the absorbance intensity of fat-and-protein-related bands increased as a consequence of an increase in fat and protein contents, these findings highlight the importance of discriminating among different regions across the entire spectral range to predict the quality attributes of buffalo mozzarella.

The absorbance areas of fat related and protein-related peaks for different cheese samples were compared with the fat and protein contents by physico-chemical analysis. Absorbance of fat-related bands decreased with a reduction of fat level in cheese as expected. The change due to protein related bands was opposite to that due to fat-related bands. Results were in agreement with proximate analysis, since a reduction in fat constituted a slight increase in protein content (Mendehall et l., 1991; Irudayaraj *et al.*, 1997).

Although the results obtained by physico-chemical analysis were in accordance with the FTIR spectroscopic findings, they did not show substantial differences between the two cheese-making processes (Table 1) and did not allow to discriminate between the two types of mozzarella.

Table 1. Mean (±SD) of the physicochemical characteristics of traditional and industrial buffalo-cheese making processes.

Parameters	*Traditional buffalo mozzarella*	*Industrial buffalo mozzarella*
pH	5,30 (±0,09)	5,22 (±0,08)
Lactic Acid %	0,47 (±0,02)	0,48 (±0,03)
Fat%	23,2 (±0,82)	22,6 (±0,57)
Protein%	16,2 (±0,38)	12,5 (±0,32)

However, it seems possible to check any variations in buffalo cheese samples analysing and comparing the absorbance values registered at the different wavelength by FTIR spectroscopy. This tool could be also used to implement the traceability of products (especially when they have to be marketed abroad) and identify substances (such as soda and bleaching) commonly used in phenomena like "Italian sounding", therefore protecting Italian products and consumer choices.

The main issue of FTIR spectroscopy application in buffalo mozzarella analysis is related to the homogeneity of samples. Although every effort was made to obtain uniform samples, the mozzarella samples used in FTIR experiments were not homogeneous: a probable reason could be the non-homogeneity of fat and bound moisture in the protein matrix, and the presence of voids in the cheese matrix. Even though several pieces from each cheese slab were analysed and results averaged in order to overcome this problem, only the conjunction of MID-infrared spectroscopy with chemometric tools can provide satisfying results for the intended purpose.

Figure 10 depicts a bivariate density of wavelength (λ) and absorbance (A) estimated from the sample for four ranges: a) 3000–2800 cm^{-1}; b) 2800–1700 cm^{-1}; c) 1700–1000 cm^{-1}. The figure shows that the absorbance in traditional products tends to be higher than in industrial products for a given wavelength. The figure also confirms that in the fingerprint region there is a clear discrimination between types of products (Cevoli *et al.* 2013; Nurrulhidayah *et al.* 2013; Reid *et al.*, 2006). For greater values of λ, the overlap of densities becomes more and more problematic. In particular, region a) is the one with the highest overlap.

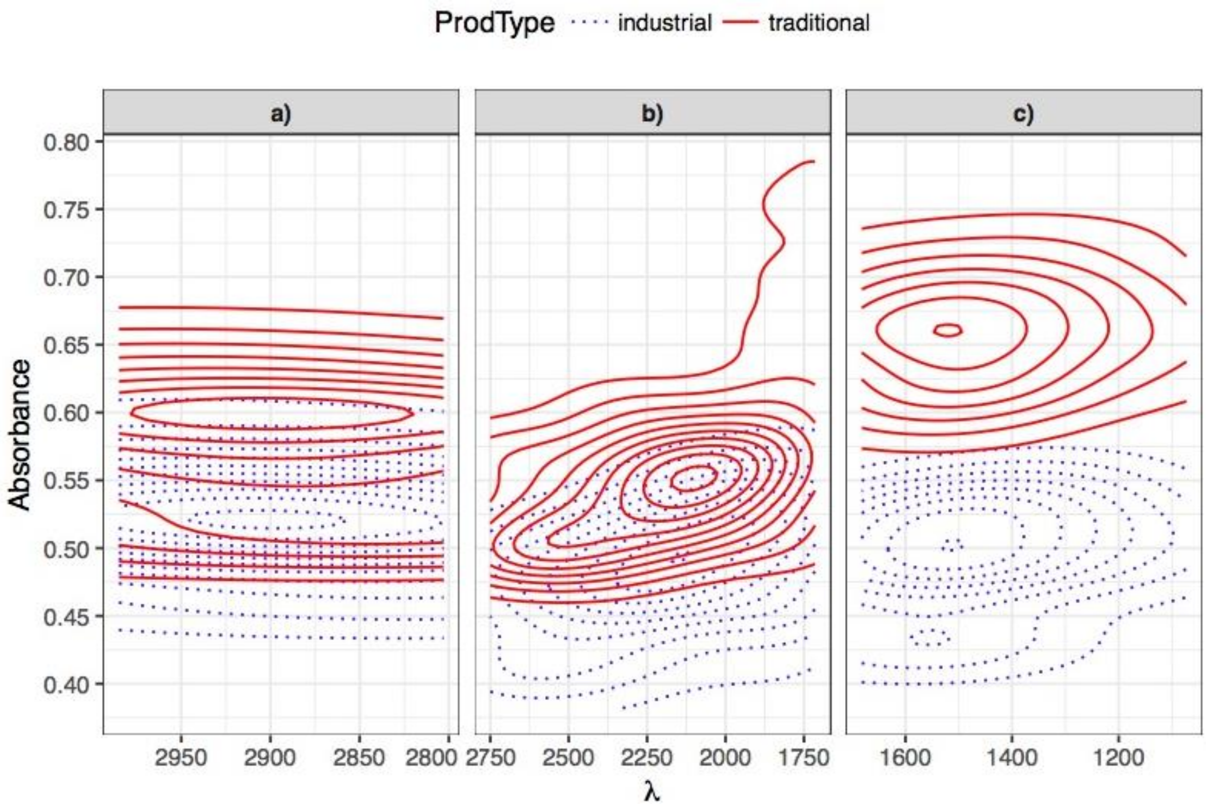


Fig. 10 Bivariate density of wavelength and absorbance for the ranges of measurement considered.

In order to discriminate between the two types of buffalo mozzarella, a logistic regression (McCullagh and Nelder, 1989) was exploited. Through such a model, it is possible to predict the probability that a unit belongs to a given category as a function of some explanatory variables. For the current study, the chosen category was the traditional DOP type of mozzarella, whereas the explanatory variables were absorbance and wavelength. Introducing also a random component accounting for the specific mozzarella on which each measurement was performed did not seem to alter the model substantially, and therefore all the measurements were finally treated as independent. In particular, the probability $\Pr(.)$ of a traditional DOP product was modelled as a function of the absorbance and the wavelength using the following specification:

$$\Pr(t \mid \mathrm{A}, \lambda) = \frac{1}{1 + \exp[-(\beta_0 + \beta_1 \mathrm{A}^4 + \beta_2 \lambda^5 + \beta_3 \lambda^6)]}$$

where t indicates the traditional category, A the absorbance, λ the wavelength, and β_i $(i = 1,2,3,4)$ are the model coefficients to be estimated.
This model seems to be able to fit the data properly in the spectral ranges considered, and account for the nonlinear patterns. This comes with almost no loss in interpretability. Table 2 shows the model summary, where all the coefficients are highly significant. The absorbance has a remarkable positive impact on t: the higher the absorbance, the higher the probability that the cheese is traditional.

Table 2. Summary of the logistic regression

Coefficient	Estimate	Std. Error	z value	Pr (>|z|)
β_0	-1.05×10^{1}	8.13×10^{-1}	−12.86	<0.001
β_1	8.40×10^{1}	6.30×10^{0}	13.32	<0.001
β_2	3.22×10^{-16}	3.34×10^{-17}	9.65	<0.001
β_3	$1{-}1.06 \times 10^{-19}$	1.27×10^{-20}	−9.66	<0.001

The effect of λ is estimated through $\hat{\beta}_2$ and $\hat{\beta}_3$. When the absorbance is kept at the mean value in the dataset (i.e. ~0.55), the behaviour of the wavelength against the odds of traditional product is the one depicted in Figure 11: the marginal effect of λ increases from 3000 cm^{-1} to 2528 cm^{-1} and decreases from 2528 cm^{-1} to 1000 cm^{-1}, being negative before 2890 cm^{-1} and after 1912 cm^{-1}. This would be noticeable in Figure 10 if one drew a horizontal line at the average value of absorbance: such a line would clearly cross the density of the traditional category on the left side of panel b), and cross the density of the industrial category in panels c), i.e. in the fingerprint area.

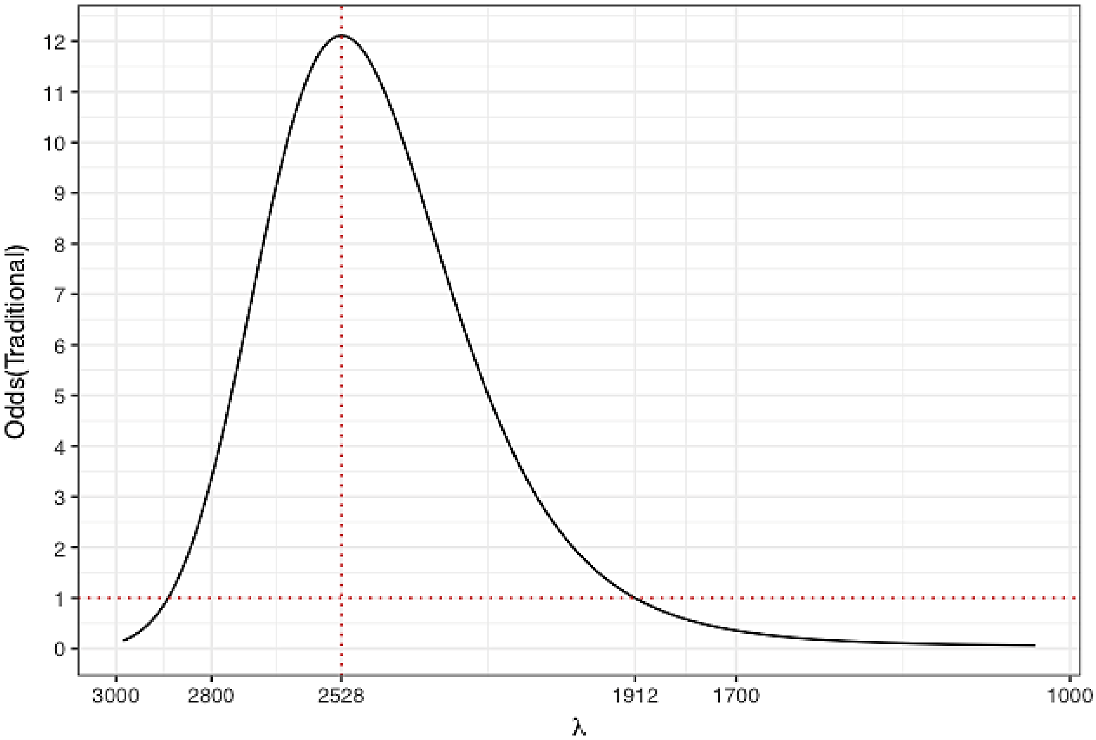


Fig. 11 Odds of a traditional product as a function of the wavelength (absorbance is kept at its mean value)

Figure 12 shows the scatterplot of absorbance against λ, with the predicted boundary separating the two categories of product. Such a boundary corresponds to a fitted probability of 50%. The figure shows that the misclassification error decreases to 0 when moving to the fingerprint area.

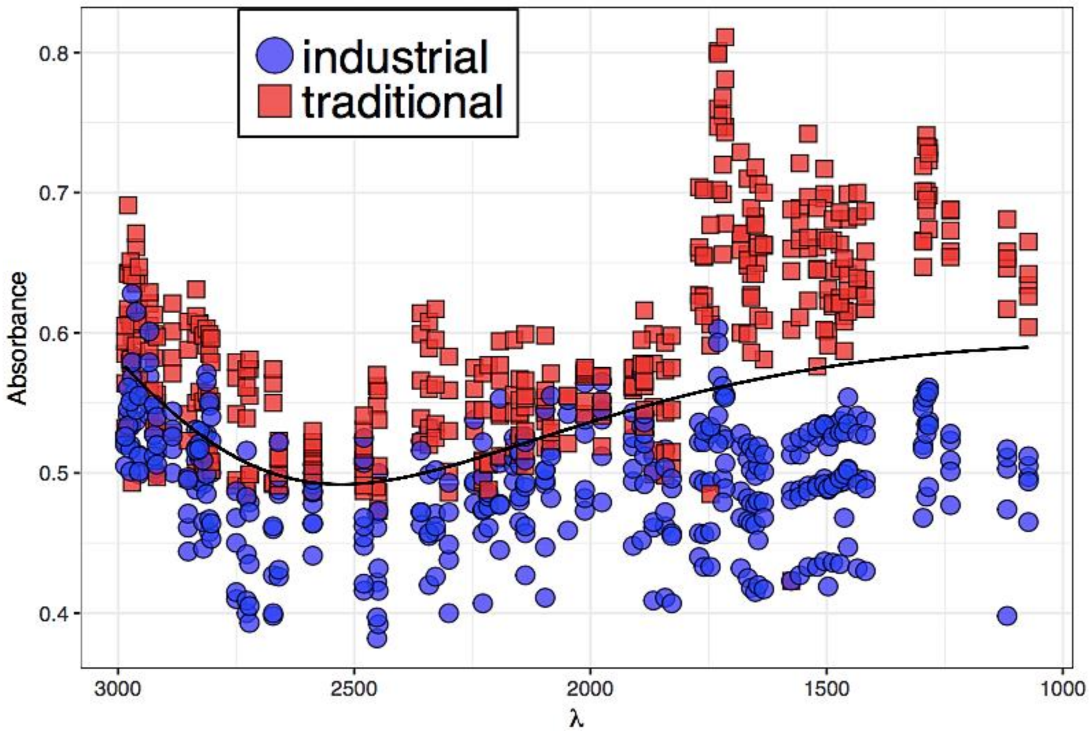


Fig. 12 Scatterplot of absorbance vs wavelength with fitted decision boundary

CONCLUSION

Results from this study showed that industrial technology has a large impact on Italian buffalo mozzarella quality, significantly affecting the texture attributes of cheeses, despite similar characteristics in composition. FT-IR spectroscopy could contribute to the development of simple and rapid protocols for monitoring complex biochemical changes, and predicting the final quality of cheese. Variation in absorbance intensity of fat and protein-related bands changed greatly due to variation in cheese-making procedure.

However, the main difficulty in the application of FTIR spectroscopy to buffalo mozzarella analysis was related to the homogeneity of samples. Although several pieces form each cheese slab were analysed and results averaged in order to overcome this problem, only the conjunction of MID-infrared spectroscopy with chemometric analysis can provide an exceptional tool to confirm cheese quality and classify products according to their manufacturing process. This could also represent an opportunity to implement the dietary product quality traceability system, and contrast the adulteration, the sophistication, and other phenomena of "Italian sounding". The worthy intent is to protect both the local economy and the consumers’ choices.

Chapter III

Quality and Consumers

The organoleptic quality of a specific product is related to the evaluation of some its intrinsic characteristics, such as the appearance, the aroma and the consistency, perceived through the sense organs; while the sensorial quality consists in the consumers acceptance of the perceivable characteristics of the product, regardless of whether the consumers are regular users of that specific "product category", or if they are simply included in the "target market" (Galvez & Resurreccion, 1992).
The difference between the two concepts is hold in the terms "perceptible characteristics". In the food field, this "expression" should underlie all the perceptions of the characteristics of a food product (safety, convenience, value, etc.) and not only those perceivable through the senses (Civille, 1991).
However, both definitions include:

- the consumer as referent;
- the use of acceptability as measure of quality;
- the relativity of the judgment reflected in the concepts of "product category" and "target market" (Cardello, 1995).

To date, no instrumental analytical method allows to evaluate the level of appreciation that the consumer will hold for a specific food product; for this reason today there is a growing interest in the use of consumer judgment and sensory analysis as a marketing decision-making tool (Garber *et al.*, 2003).

3.1 SENSORY ANALYSIS

The sensory science is “a scientific discipline used to evoke, measure, analyse and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing" (Stone and Sidel, 2004). This sciense is very important for the development of specific methods to evaluate food products in a problem-solving perspective.
It is based on a standardized analytical methodology and involves a group of selected tasters (panel test), in which everyone acts as a measuring instrument like any other laboratory instrument. The main objectives of the sensory analysis are the following:

- to study the relationship between the sensory characteristics and the sensations that they stir up both in terms of quality (definition of sensation) and quantity (intensity of perceived sensation);

- to outline a sensorial profile able to describe a specifical product in a univocal and objective way.
- sensorial characterization of the product (qualitative and quantitative identity);
- to analyse and validate the product shelf-life;
- to study the sensory properties of a food product, already present on the market, to enhance its merits or correct its defects (quality optimization);
- the development of a new product to be placed on the market, in order to acquire in advance the consumer acceptability and preferences (consumer science);
- enhancement of local products;
- checking the conformity of the product with the specific rules laid down in the EC Regulations and the production disciplinaries (PDO, PGI, ..).

Specifically, the sensory tests can be divided in two types: analytical sensory test, including discrimination and descriptive methods, and affective or hedonic tests, such as those invoved in assessing consumer liking or preferences (Lawless and Claassen, 1993).

The analytical sensory test allows to describe in objective terms the sensorial characteristics of a product through the measurements, performed by a trained panel, of the intensity of specific attributes concerning the various aspects of the perceivable quality.

In these cases, panelists are selected based on having average to doos sensory acuity for the cricial characteristics (tastes, smells, textures, etc.) of products to be evaluated. They are familiarized with the test procedures and may undergo greater or lesser amounts of training, depending on the method. In the case of descriptive analysis, they adopt an analytical frame of mind, focusing on specific aspects of the product as directed by the scales on their questionnaires (Stefanowicz, 2013). Generally, they are asked to put personal preferences and hedonic reactions aside, then they have “only” to specifiy what attributed are present in the product and at what levels of sensory intensity, extent, amount or duration (Stefanowicz, 2013).

To achieve this purpose rigorous descriptive sensory methods are applied (UNI EN ISO 11035: 1994). The applied method is that of quantitative descriptive analysis with a consensus vocabulary that has been developed following the indications of the UNI EN ISO 13299: 2016 standard.

Only by means of descriptive tests it is possible to outline the so-called "sensory profile", which are able to describe in a univocal and objective way the product under analysis (Pagliarini, 2002) and to provide important information on how certain processes and production parameters affect its perceptible organoleptic characteristics.

Different types of tests can be conducted: "profile verification", consensual profile, flash profiling, nipping, orientation tests, discriminating tests. Another field of application is linked to the analysis of the correspondence between a product and a standard.

The hedonic sensory analysis, instead, focuses on subjective impressions to evaluate, for example, the popularity of a product or the consumers preference. Although using qualitative and quantitative methods, hedonistic evaluations, unlike analytical ones, are carried out by consumers and use tools such as: preference mapping, focus groups, and interviews.

In this case, consumers perceive the product as a whole pattern. Also if their attention is sometimes captured by a specific aspect of a product (especially if it is unpleasant), their reactions to the product are often immediate and based on the integrated pattern of sensory stimulation from the product and expressed as liking or disliking (Stefanowics, 2013). This occurs without a great deal of thought or dissection of the product's specific profile. In other words, consumers are effective at rendering impressions based on the integrated pattern of perceptions. In such consumer tests, participants must be chosen carefully to ensure that the results will generalize to the population of interest. Participants should be frequent users of the product, since they are most likely to form the target market and will be familiar with similar products. They possess reasonable expectations and a frame of reference within which they can form an opinion relative to other similar products they've tried.

In the last decades, many efforts have been made to identify the correlation between analytical-instrumental and sensorial data (Garcia Loredo and Guerrero, 2011), as well as to highlight the validity and reliability of sensory analysis (Cartier *et al.*, 2006).

Although the judgments expressed on the hedonistic and sensory properties of agri-food products may be influenced by several factors, including some intrinsic characteristics of the product and other aspects related to subjective perceptual and cognitive mechanisms of man (Cardello et Sawyer, 1992; Prescott, 1999; Köster EP , 2007, Brattoli *et al.*, 2011), many studies have succeeded in demonstrating the importance of adopting both approaches, one in support of the other, in order to have a clearer and more complete picture to correctly interpret the data, and predict results (Meullenet *et al* 1998 Angerosa F., 2002; Benito *et al.*, 2010; Moussaoui *et al.*, 2010).

The correlation between the hedonistic and analytical analysis data with the phsico-chemical measurements results allow to identify the main factors and the components that influence the acceptance of a product and to highlight the differences between foods belonging to the same category, but with different origin and production process.

3.2 CONSUMER SCIENCE TOOLS

The use of sensory acceptability as measurement parameter of quality, consumer science, sometimes presents limits (Garber *et al.*, 2003) as it is not always sufficient to satisfy all the consumer needs and therefore to guarantee the choice (or the preference) of one product over another (Peri, 2006). For this reason, different parameters (expectation, acceptability, preference, willingness to pay, choice) are today used as a measure of quality intended as satisfaction of consumer needs.

Consumer tests aim to evaluate the differences between products and how these affect the tastes and choices of consumers who find in food products a hedonistic satisfaction.

The data collected from consumer tests have practical value in reducing the uncertainty level in decisions to be made about the product (improvement, development, optimization); these tests are therefore of great interest, especially in the field of marketing.
In the consumer test, the interviewees, identified as representative samples, are invited to express their opinion, expectations, satisfactions, preferences and their willingness to pay for a food product (Peter & Olson, 2008).
Consumer tests are characterized by the execution mode and the place where they are carried out (Resurreccion, 1998):

- The 'laboratory test' offers the advantage of being able to use a sensory evaluation laboratory equipped with all the necessary, thus minimizing the variability due to environmental conditions. On the other hand, the panelists sampling is more complicated, as well get to the analysis place could be a problem.
- The term “central location test” refers to consumer tests conducted in specially adapted facilities in convenient locations where larger groups of people can be gathered (supermarkets, food fairs etc.). This type of testing is usually used for research into new or modified products, though it also finds successful application in testing advertising or packaging materials. Central location tests employ both traditional – quantitative and qualitative – research techniques, as well as modern visual techniques (“eye-tracking”, “t scope”). In this type of test, data is gathered in the same, standardised conditions. Conducting interviews with respondents in the same space and at approximately the same time ensures control over external factors with the potential to impact the test results. Besides, it offers the advantage of an easy retrieval of the sample, but the number of questions that can be asked is limited by the time availability of the interviewed subjects, and the informations that can be obtained are therefore reduced.
- The "home use test" is another type of consumer test and it involves participants evaluating products in their own homes, or more generally, in a natural usage environment and normal conditions of use. This approach results in more realistic outcomes on product satisfaction, usage, and potential improvement areas. It usually represents a way to test a product with real consumers before moving forward with its launch on the market. Other advantages of this test are: the possibility of comparing two or more samples, more time to obtain information on certain characteristics of the product, the possibility of evaluating the effect of repeated or prolonged use of the product.

  The disadvantages are instead related to the maximum number of products that can be evaluated, the possibility of not receiving answers, the high costs and the possibility of errors in preparation phases that can lead to a considerable variability of analysis.
- Finally there is also the "simulated test market", based on a marketing research technique in which consumers are exposed to staged advertising and purchase decisions to observe their response to a new product. This test offers some

advantages: it can control the environmental condition and better explore the consumer behaviours. On the other side, also in this case, the subjects sampling and the analysis place can constiture problematic aspects.

All types of tests analysed share the following limits:

- the difficulty of identifying the sample of the target market population for the test;
- the difficulty of having a sufficient number of consumers to represent the entire population;
- the limited amount of information that can be requested and obtained from the consumer;
- the repeatability of the data.

Extreme care must be taken by those who design, execute, interpret and report data collected through tests performed by consulting consumers, in order to ensure the highest possible standards of validity and reliability (Schutz, 1999).

3.3 PROBLEM SOLVING

Consumer tests help to identify the best solutions for the problems that occur throughout all the agri-food chain (problem-solving approach), from the study of the product until its consumption, and its action field extends from the control quality to the market research (Martens, 1999). Consequently, they have achieved great success in the food industry in the fields of quality assurance, research and development (R & D) and product / process optimization (Resurreccion, 1998).

Today, particular attention is given to the relative importance study of the relative importance of sensory and non-sensory factors (demographic, cultural and motivational) on the satisfaction and preference of consumers.

The research includes studies on: - basic sensory and motivational aspects; - the effects of sensory, psychological, cultural, geographical and environmental variables on consumer preferences; - evolution of preferences; - experts and consumers quality perceptions; - correlations between measured and perceived quality; - quality assurance systems based on consumer tests; - relations between acceptability and preference.

Nevertheless it needs to be pointed out that the consumer test results doesn't have a "universal value" and that their application is limited to the sample interviewed or, anyway, to a particular segment of consumers.

At the same time, it must be recognized that such tests certainly have a practical value in reducing the uncertainty level in decisions to be made regarding a product in terms of improvement, development, quality control, price definition, market strategy (Peter & Olson, 2008).

With reference to this last point, in order to enhance the quality of certain foods, and to obtain reproducible results, it is necessary to promote information campaigns that help consumers in acquiring the necessary knowledge to discriminate similar products, as well as

to organize training sessions so that they are able to identify the main descriptors of the food matrices, the merits and defects of a product, while developing also a suitable language.
Many market analysis show significant misinformation of the consumers, both in relation to the products currently on the market, both in relation to their production process.
The consumers must be informed through a well-targeted communication that illustrates the complexity of the production chain and help them to choose with greater awareness. When consumers will be able to discriminate the products and the processes with which they are obtained, they will also be able to quantify the amount they are willing to pay more for a food product that they consider qualitatively better.

3.4 CASE STUDY II

IMPACT OF ORGANIC AND PDO LABELS IN THE PERCEPTION OF OLIVE OIL SENSORY QUALITY

A BRIEF INTRODUCTION

Olea europaea L. is one of the most ancient and important crops in the Mediterranean area, widely known especially for its principal product: the olive oil.

Olive oil is internationally appreciated for its nutritional value and health benefits; with its organoleptic characteristics, fine aroma and pleasant taste it has always represented a valuable market for Italy, which even in 2017 was confirmed as the first importer, second largest producer and exporter in the world.

In terms of olive-oil production, Italy ranks second in the world (after Spain), exporting 400.000 tonnes of olive oil per year, mainly represented by extra-virgin and virgin olive oils. Specifically, it is expected to reach 320 thousand tons by the end of crop year 2017/2018 (EC, 2017).

As regards olive-oil consumption, Italy is the world leader with a consumption of 600.000-700.000 tonnes, corresponding to about 12 kg per head of population. Therefore, taking into account the internal consumption and the amount of olive oil exported, Italy must necessarily import a large amount of olive oil, usually more than 500.000 tonnes/year.

The uncertainty in the national production, the competitive pricing of other exporters and the abundant availability of the product in other countries have led in recent years to an increase in olive oil imports, often characterized by lower quality, and in more frequently occurring of episodes of adulteration and counterfeiting.

This unfair competition not only discourages producers but also misleads consumers, so European Union created labels known as PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication), already described in the previous chapter to promote and protect traditional food products. Their directives (2081- 2082/1992) complement the directive on Organic Farming (2092/91) (Giraud, 2003). Specifically until the 27th of March 2016, according to the EC's DOOR database (Database Of Origin & Registration), Italy had registered 42 PDO olive oils (EC, 2016). However, also if food labelling is an important tool for consumer's perception of sustainability and quality of a product, can lure or distance consumers. According to recent studies, Italian olive oil consumers seem to be positively affected by PDO and Organic labels (Di Vita *et al.*, 2014; Vecchio R. *et al.,* 2011), although other authors argue that there is a different influence of quality and sustainability labelling on the purchase intentions and sensory acceptance.

National and regional products with PDO certification are preferred mainly for their higher perceived quality and respondents' interest to support the domestic producers (Velčovská & Del Chiappa, 2015), while food products with EU organic logo are often considered of inferior quality or even a fraud (Delmas and Grant, 2014).

Overall, although there is some knowledge and awareness about organic products, consumers are not consistent in their interpretation of what is organic. Also if, some consumers typically understand the broad issues about organic foods, many tend not to understand the complexities and niceties of organic farming practices and organic food quality attributes.

In the last years, uncertainty regarding the true attributes of organic, and skepticism about organic labels (part of which stems from reported cases of mislabeling, and product misrepresentation), and partly because of nonuniform organic standards and certification procedures, held some consumers back from purchasing organic food products (Yiridoe *et al.*, 2005). However, today there has been a growing concern for human health and safety, which is a key factor that influences consumer preference for organic food, which is motivating consumers to buy organic food as insurance and/or investment in health.

The authors thought it might be interesting to investigate the perception of quality and sustainability labelling of olive oils in a country of producers, connoisseurs but especially of habitual consumers. Specifically, in the central-southern regions of Italy there is the greatest number of olive oil producers and the highest presence of oil mills, in a context where small scale farming is widespread, the consumption of self-produced olive oil is relevant, as well as the purchase of local olive oil since the consumers are in direct proximity to the production market.

This study aimed to investigate the behaviour and the habits of the Italian consumers from central-southern Italy in relation to extra olive oil consumption, focusing on the impact of quality and sustainability labelling (respectively PDO and EU organic certification), on purchase intention and quality perception of products labelled by the quality and sustainability criteria.

EXPERIMENTAL PART

In the first part of this survey a specific questionnaire (see Appendix 1) was submitted to a random sample of people from central-southern Italy, by using internet, in order to collect socioeconomic information and analyse the behaviour of extra-virgin olive oil consumers, and their perception of quality attributes. In the second part, after completing the questionnaire, ten experts (which are part of a sensory tasting panel of olive oil), ten semi-experts and ten habitual consumers of olive oil tested three Italian olive oil samples: an extra virgin olive oil (EVOO) without quality or sustainability certification/logo), an organic extra virgin olive oil and a PDO extra virgin olive oil.

The samples were analysed twice: through a blind test first, and a normal one then. During the tasting, the judges answered some questions, then the answers obtained were thus statistically analysed and compared, while the three sample of olive oils were characterised also from a physico-chemical point of view.

The following questionnaire was submitted to 130 consumers from central-southern Italy to acquire information about their behaviour regarding the purchase of extra-virgin olive oil and their perception of its quality and sustainability attributes. The answers were obtained by an internet-based survey, following standard guidelines for web-based surveys: the questionnaire was self-administered included multiple choice questions, two open-ended questions and did not allow consumers to proceed to a new section until they had completed the preceding section (Delgado and Guinard, 2011).

The questionnaire was divided in three parts: in the first one multiple choice demographic questions were included, such as gender, age, educational level and family income (it was not obligatory to answer this last question).

I PART – DEMOGRAPHIC SECTION

1.	**Gender**	❑ M	❑ F			
2.	**Educational Level**	❑ Middle School	❑ High School	❑ Degree		
3.	**Age**	❑ 18-29	❑ 30-39	❑ 40-49	❑ 0-59	❑ +60
4.	**Family income(€)**	❑ < 25.000	❑ < 50.000	❑ < 75.000	❑ +75.000	

In the second part of the questionnaire, were included the following variables: frequency of olive oil consumption, places where consumers buy olive oil, reasons to consume olive oil, factors influencing olive oil purchasing and the importance of olive origin, categories of olive oil purchased and specifically the interest in organic and PDO olive oil, ability to recognize the EU organic and PDO logos among others proposed, willingness to pay for olive oil with EU organic certification, perception and preferences related to olive oil attributes (fruity, bitter, pungent).

Furthermore, respondents were asked to describe with three adjectives a generic PDO product and an Organic foodstuff and to choose an olive oil among those indicated in the table.

II PART – OLIVE OIL CONSUMPTION

5.	**Consumption Frequency**	❑ Once a year	❑ 3 or 4 times a month	❑ 2 or 3 times a week	❑ Daily
6.	**Purchase place**	❑ Supermarket	❑ Specialised Store	❑ Local Producer	❑ Own Production
7.	**Reason to buy**	❑ Healthy	❑ Tasty	❑ Good Seasoning	❑ Naturalness
8.	**Most Important Factor**	❑ Packaging ❑ Origin	❑ Experience ❑ DOP/EU organic certification	❑ Label	❑ Price

9. Less Important Factor	❑ Packaging ❑ Experience ❑ Label ❑ Price ❑ Origin ❑ DOP/EU organic certification
10. Is the origin of the olives an important factor when buying olive oil?	❑ Yes ❑ No
11. You usually buy olive oil, whose origin is…	❑ Regional ❑ National ❑ European ❑ Extra-EU
12. Generally, are you interested in PDO and / or Organic products?	❑ Yes ❑ No

13. Would you recognize the Organic production logo of the European Union among them?

❑ ❑ ❑ ❑ ❑ ❑

14. Would you recognize the PDO European logo?

❑ ❑ ❑ ❑ ❑

15. Have you ever bought Olive oil with EU Organic certification?	❑ Yes ❑ No
16. If so, how often?	❑ Once/twice a year ❑ Once/Twice a month ❑ Weekly
17. Have you ever bought Olive oil with PDO European	❑ Yes ❑ No
18. If so, how often?	❑ Once/twice a year ❑ Once/Twice a month ❑ Weekly

19. Which attributes come to mind when you think of Organic food products? Please, provide three answers.

1. **2.** **3.**

20. Which attributes come to mind when you think of PDO food products? Please, provide three answers.

1. **2.** **3.**

21. How much are you willing to pay more for Organic olive oil?	❑ Up to 10% ❑ Up to 20% ❑ Up to 50% ❑ More than 50%

22. Do you consider positive or negative the following attributes of olive oil?	***Fruity***	❑ Positive	❑ Negative
	Bitter	❑ Positive	❑ Negative
	Pungent	❑ Positive	❑ Negative

23. If you had to choose between two olive oils: one characterized by pronounced hints of fruity, bitter and spicy (A) and one in which such attributes are barely perceptible(B), which would you buy?	❑ A	❑ B

24. ***Finally, which one of the following olive oils would you buy?***

Sample	***Price*** (€)	***EU Organic Certification***	***PDO Certification***	***Origin***
1.	12,00	✓	✗	Italian
2.	12,00	✗	✓	Local
3.	9,50	✗	✓	Italian
4.	9,50	✓	✗	Local
5.	8,00	✗	✗	Local
6.	8,00	✗	✗	Italian

This questionnaire was subsequently administered to ten experts, ten semi-experts and ten normal consumers, but in this case a third part was included through which they expressed their opinions (and preferences) on three samples of olive oil: an extra virgin olive oil (EVOO) of a well-known brand, but without quality or sustainability certification/logo, an EU organic extra virgin olive oil and a PDO extra virgin olive oil, produced by the same olive farm and purchased from a specialised store.
The characteristics of each oil present on the label are summarized in the following table (Table 3):

EVOO	organic EVOO	PDO EVOO

EVOO

Nutrition Information Typical Values 100ml	
Energy	**3389kj/824kcal**
Total Fat:	**91,6g**
➢ Saturated Fat	**14g**
Total Carbohydrate:	**0g**
➢ Sugars	**0g**
Dietary Fiber	**0g**
Protein	**0g**
Salt	**0g**

CHEMICAL-PHYSICAL SPECIFICATIONS	LEGAL LIMIT	*LIMIT SET BY THE *BRAND*
Acidity (%)	≤0,8	≤0,5
Peroxides (mEq O_2/Kg)	≤20	≤10
Ethyl Esters (mg/kg)	≤35	≤20
Uv Absorption		
K232	≤2,50	≤2,10
K270	≤0,22	≤0,15
ΔK	≤0,01	≤0,005
Waxes (mg/Kg)	≤150	≤100

organic EVOO

Nutrition Information Typical Values 100ml		%DRV (Daily Reference Value) Per 100ml
Energy	**3389kj/824kcal**	
Total Fat:	**91,6g**	
➢ Saturated Fat	**14g**	
➢ Monounsaturated Fat	**69,6g**	
➢ Polyunsaturated Fat	**8g**	
Total Carbohydrate:	**0g**	
➢ Sugars	**0g**	
Dietary Fiber	**0g**	
Protein	**0g**	
Salt	**0g**	
Vitamin E	**20,5g**	**171**

PDO EVOO

Nutrition Information Typical Values 100ml	
Energy	**3389kj/824kcal**
Total Fat:	**91,6g**
➢ Saturated Fat	**14g**
Total Carbohydrate:	**0g**
➢ Sugars	**0g**
Dietary Fiber	**0g**
Protein	**0g**
Salt	**0g**

Table 3. Nutritional labelling of olive oil samples used in the panel tests.

The panelists analysed these samples twice: firstly with a blind test, then with a normal one.

III PART – *a) Panel Test (Blind Test)*

Parameters	***Sample A***	***Sample B***	***Sample C***
How **fruit** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
How **bitter** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
How **pungent** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
Would you buy it? (Yes/No)	❑ **Yes** ❑ **No**	❑ **Yes** ❑ **No**	❑ **Yes** ❑ **No**
Willingness to pay (WTP) for this sample Select one of the following options:	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€
Which sample did you prefer? Order numerically the samples according to your preferences (**1**,**2**,**3**: "1" the most appreciated, "3" the less appreciated).	❑	❑	❑

III PART – b) Panel Test (Normal test)

Parameters	***EVOO***	***PDO***	***EU Organic***
How **fruit** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
How **bitter** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
How **pungent** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
Would you buy it? (Yes/No)	❑ **Yes** ❑ **No**	❑ **Yes** ❑ **No**	❑ **Yes** ❑ **No**
Willingness to pay (WTP) for this sample Select one of the following options:	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€
Which sample did you prefer? Order numerically the samples according to your preferences (**1**,**2**,**3**: "1" the most appreciated, "3" the less appreciated).	❑	❑	❑

In order to understand whether the quality and organic labelling can affect the flavor perception and purchase intention of olive oils, sensory acceptance and purchase intention tests were performed on two sessions, through the third part of the questionnaire showed above.

The sensory evaluation was performed in individual cabins in the sensory analysis laboratory in the Faculty of Economics and Law of University of Cassino and Southern Lazio. Sampled were served at 25°C in cobalt blue tasting glass to consumers, who tasted them in two following step, according to a randomized complete block design (MacFie *et al.*, 1989).

Green Granny Smith apples and very sparkling water were provided to cleanse palate in between tastings.

A blind test was carried out in the first evaluation session, and in the second session, judges were informed about the peculiarities of each sample. In both cases the judges had to compiled the evaluation forms. After answering the questionnaire, consumers received a card with explanations about the products tested and their characteristics.

In this occasion we showed them also the results obtained by the analysis of the physicochemical quality parameters of the samples. These parameters: Free acidity, peroxide value, UV absorption characteristics, K270 and K232, were determined following

the analytical methods described in Regulation EEC/2568/91 of the Commission of the European Union, in order to characterise the samples but also to guarantee that they were chemically free from defects.
Finally, the answers were elaborated and analysed.

Results

In the first part of our research we submitted the questionnaire to 130 consumers from central- southern Italy by using Internet and the data collected with reference to demographic section are shown below (Fig. 13). The sample is mainly represented by young people, whose age is between 18 and 40 years: this is probably due to their greater familiarity in using internet, compared to the middle-aged persons and elderlies. In general, family income is medium-low, while there are no significant data in relation to the gender.

I PART – DEMOGRAPHIC SECTION

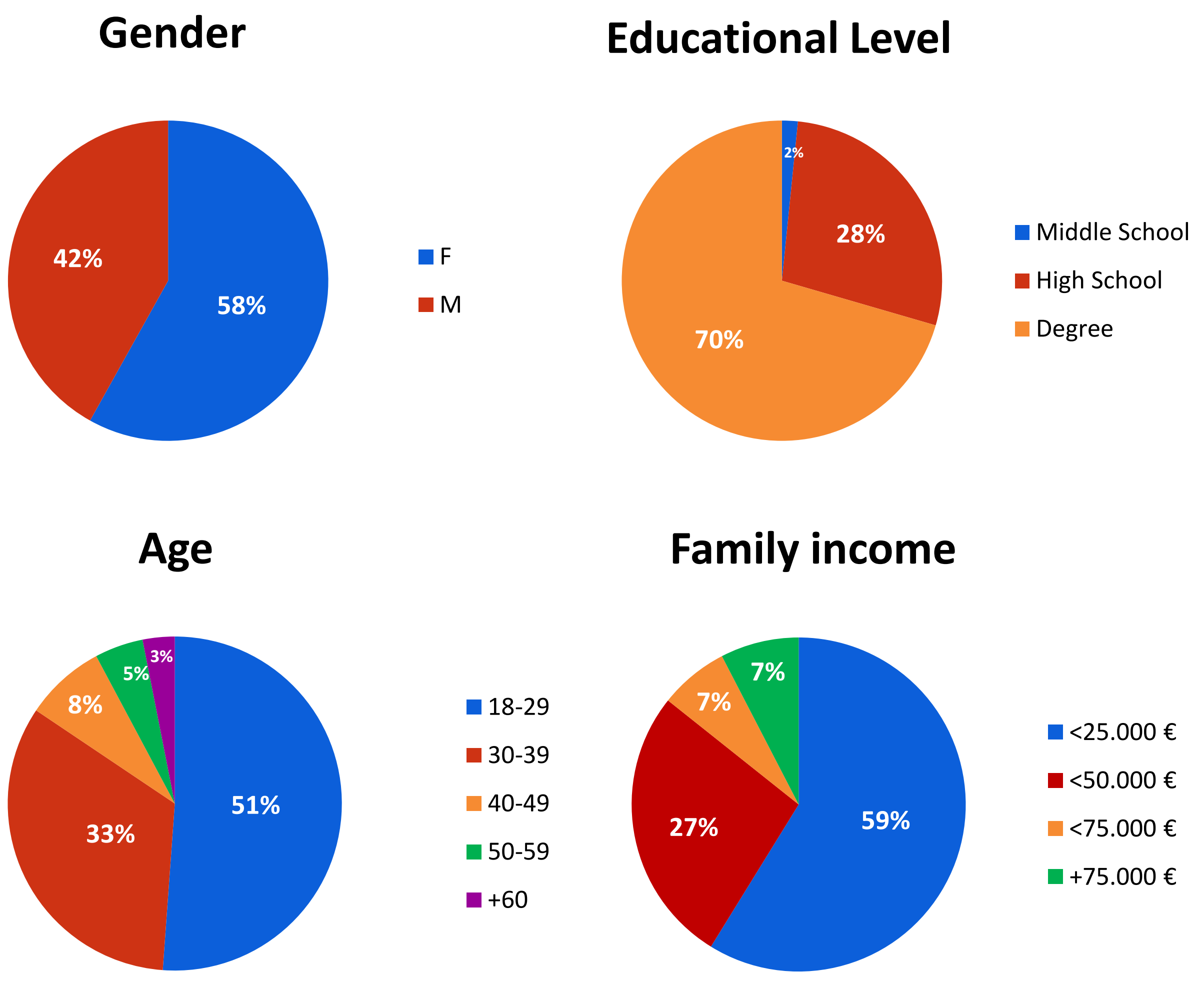


Fig. 13 Data obtained from the administration of the questionnaire in relation to "gender", "educational level", "age" and "family income".

II PART – OLIVE OIL CONSUMPTION

Respondents state that they mainly consume their own olive oil (37%) or that bought it from local sellers (38%). Only 23% of them buy olive oil at the supermarket. This result is justified by the greater number of olive oil producers and by higher presence of oil mills in the central-southern regions of Italy. Consumers in fact are in direct proximity to the production market, thereby facilitating the purchase of local olive oil. Besides, in a context where small scale farming is widespread, the consumption of self-produced olive oil is relevant.

According to results, the reasons for buying olive oil are different: because it is a natural product, it is healthy, tastes good and it is certainly a good seasoning for pasta and salads. Moreover, it emerges that the most important factor at the time of purchase is the "origin", whilst the less important is the "packaging".

All the respondents declared that the origin of the olives is important; the 59% of them usually buy regional olive oil, while the others buy national products. 80% of respondents are interested in organic products, also if only 66% are able to recognize the EU organic farming logo, and only half of those questioned buy them. Among those who declare to buy organic olive oils, 51% do it once/twice a year, 38% once/twice a month, 11% once a week.

On the other hand, for the PDO products, 54% of respondents recognized the corresponding logo, and among those who did wrong, 18% confused the PDO label with that of the "Protected Geographical Indication".

Nevertheless, 60% of respondents buy PDO olive oils, but among them 59% with a frequency of 1-2 times a year and 30% with a frequency of 1-2 times a month. 77% of respondents indicated that they were willing to pay less than a 20% premium for organic extra virgin olive oils as compared to other products without labels.

Subsequently, the consumers described the "organic" and "PDO" terms, having only three adjectives available for each one.

In the following table (Table 4), we have tried to group all the adjectives into categories and, in order to highlight the most frequent answers, we decided to create specific "word clouds" (Fig. 14)

	% of the *Total answers*	
Adjectives	**PDO**	**Organic**
Healthy	6,6	**24,4**
Food fraud	0,9	2,8
Untreated	2,7	**30,8**
Best or Better quality	**16,0**	7,0
Safe	**33,4**	9,8
Eco-friendly	0,9	**15,4**
Certified	**15,1**	1,1
Expensive	0,9	1,7
Local	10,8	2,5
Traditional	5,1	1,1
Limited	5,4	1,4
Others…	2,1	2,0

Table. 4 Adjectives provided by respondents to define the terms "PDO" and "Organic"

Fig 14. The word cloud on the left was created with the adjectives provided for the term "PDO", on the right instead there is the word cloud related to the "organic" term.

As can be seen above, PDO products suggest ideas of "safety", "certification", "quality" and "local production". On the other hand, instead, the organic brand gives the idea of an "untreated", "safe", "healthy" and "environmentally friendly" product.

Also if numerous studies have shown that the main reason for the purchase of organic products is the belief that these products are better and safer than the conventional equivalents (Vasileva *et al.,* 2018; Bonti Ankomah and Yiridoe, 2006; Hemmerling *et al.,* 2015; Naspetti and Zanoli, 2009), in some specific food sectors the concept that the organic product has a lower quality than the conventional one still predominates (de Andrade *et al.*, 2017; Delmas and Gran, 2014; Rauber, 2006). In the case of the wine for example, some certified wineries prefer not to display the environmental certifications in label (de Andrade *et al.*, 2017).

With reference to the knowledge on the positive attributes of the olive oil, 68% of respondents identified the fruitiness as a quality and 65% also the spicy. On the contrary, only 54% recognized that bitterness is not a defect. It's very interesting especially if we consider that in a recent Italian study, Di Vita *et al.* showed that, for the majority of consumers, bitterness and pungency are negative drivers of liking (Di Vita *et al.*, 2013).

However, 49% of consumers said they prefer strong intensities of the three attributes (fruity, bitterness and pungency), despite being convinced that bitterness is a defect. If we consider that the vast majority of consumers have said they produce olive oil or, at most, buy it from local producers, this result may seem strange. The olive oil producers not only know which are the positive attributes of olive oil but, moreover, they prefer strong intensities of such attributes; they know, in fact, that higher intensities correspond to a higher quality level of the product.

In other words, a high quality olive oil is generally very fruity, bitter and pungent. The fact that the respondents have not been able to recognize the bitterness as a positive attribute of olive oil and they prefer mild intensities of fruity, bitterness and pungency could therefore seem contradictory. Perhaps the explanation may lie in the fact that, having probably never participated in tasting and training courses, they are not able to correctly quantify the intensity of the attributes they perceive.

Finally, the answers provided by the respondents regarding purchase preferences (with reference to the last question), are summarised in the following table (Table 5):

INTERNET USERS

Sample	*Price* (€)	*Certification*	*Origin*	*% Preference*
1.	12,00	Organic	Italian	4
2.	12,00	PDO	Local	22
3.	9,50	PDO	Italian	8
4.	9,50	Organic	Local	**37**
5.	8,00	✗	Local	**28**
6.	8,00	✗	Italian	2

Table 5. Percentages of Olive oil purchase preferences among the respondents.

The recurring attribute in their choices is "local". This parameter seems more important than the "price", given that only 2% of the respondents would buy Italian olive oil paying it € 8.

The participants would buy PDO oil but only if it is local, so even in this circumstance the "local" term is more influential in the choice. The price does not seem so important, consistently with the answers given to questions 8 and 9 of the questionnaire; in fact, only 6% of respondents considered it the most important parameter in their choice, while 22% considered the price the least influential factor.

This is in line with the results showed by previous studies. According to Caporale *et al.*, (2006), Fotopoulos & Krystallis (2001), Stefani, Romano & Cavicchi (2006), consumers who are experienced, local, or familiar with a particular region of origin tend to consider region a key factor that drives purchasing and preference, while these factors do not seem to influence urban, less knowledgeable, and less experienced consumers. Besides, also Scarpa and Del Giudice (2004) sustained that the origin of the product is of importance and that there is a bias in preferences towards local products.

Similar results were demonstrated in a study by Di Vita *et al.* (2013), revealing that the three main factors affecting consumer preferences toward olive oil involve its area of origin, geographical designation (PDO and PGI), organic certification and price. With regard to the price factor, consumers from traditionally non-olive oil producing countries, consider price to be an indicator of quality. Other studies indicate that Italian olive oil consumers are positively affected by PDO and BIO (Biological) labels (Di Vita *et al.,* 2013; Vecchio & Annunziata, 2011) and that the PDO label is a more important factor than price (Fotopoulos & Krystallis, 2001; Resano *et al.,* 2012).

In another investigation performed in Andalusia (Spain) in a sample of 439 olive oil consumers, results demonstrated that origin labelling (PDO labelling), affected the preferences of most consumers [25].

In our study, the Organic label can be a positive factor in the consumer choice, in fact, consistent with the answers provided to the question on the "willingness to pay", consumers would spend 1.50€ more to buy an Italian product with the EU organic label.

Usually the most sustainable products are considerably more expensive than traditional products, and consumers who do not care about sustainability are reluctant to search for information about the long-term earning potential for growers associated with more sustainable products. However, there are consumers willing to pay more for them (Gleim *et al.*, 2013; Ritter *et al.*, 2015; Tseng & Hung, 2013). In Greece, demand for organic olive oil is positively affected by the socio-economic characteristics of consumers, as well as high income and employment status (Tsakiridou *et al.*, 2006). Spanish consumers, on the other hand, whose social profile is similar to that of Greece, seem to be less interested in organic certification but are highly concerned about the origin of product (D'Amico *et al.,* 2002). Panico *et al.* (2014) investigated consumer preferences in extra virgin olive oil in Italy and according to its results market segmentation shows that there are consumers who are particularly sensitive to origin and organic certification as well as labelling clarity.

In the second part of the present research, we administered the same questionnaire to 10 experts, 10 semi-experts and 10 usual consumers of olive oil before conducting, with them, blind and no-blind panel tests, as previously described.

The results are summarised in the table below (Table 6a):

I PART (DEMOGRAPHIC SECTION) & II PART (OLIVE OIL CONSUMPTION)

	EXPERTS	SEMI-EXPERTS	NOT EXPERTS
DEMOGRAPHIC SECTION	- Age range of 30-70 years (homogeneous age distribution) - 50% women, 50% men - 60% Graduated - Medium – low family income	- Age range of 18-70 years (70% concentrated in the 18-30 and 40-50 ranges). - 70% men - 50% Graduated - Medium – low family income	- Age range of 18-40 years - 60% men - 50% Graduated - High – medium family income
CONSUMPTION	- Daily consumption, as olive oil is considered healthy - 50% produce the olive oil that consume; the others buy olive oil from local producers.	- Daily consumption, because it is healthy but also for its good taste. - 70% produce the olive oil that consume; the others but it from local producers	- Only 70% of respondents consume olive oil every day. it is a good seasoning, it is healthy and tastes good. - Among them, 50% buy it from local producers, 30% consume their own olive oil, 20% buy it from supermarkets.

KEY FACTORS	- The key factor for the purchase is the experience (for 60% of respondents), while the least important factor is the packaging. (Only one person stated that the least important factor is the price).	- Experience seems to be the most important factor in the purchase guide, followed by the label. Packaging and price are the least important factors. - 20% state that the origin is the least important factor.	- At the time of purchase the key foods are: experience (40%), brands (30%), and origin (20%). Less important: packaging and the price (30%).
ORIGIN	- The origin of the olives is fundamental and the oil purchased has generally regional origin (60%), or at most, national origin. Experts do not buy EU or extra-European olive oils.	- The olives origin is fundamental (in disagreement with the previous answer). - Generally, they buy national olive oil (70%), or at most, olive oil of regional origin (30%).	- The origin of the olives is important; - they buy national (60%) or regional (40%) products.
ORGANIC PRODUCTS	- 60% are not interested in organic products; - However, 70% recognize the logo and admit to buying organic olive oils with different frequencies: - 1-2 times a year (57%); - weekly (14%).	- 80% are interested in organic products and recognize correctly the logo. - 70% buy organic olive oils and among them: 71% with frequency of 1-2 times a year, 14% monthly.	- About half of the respondents claim to be interested in organic products - 70% recognise the logo - 40% buy organic olive oils, but very rarely.
PDO PRODUCTS	- Only 80% of the experts recognize the PDO mark and purchase PDO olive oils 1-2 times a year (63%), or 1-2 times a month (25%).	- All respondents recognize the logo of the protected designation of origin. - 60% buy PDO olive oils, of which 71% 1-2 times a year, 14% 1-2 times a month.	- 80% recognise the DOP logo; - 40% buy DOP olive oils but with low frequencies.
WTP	10-20%	20-50%	20-50%
POSITIVE ATTRIBUTES	- They know the positive attributes of olive oil. - Surprisingly, 40% of them prefer products with low intensity attributes.	- They know the positive attributes of olive oil; - they prefer olive oils in which the intensity of positive attributes is greater.	- Most people know that fruity and spicy are positive attributes, whereas about 60% of panelists consider bitterness to be a defect. - However, they prefer oils in which these attributes are intense.

Table. 6 (a) Data obtained from the administration of questionnaire to experts/semi-esperts/non-experts

With reference to the last question of the questionnaire, we have schematized the answers of respondents in the Table 6 (b) and discussed the results below:

Sample	Price (€)	Certification	Origin	% Preference EXPERTS	SEMI-EXPERTS	NON-EXPERTS
1.	12,00	Organic	Italian	70	50	
2.	12,00	PDO	Local	10	10	60
3.	9,50	PDO	Italian		10	20
4.	9,50	Organic	Local	10	20	20
5.	8,00	✗	Local	10		
6.	8,00	✗	Italian		10	

Table 6 (b). Preferences regarding olive oils that experts, semi-experts and non-experts would buy.

EXPERTS - The preference of the experts fall on the Italian Organic olive oil (12,00 €) even if previously they declared to prefer local oils to the national ones.
Besides, the "biological" factor has more influence than the “origin” on the choice, in fact, they are willing to spend more for a national product, despite do not consider the price a negligible variable.
Although 80% of experts would buy an organic product, it must be emphasized that all local products would be purchased by at least one person.
SEMI-EXPERTS - 70% of the respondents would buy an organic product; and among them, 50% would buy the Italian olive oil (12,00 €) while 20% the local one that costs 9.50 €. This result is consistent with the answers given to question 11, concerning the origin of purchases.
The PDO products get 20% of the preferences, and all the Italian products, present in the table, receive at least one preference.
NON-EXPERTS - 80% of the respondents would buy a PDO product; and among them, 60% would buy the local olive oil (12,00 €) while 20% the national one that costs 9.50 €.
Besides, 20% of non-experts would buy the organic local olive oil (9.50 €)
The denomination of protected origin and the local origin seem to be the most important parameters. However, the "organic" factor is not such an important component because consumers would not spend 12€ for an Italian organic oil. On the contrary, they would spend the same amount for a local PDO olive oil despite, at Question 11 of the questionnaire, they said they prefer national products. This may seem strange if one considers that only 40% of respondents said they buy PDO products.

With reference to the attributes used to define a "organic" product, observing the table 7, we can see how both experts, and semi-experts that non-experts have used words like "healthy", "untreated" and "safe" (Table 7). On the contrary to define the "PDO" term, experts and semi-experts used concepts like "safety" and "certification", while the non-experts have used words as "quality" and "local origin", in addition to "safety" (Table 7). In this case, the answers provided by the non-experts are in line with those obtained in the first phase of the research (from the 130 persons which answered on line to the questionnaire), although the term eco-friendly disappears.

	EXPERTS & SEMI-EXPERTS		NON-EXPERTS	
ADJECTIVES	**DOP**	**Organic**	**DOP**	**Organic**
Healthy	2,3	24,5	6,7	33,3
Food fraud	-	-	-	-
Untreated	-	45,3	-	46,7
Best or Better quality	9,1	3,8	16,7	3,3
Safe	31,8	15,1	43,3	13,3
Eco-friendly	-	7,5	-	-
Certified	38,6	-	-	-
Expensive	-	1,9	10	3,3
Local	-	-	16,7	-
Traditional	-	-	3,3	3,3
Limited	-	-	-	-
Others…	2,3	1,9	3,3	-

Table 7. Adjectives provided by experts/semi-experts and non-experts to define the terms "PDO" and "Organic"

After analysing the answers obtained from the first and second sections of the questionnaire, we elaborated the data recorded in the third part of the questionnaire, related to the panel tests.

Before to carry out the normal test, we have informed the panelists about the type of olive oil they would have taste shortly thereafter.

The data collected in the two panel tests, blind (B) and not blind (N) are shown in the figure below (Fig.15).

		COMPARISON																	
		Fruit (1-5)			Bitter (1-5)			Pungent (1-5)			Would you buy			WTP			Preferences		
		Bio	DOP	EVOO	Bio	DOP	EVOO	Bio	DOP	EVOO	Bio	Dop	EVOO	Bio	Dop	EVOO	Bio	DOP	EVOO
E1	B	2	3	1	2	2	1	2	2	1	no	yes	no	6-8	6-8	3-5	2	1	3
	N	2	3	1	2	2	1	2	2	1	no	yes	no	6-8	6-8	3-5	2	1	3
E2	B	2	2	1	2	1	1	3	2	1	yes	no	no	9-11	9-11	3-5	1	2	3
	N	2	2	1	2	1	1	3	2	1	yes	no	no	9-11	9-11	3-5	1	2	3
E3	B	3	2	2	2	1	1	2	2	2	no	no	no	6-8	6-8	<3	1	2	3
	N	3	2	2	2	1	1	2	2	2	no	no	no	6-8	6-8	<3	1	2	3
E4	B	2	2	1	2	2	1	3	2	1	yes	no	no	6-8	6-8	<3	1	2	3
	N	2	2	1	2	2	1	3	2	1	yes	no	no	6-8	6-8	<3	1	2	3
E5	B	2	3	1	3	1	1	4	2	1	no	yes	no	9-11	9-11	<3	2	1	3
	N	2	3	1	3	1	1	4	2	1	no	yes	no	9-11	9-11	<3	2	1	3
E6	B	2	3	2	2	1	1	3	3	2	no	no	no	6-8	3-5	<3	1	2	3
	N	2	3	2	2	1	1	3	3	2	no	no	no	6-8	3-5	<3	1	2	3
E7	B	2	3	2	3	2	2	3	2	2	yes	no	no	9-11	3-5	3-5	1	2	3
	N	2	3	2	3	2	2	3	2	2	yes	no	no	9-11	3-5	3-5	1	2	3
E8	B	3	4	2	1	2	1	2	2	1	no	yes	no	6-8	6-8	<3	2	1	3
	N	3	4	2	1	2	1	2	2	1	no	yes	no	6-8	6-8	<3	2	1	3
E9	B	3	3	1	3	2	1	3	3	1	yes	yes	no	6-8	6-8	<3	2	1	3
	N	3	3	1	3	2	1	3	3	1	yes	yes	no	6-8	6-8	<3	2	1	3
E10	B	2	3	1	2	1	1	3	2	1	yes	no	no	9-11	6-8	<3	1	2	3
	N	2	3	1	2	1	1	3	2	1	yes	no	no	9-11	6-8	<3	1	2	3
S1	B	2	4	2	2	3	2	1	3	1	no	yes	no	<3	3-5	<3	2	1	3
	N	3	4	2	2	4	3	2	4	3	no	yes	no	3-5	6-8	<3	2	1	3
S2	B	3	5	4	4	5	2	5	5	2	yes	yes	no	6-8	9-11	<3	2	1	3
	N	3	5	4	4	5	2	5	5	2	yes	yes	no	6-8	9-11	<3	2	1	3
S3	B	3	4	3	3	5	5	5	5	5	yes	yes	no	6-8	6-8	3-5	2	1	3
	N	3	4	3	3	4	3	4	5	4	no	yes	no	<3	9-11	<3	2	1	3
S4	B	4	4	4	3	4	4	4	4	4	yes	yes	yes	9-11	9-11	9-11	1	2	3
	N	4	4	4	4	4	3	4	4	3	yes	yes	yes	9-11	9-11	9-11	2	1	3
S5	B	3	3	1	3	4	1	2	4	1	yes	yes	no	6-8	9-11	<3	2	1	3
	N	3	2	2	4	2	2	2	2	2	no	no	no	<3	<3	<3	1	2	3
S6	B	2	3	5	2	4	4	3	4	4	yes	yes	yes	3-5	6-8	6-8	3	2	1
	N	3	4	2	4	4	4	4	4	4	yes	yes	no	6-8	6-8	3-5	2	1	3
S7	B	1	2	2	3	3	3	5	4	4	yes	yes	no	12+	12+	<3	2	1	3
	N	2	3	2	2	3	2	4	2	2	yes	yes	no	12+	9-11	<3	1	2	3
S8	B	4	3	2	4	4	4	3	3	3	yes	yes	no	9-11	9-11	<3	1	2	3
	N	3	4	3	4	4	3	3	4	4	yes	yes	yes	9-11	12+	9-11	2	1	3
S9	B	3	4	4	1	2	1	3	4	1	yes	yes	no	6-8	6-8	<3	2	1	3
	N	2	3	3	3	3	2	1	4	3	yes	no	yes	3-5	6-8	3-5	2	1	3
S10	B	3	4	2	3	3	2	3	4	2	yes	yes	no	9-11	12+	3-5	2	1	3
	N	3	4	2	3	3	2	3	4	2	yes	yes	no	9-11	12+	3-5	2	1	3
NE1	B	3	2	4	4	4	2	5	3	4	yes	yes	yes	9-11	6-8	6-8	1	2	3
	N	2	3	3	4	3	3	1	1	1	no	no	no	<3	3-5	<3	3	1	2
NE2	B	3	4	4	4	4	3	3	3	2	no	yes	yes	6-8	6-8	9-11	3	2	1
	N	3	4	4	4	3	3	3	3	2	no	yes	yes	6-8	9-11	9-11	3	2	1
NE3	B	3	3	4	2	4	1	2	3	1	yes	no	yes	6-8	3-5	6-8	2	3	1
	N	3	3	4	3	3	1	4	2	2	no	no	yes	3-5	6-8	9-11	3	2	1
NE4	B	4	3	3	4	2	3	5	4	3	no	no	no	9-11	6-8	6-8	1	3	2
	N	2	4	3	4	4	1	3	4	2	yes	yes	yes	6-8	9-11	9-11	3	2	1
NE5	B	2	1	3	2	1	3	2	1	4	no	no	yes	3-5	<3	9-11	2	3	1
	N	3	4	4	4	4	4	3	5	4	no	yes	yes	6-8	9-11	9-11	3	1	1
NE6	B	2	3	1	2	3	1	3	4	2	yes	yes	yes	3-5	6-8	<3	2	1	3
	N	0	2	1	4	3	2	4	2	2	yes	yes	yes	6-8	3-5	<3	1	2	3
NE7	B	3	3	2	2	3	2	3	2	3	no	no	yes	3-5	3-5	6-8	2	3	1
	N	3	2	2	2	3	2	2	2	2	no	no	no	6-8	6-8	6-8	3	2	1
NE8	B	3	3	1	2	3	1	3	4	2	no	no	yes	3-5	<3	3-5	2	3	1
	N	3	3	2	4	4	2	3	4	3	yes	yes	yes	6-8	3.5	3-5	1	2	3
NE9	B	4	3	1	2	3	1	3	3	1	yes	yes	yes	6-8	3-5	6-8	1	3	2
	N	4	3	4	4	4	2	3	3	2	yes	yes	yes	9-11	6-8	6-8	1	3	2
NE10	B	3	4	4	4	4	2	4	4	4	yes	yes	yes	6-8	6-8	6-8	2	1	3
	N	3	4	4	4	3	1	3	4	2	yes	yes	yes	3-5	6-8	6-8	3	1	2

Fig. 15 Third section of the questionnaire with the results of the blind (B) and normal (N) panel tests. The abbreviations "E1" to "E10" correspond to the experts; S1-S10 to the semi-experts; NE1-NE10 to the non-experts.

First of all, we analysed the first three points related to the olive oil attributes ("fruity", "pungency" and "bitterness"), to understand if the panelists had provided the same answers after the normal test, and verify, thus, their coherence (Fig. 16, 17, 18).

Fig. 16 Comparison of results of the blind and normal tests obtained for the "fruity". Blue circle: there were no differences in the responses between the two sensory sessions. Red triangle: score obtained in the normal test is lower than that recorded in the blind session. Green square: score obtained in the normal test is higher than that obtained in the blind session. The number contained within the triangles or squares symbolises the difference in the scores

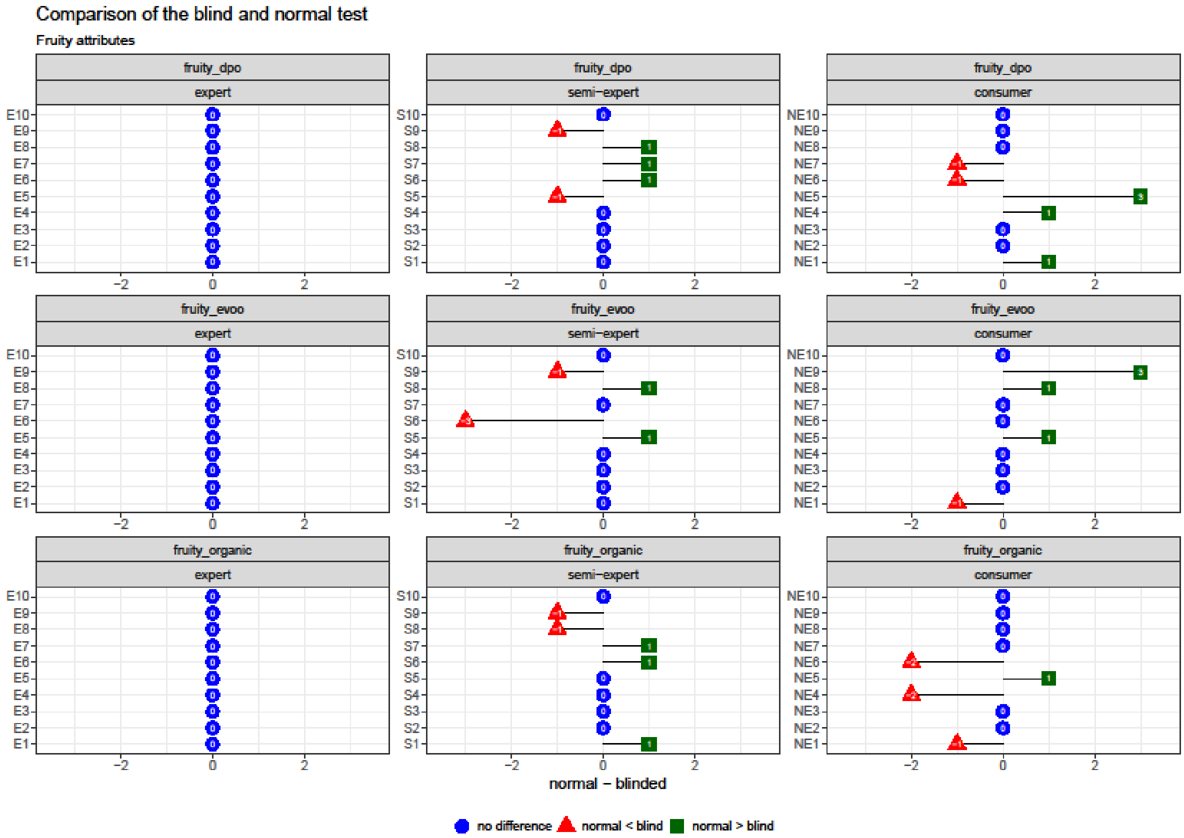


Fig. 17 Comparison of results of the blind and normal test obtained for the "bitterness".

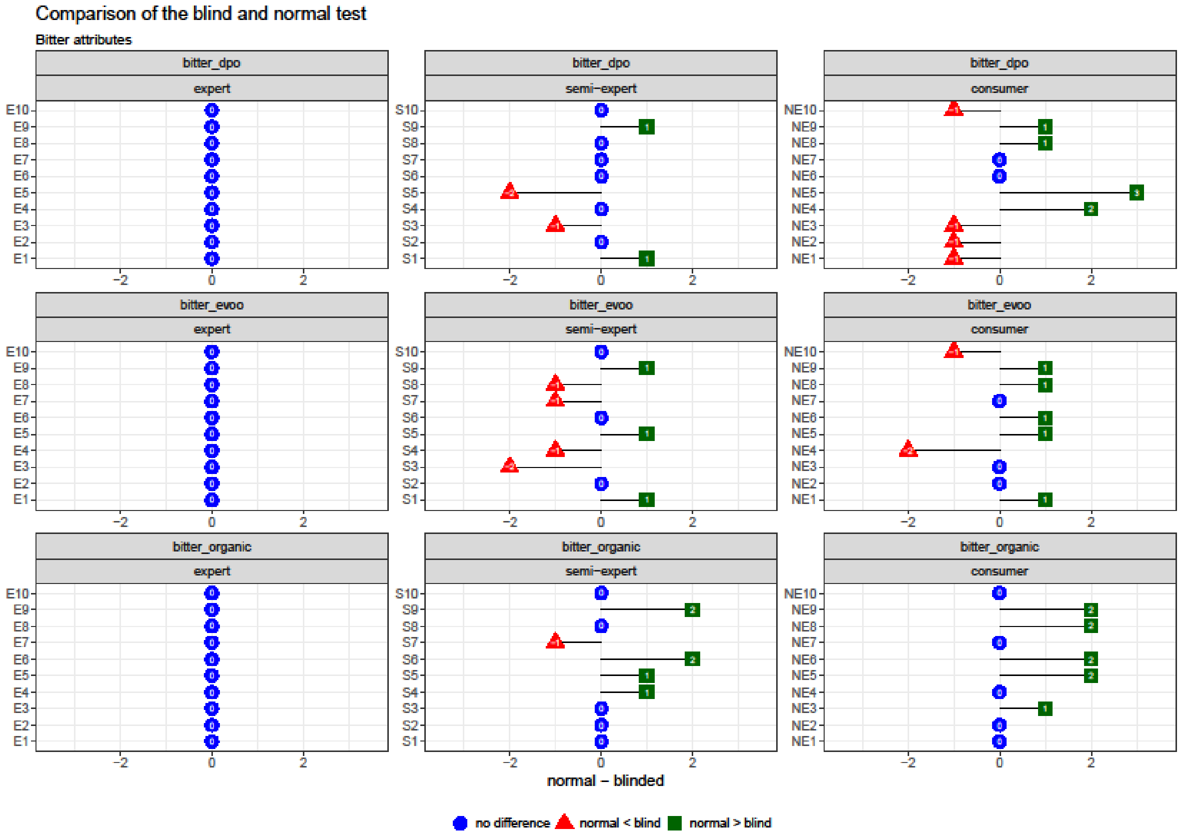
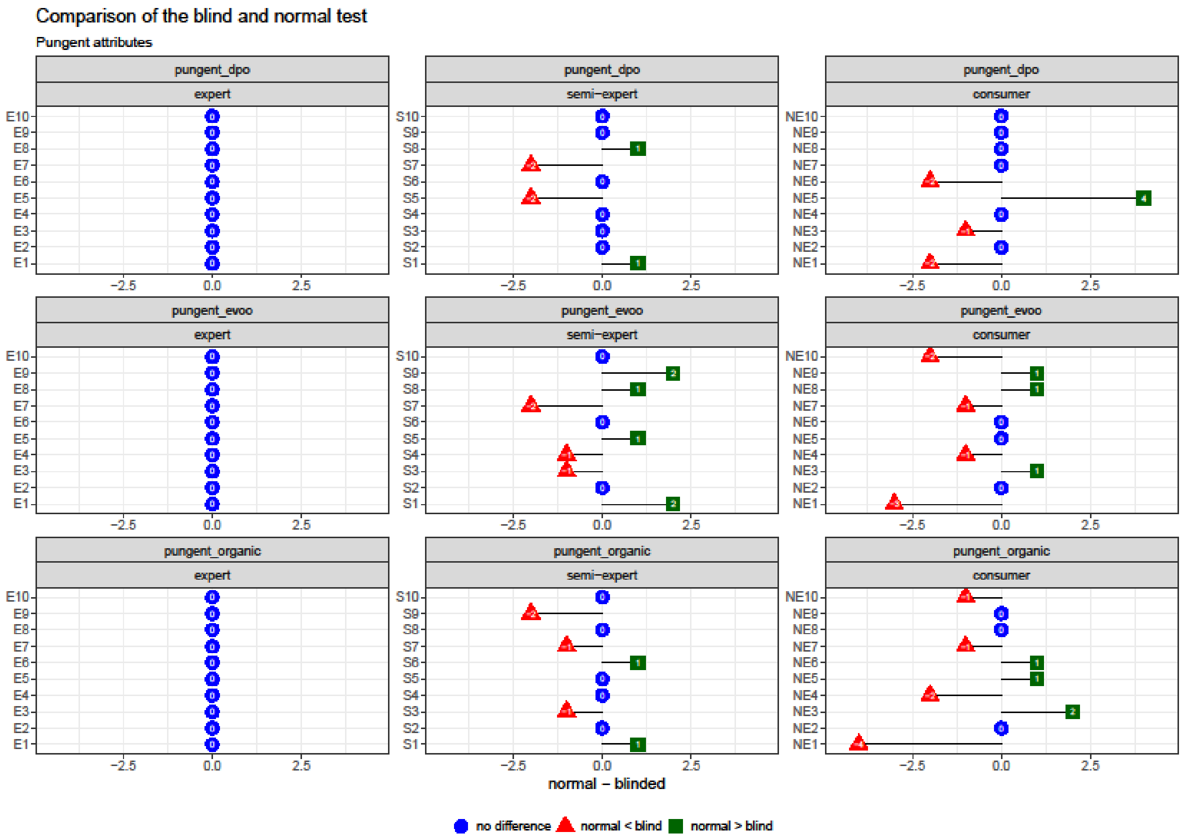


Fig. 18 Comparison of results of the blind and normal tests obtained for the "pungency".



From the comparison of the three figures (figures 16, 17, 18), we can immediately observed that the expert judges have been always consistent in the answers provided; on the contrary, there is more or less the same variability among the answers of the semi-experts and non-experts. This suggests that habitual consumer is quite informed, given that his answers are sometimes similar to those provided by semi-expert.

However, also if the number of variations could be the same, there are quantitative differences between the scores assigned by non-experts and semi-experts (which have achieved the certificate of physiological suitability to the extra virgin and virgin olive oil tasting).

With reference to the “fruity”, we can see how this attribute is the most easily recognisable both from semi-experts and non-experts (fig.16). In particular, there are no significant differences between the semi-experts, but most of them changed their mind when they became aware of the olive oils characteristics, with an increase of the "fruity" score (fig. 16).

SEMI-EXPERTS - With regard to the evaluation of the PDO olive oil, and specifically to bitter and pungent attributes, in both directions we noted a minimal and balanced score "correction". However, all the corrections of two points were negative (the values passed from a higher score to a lower one).

With reference to the bitterness and the pungency of EVOO product, the comparison is not very informative. We can note a lot of score changes after the normal test, well balanced between them. Overall, panelists were positively influenced by the sample identity revelation seven times, whilst in another seven cases their opinions were negatively affected.

With regard to the "bitterness" parameter of the biological sample, four panelists changed their minds with an increase of the score (positive influence). On the contrary, as regards the pungency of the same sample, the scores assigned by the semi-experts in the two sensory evaluations resulted more balanced. However, considering all the attributes, were recorded 9 increases in the score, and 6 decreases for organic olive oil, with a total positive influence.

NON-EXPERTS - To correctly analyse the scores assigned by non-experts and then understand in which way the labels can influence their opinion, we must take into account that not all of them know which are the positive attribute of the olive oil. It’s interesting to note how two judges, in evaluating the fruity of organic olive oil, changed their minds with a decrease of their scores of two points, despite knowing that it is a positive attribute (negative influence).

With reference to fruity, in two cases we observed a score change of three points, but in the first case (PDO product) the judge (NE5) has considered the fruity as a defect of the product (negative influence of PDO label); while in the second case (EVOO), the panelist NE9 was aware that the fruity is a positive attribute (positive influence).

Analysing the bitterness of organic oil, we noted that five persons remained of the same opinion, while the others increased their score, also if only two of them were aware that the bitterness is a quality attribute (positive influence). For the same reason, with reference to the bitterness of EVOO, we can note that three person provided coherent answers, while

five panelists were positively influenced also if their scores changed in both directions (NE1, NE4, NE5, NE6 and NE10).
With regard to the bitterness of PDO olive oil, five judges were negatively influenced, while three in a positive way, and in one case the score changed by three points.
Regarding the pungency of EVOO and organic samples, the situation resulted balanced; two judges changed opinions with a significant decrease of their scores, despite knowing that the pungency is a positive attribute (negative influence). In the case of PDO olive oil, three non-experts were negatively affected, while the score associated to NE5 was increased of 4 points (positive influence).

With reference to the other parameters analysed (propensity to purchase, price and order of preference), we found some interesting differences between the two panel tests.
EXPERTS **-** we didn't detect any variation, in fact nobody would buy the EVOO which is always the third in order of preferences. We have also noticed that four experts would buy the PDO olive oil, preferring it to the other samples and paying 6-8€ for a 50mL bottle.
Six judges preferred organic olive oil and four of them would have bought it, paying a sum between 9 and 11€.
SEMI-EXPERTS - As for the propensity to purchase, almost all would buy PDO and organic olive oil, many less are those who would buy EVOO (20-30%). Out of thirty answers provided, seven of them were changed in the no blind test, (i) five passed from “yes” to “no”(negative influence): two about the organic olive oil, two about the PDO olive oil and one about the EVOO; (ii) two panelists changed their answers from “no” to “yes” (positive influence) with reference to the extra virgin olive oil.
Regarding the price, there are considerable variations in the answers: in general, panelists wouldn't pay more than 3€ for a bottle of EVOO whilst more than 60% of them would pay more than 12€ for a bottle of PDO olive oil.
During the no-blind test, only 50% of the panelists provided answers consistent with those given previously.

- As for the biological type, three people changed their mind with a consequent decrease of the price they would be willing to pay for this product while, on the contrary, in two cases we recorded an increase of the price.
- About the PDO olive oil, three people said they were willing to pay more, two less and in one of these two cases, with a significant decrease of the price. This latter one also lowered the value of positive attributes and he stated that he would not even buy this product.
- As for the EVOO, we have recorded two increases and two price drops.

In the blind test seven judges preferred the PDO olive oil, in the normal test eight panelists said to prefer it, but only five of them have gave the same answer before and after.
This result is certainly interesting if we think that in previous answers of the questionnaire the semi-experts showed a clear preference for organic olive oils. Maybe, in this

circumstance, the PDO olive oil used for the sensory test has particularly satisfied the tastes of the semi-experts panel.

NON-EXPERTS – We can immediately note that there is a lot of variability in the answers.

With regard to the propensity to purchase, there is a great consistency in the answers provided about the EVOO sample; seven panelists in fact would buy it before and after the no blind test.

As for the PDO olive oil, four non-experts would buy it consistently with the answers previously given, two wouldn't buy it; the others changed their minds, three of them positively and another in a negative way.

With regard to the organic olive oil, three persons, both in the blind test and in the no-blind test, said that they would buy it, while other three panelists wouldn't. At the same time, two judges changed their minds positively in the no blind test and two in a negative way.

Price - As for EVOO, the prices suggested by consumers are varied; however in both test, seven people given consistent answers.

As far as PDO olive oil is concerned, only one person confirmed his answer, seven panelists changed their minds, with an increase of the price. In this case there is a positive influence of the PDO brand.

With reference to the organic olive oil, only one person remained of his idea, four judges have lowered the price they would be willing to pay, whilst five persons have increased it.

Preference - Four people do not change their minds by showing preference for EVOO. This result is a bit contradictory: EVOO presents slight intensity of the positive attributes and 60% of the non-experts answered the question 23 of the questionnaire saying to prefer higher intensities.

Five persons are positively influenced by the PDO logo; on the contrary, with reference to the organic brand, six persons changed their mind in a negative way and only two positively.

With reference to the figure 15 (preferences), experts have shown greater consistency in their answers, in fact they would buy the organic olive oil as they had previously said. Semi-experts have preferred the PDO olive oil, unlike as they previously said, but their preferences could be linked to the quality of the PDO olive oil type that they have tested.

However, all of them put the EVOO sample in third place, probably because they considered it less good than others. Our laboratory results suggest that the acidity of this sample was effectively higher than that of the other olive oils (Fig.19)

	Acidity (%)	Peroxide	ΔK	K_{232}	K_{270}
Organic Olive Oil	0,29	9,37	<0,01	<2,5	<0,22
PDO Olive Oil	0,32	8,73	<0,01	<2,5	<0,22
EVOO	0,49	8,9	<0,01	<2,5	<0,22

Fig. 19 Results of the chemical analysis carried out on the three olive oil samples.

Chemical analysis suggested that all the olive oils were extra-virgin and that they haven't been adulterated.
Summing up, the tests show that the experts have a certain awareness and experience, such that they are able to distinguish different olive oils and to recognise the quality of a specific sample, also if they wouldn't bought it because it doesn't meet their personal taste.
Semi-experts are influenced by brands, but not always in the same way.
The non-experts are positively influenced by the presence of the PDO brand and in an ambiguous (rather negative) way by that organic. Compared to the other two categories, they appreciate the EVOO sample without brands; they would buy the olive oils at various price, often too low for a product of quality, showing little knowledge about the olive oil value.
Also for this reason, once the test was finished, we delivered to all respondents, brochures with valuable information in order to clarify: - differences in the production processes of olive oil; - aspects on production specifications of PDO products; - aspects related to organic production; - the positive attributes of the oil and its main defects; - the olive oil quality-price ratio in the agrifood sector. Finally, a focus group was carried out to understand what initiatives could be taken to support the consumers in order to make them more awared and capable of making informed choices.

CONCLUSION

The sample interviewed online, composed mostly of young graduates, with a low average income showed preferences for local olive oils. Accoding to our results, the PDO label and the organic products have a positive influence on their purchase choice, while the price is not a decisive factor.
However, it should be investigated, if the same happens at the time of purchase, for example in a supermarket.
With reference to the second part of our study, all the categories of consumers (experts, semi-experts and non-experts) declared to be interested in PDO and organic olive oils. They recognise the logos of the PDO and organic products; moreover, they said to buy this kind of products, but with rather low frequencies. Experts and semi-experts know the merits of olive oil. Non-experts show good knowledge, although they didn't know that bitterness is a positive attribute and they aren't able to quantify perceived attributes.
The questionnaire results showed that experts and semi-experts would buy an organic oil, even spending more. Non-experts, on the other hand, would be willing to spend more money just for a PDO olive oil, that they consider better from the qualitative point of view.
The comparison of the answers obtained from the two panel tests showed that: - the experts gave coherent answers with what they had said in the questionnaire; - the semi-experts have been positively influenced by the PDO brand contrary to what they had

declared in the questionnaire ; - finally, non-experts would buy EVOO, although they have been positively influenced by the PDO brand and negatively by the Organic one.

The factors influencing consumer choices are different, and only knowledge and experience can help them make consistent and aware choices. It is therefore necessary to sensitise consumers, so that they can understand if their "purchase behaviour" really coincides with their opinions and convictions, and conduct information campaigns in order to overcome their skepticism towards quality of organic products.

CHAPTER IV

Nutritional quality, diets and consumers

The nutritional quantity of food must be guaranteed at every level of the production process because, through the diet, sufficient nutrients must be provided to meet the metabolic requirements, giving the consumer a feeling of satiety and well-being. Recent knowledge supports the hypothesis that, beyond meeting nutritional needs, diet may modulate various physiological functions and may play detrimental or beneficial roles in some diseases (Koletzko and others 1998). There is a threshold of a new frontier in nutrition sciences and indeed, at least in the western countries, concepts in nutrition are expanding from the past emphasis on survival, hunger satisfaction and prevention of adverse effects to an emphasis on the use of foods to promote a state of well-being, improve health, and reduce the risk of diseases.

The last decades have seen a further progression to the concept of nutrition in disease prevention, given added impetus with the developing field of gene-nutrient interactions and recognition of the potential importance and individualised nutrition (Arab, 2004; German *et al.*, 2005). Recently, the concept of a poly-meal, designed using foods identified from the literature as having cardio-protective effects, has been proposed as a mechanism for disease prevention (Franco *et al.*, 2004).

For the majority of the population in developed countries, the emphasis of nutrition advice is on balanced nutrition. Undernutrition can affect every system in the body and is mainly restricted to illness (whether poor nutrition leads to disease or disease adversely affects nutritional status) compare with unbalanced nutrition brought about by overconsumption (Cooper, 2007). In the contest of the balanced nutrition, national surveys of food and nutrient consumption have shown, that in the majority of developed countries, total energy intake has declined since the mid-1970s. Over the same period the relative contributions of carbohydrate and fat to total energy intake have fallen and risen, respectively. The consumption of fruits and vegetables by omnivores has remained generally low.

Current recommendations have focused on reducing the proportion of daily energy from fat, increasing the proportion of energy from carbohydrate and increasing fruit and vegetable consumption (Cooper, 2007). Dietary behavioural changes, such as increasing consumption of fruits, vegetables, and whole grains, and related changes in lifestyle, are practical strategies for significant reduction of the incidence of cancer (Fogelholm *et al.*, 2000). Current targets for a reduction in the total fat content of the diet are accompanied by recommendations to reduce the proportion of saturated fatsin the diet in favour of unsaturated forms; the total fat intake should be less than 30%, and the saturated fat intake should not exceed 10% of the total calories for healthy people (Hadnall *et al.* 1991).

In making recommendations on food and nutrient consumption, governments across international boundaries have generally adopted a similar approach with broadly similar objectives.
Consumers are beginning to show preference to low-fat heart-friendly food products, paying increased attention to labels. For this reason, the European authorities established new labelling rules, which enable the citizens to get comprehensive information about the content and composition of food products, so they can make an informed choice while purchasing their foodstuffs.

4.1 NUTRITIONAL LABELLING

The label contains information on the nutritional content of the product and provides a series of indications to understand how different foods contribute to a correct and balanced diet. The Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on "the provision of food information to consumers" updates and simplifies the previous food labelling rules. The purpose of this innovation is to further protect consumer health and ensure a clear and transparent information.
The label shows: name of the food, list of ingredients with indication of allergens and the origin of vegetable oils and fats, durability of the product, conditions of conservation and use, origin country and place of provenance, nutritional declaration and complementary indications.
Nutrition labelling is information found on the labels of prepackaged foods.The legislated information includes:

- the Nutrition Facts table, that provide information about: calories, core nutrient, % Daily Value (% DV) of nutrients. All of the information in the Nutrition Facts table is based on an amount of food. This amount is always found at the top of the Nutrition Facts table.
- the ingredient list
- some optional nutrition claims

Nutrition labels describe the nutrient content of a food and are intended to guide the consumer in food selection. The nutrition information provided must be selected on the basis of consistency with dietary recommendations. Selection of the specific nutrients or food components to be listed should further take into account label space, the analytical feasibility of measuring the particular nutritional component within the food matrix, and the relative costs of such analyses. Nutrition information provided on labels should be truthful and not mislead consumers. At the same time, labelling regulations should provide incentives to manufacturers to develop products that promote public health and assist consumers in following dietary recommendations (Van den Wijngaart, 2002).
It is likely that in many countries, there would be some segments of the population that would benefit from information about the composition of foods. In these cases, countries

should consider the need to provide for appropriate labelling and its presentation relative to existing guidelines and approaches. As nutrition-labelling efforts have evolved, different approaches and legal requirements have been established. These create difficulties in developing and harmonizing nutrition information listings, which have broad international applications. For these reasons, the Codex Guidelines on Nutrition Labelling play an important role to provide guidance to member countries when they want to develop or update their national regulations and to encourage harmonization of national standards with international standards. These Guidelines are based on the principle that no food should be described or presented in a manner that is false, misleading or deceptive.

The Guidelines include provisions for voluntary nutrient declaration, calculation and presentation of nutrient information. The Guidelines on Claims establish general principles to be followed and leave the definition of specific claims to national regulations. Definitions are provided for a number of claims (nutrient content, comparative claims, nutrient function claims) as well as general requirements concerning consumer information in relation with claims. Nutrition labelling by itself cannot solve nutrition problems. It should be seen as one of the elements of nutrition policy and should be envisaged in the larger perspective of consumer education, which in its turn is part of an overall development policy. Exchange of information at the regional and sub-regional level is important, as each country can learn from the experience of others and regional co-ordination and co-operation can be developed.

4.2 NUTRITION CLAIMS, HEALTH CLAIMS AND NEW TRENDS

Union rules on nutrition and health claims have been established by Regulations (EC) No 1924/2006 (and subsequent modification No 1047/2012). The Regulation started to apply on 1 July 2007. This regulation is the legal framework used by food business operators when they want to highlight the particular beneficial effects of their products, in relation to health and nutrition, on the product label or in its advertising.

The rules of the Regulation apply to different types of claim:

- Nutrition claims, which state, suggest or imply that a food has particular beneficial properties due to its composition (regarding energy or a particular nutrient). Examples of this type of claim will be: ‘source of’, ‘free of’, ‘high’, ‘low’ or ‘reduced’ in calories or a particular nutrient (Codex Alimentarius Commission, 1997).
- Health claims, which state, suggest or imply that a relationship exists between a food or one of its components and health. This type of claim mentions the physiological function of a constituent such as ‘calcium can help build strong bones’, "Vitamin D is needed for the normal growth and development of bone in children". The claim must be based on generally accepted scientific data and be well understood by the average consumer.

- The third type are ‘Disease risk factor reduction’ claims. They are a specific type of health claim, which state that a food or one of its components significantly reduces a risk factor for human disease. For example, phytosterols can help reduce blood cholesterol, hereby reducing a risk factor for cardiovascular disease. For the first time, mention of disease will be allowed on food, but only after approval by the European Food Safety Authority (EFSA).

The objective of those rules is to ensure that any claim made on a food's labelling, presentation or advertising in the European Union is clear, accurate and based on scientific evidence.

Food bearing claims that could mislead consumers are prohibited on the EU market.

This not only protects consumers, but also promotes innovation and ensures fair competition. The rules ensure the free circulation of foods bearing claims, as any food company may use the same claims on its products anywhere in the European Union. There are different procedures managed by the Commission for the various types of claims, with regard to their authorisation.

A public EU Register of Nutrition and Health Claims lists all permitted nutrition claims and all authorised and non-authorised health claims, as a source of reference and so that full transparency for consumers and food business operators is ensured. The European Union has continually to deal with new eating habits and food trends that have a strong impact on health, environment and society.

To date there are still important open questions concerning the health claims and labelling regulations of vegan foods, functional foods, prebiotics, probiotics, and synbiotics (synergistic combinations of pro- and prebiotics).

4.2.1 VEGANISM

Until today, in the EU there is no legally binding definition or regulation of the use of the term ‘vegan’ . Article 36 of the European Regulation (EU) No. 1169/2011 (paragraph 3 b) lays down that the European Commission should issue an implementing act defining the requirements for the voluntary labelling of food suitable for vegans or vegetarians. However this has not happened since there is no deadline for the implementing, despite the numerous initiatives from vegetarian and vegan associations, consumer protection organizations, which highlight the importance of a uniform definition and regulation for the labelling of vegan food, especially in the light of increasing numbers of vegan consumers (Gerke & Janssen, 2017).

On the other hand, the number of vegans in Europe has greatly increased, and in Britain it has risen by more than 360 per cent over the past decade, according to a new survey that shows record numbers of people are avoiding food derived from animals. Furthermore in Great Britain some 542,000 people aged 15 or over – more than one per cent of the population – have adopted a plant-based diet, up from 150,000 in 2006. According to the

Vegan Society, the survey proves that veganism is now one of Britain's "fastest growing lifestyle movements".

A vegan can be described as someone who "chooses not to consume any animal foods, including meat, poultry, game, fishes, shellfishes, dairy products, eggs, and honey" (Appleby, 2016). This interpretation of veganism can be expanded, as noted by The Vegan Society, to encompass "a way of living which seeks to exclude, as far as is possible and practicable, all forms of exploitation of and cruelty to animals for food, clothing, or any other purpose" (The Vegan Society, 2017). Both of these definitions highlight the exclusion of animal and animal derived foods from one's diet, but the second importantly notes that veganism can further limit the utilization of any animal-based products as a part of a multifaceted lifestyle choice. In this sense, the significance of veganism expands beyond solely that of a diet to serve as an everyday behavioral practice.

Usually, a vegetarian diet is associated with many health benefits because of its higher content of fiber, folic acid, vitamins C and E, potassium, magnesium, and many phytochemicals and a fat content that is more unsaturated. Neverthless, compared with other vegetarian diets, vegan diets tend to contain less saturated fat and cholesterol and more dietary fiber. Vegans tend to be thinner, have lower serum cholesterol, and lower blood pressure, reducing their risk of heart disease (Craig, 2009). However, eliminating all animal products from the diet increases the risk of certain nutritional deficiencies. Micronutrients of special concern for the vegan include vitamins B-12 and D, calcium, and long-chain n-3 (omega-3) fatty acids. Unless vegans regularly consume foods that are fortified with these nutrients, appropriate supplements should be consumed. In some cases, iron and zinc status of vegans may also be of concern because of the limited bioavailability of these minerals (Craig, 2009).

4.2.2 FUNCTIONAL FOOD

The term "functional food" was introduced for the first time in the mid-eighties in Japan. Since then, there has been a very marked increase in interest both from a scientific point of view, but also from a commercial point of view.

Until today, in the European Union, no legislation is operative on functional foods, so there is no legal value concerning their definition. Despite this, according to literature we can define the Functional Food, all the foods or food ingredients that exert a beneficial effect on human health and/or reduce the risk of chronic disease beyond basic nutritional functions (Huggett and Schliter, 1996, Katan, 2004). For healthy growing and life, must daily diet contain a healthy functional food ingredients. Consumers judge food products not only in terms of taste and nutritional needs, but also in terms of the ability to improve their health and well-being (Katan, 2004). Functional foods and functional food ingredients exert a beneficial influence on body functions to help improve well-being and health and/or reduce the risk of chronic diseases. Functional food can be produced by addition of health-promoting

component(s), by reducing/removing harmful components and/or by modifying the nature or the bioavailability of specific components (Ashwell 2002).
To promote the use of any particular functional food its beneficial effects must be communicated to consumer. This is usually done through the use of nutrition and health claims in the labelling and advertising of foods (Pravst,2012). In this context, functional foods in Europe are affected by the Regulation (EC) No 1924/2006 on nutrition and health claims made on foods (Regulation E.C. 2007). The regulation applies to all nutrition and health claims made in commercial communications, including trademarks and other brand names which could be construed as nutrition or health claims. The general principle is that claims should be substantiated by generally accepted scientific data, non-misleading and pre-approved on the EU level (Pravst, 2012). Additionally, claims shall not give rise to doubt about the safety of other foods or encourage excessive consumption of a food.
To be successful, functional food has to adhere to the following positioning: the health benefit has to appeal to a mass market and address general well-being issues; the health benefit has to be well communicated, either through understandable health claims, or through an active ingredient which is readily understood; the product must be competitive on all platforms, and not rely solely on its health benefits; it must also offer taste, convenience, and appropriate pricing. Although functionality allows for higher margins, it does not guarantee success by itself. Other aspects, such as brand name loyalty, advertising, promotion, quality control, competitors, and economic factors are also important.
Functional foods are certainly appealing, but high-level claims that do not comply with current food regulations are often being used, particularly in advertising. Experts agree that many of the claims currently used are potentially misleading (William & Gosh, 2008; Marette, 2017). Consumers' main scepticism regarding functional foods resides in the veracity of health claims, in lack of strong regulation and in the low and often inadequate control of their claimed properties.
Legislation concerning this matter is progressing at an extremely low pace and currently only Japan, the U.K., U.S.A., and Scandinavian countries have managed to make notable progress. Moreover, the labelling of functional foods is far from informative, providing scanty information about nutritional value, storage, and cooking recipes (Arvanitoyannis *et al.*, 2005; Williams & Ghosh, 2008). It is anticipated that technological advances in the food industry, in conjunction with extensive clinical trials and governmental control, will eventually guarantee the credibility of health claims and ensure consumers' confidence in functional foods.

Linseed As Potential Techno-Functional Ingredient

Since ancient times, common flax (Linum usitatissimum L.) has been cultivated both as fibre and oilseed crops. Linseed (or flaxseed) has usually been used in industrial applications as linen textile or drying oil in paints and varnishes (Berglund, 2002). Besides, its medicinal applications as antitumoral and anti-inflammatory remedy were also known since antiquity (Tolkachev & Zhuchenko, 2000). Flax is an attractive nutrition crop because of the high content of alpha-linolenic acid in the flaxseed oil and its dietary fibre and high quality proteins; specifically, flaxseed contains 40– 45% oil, 20–25% fibre, 20–25% proteins, and 1% lignans secoisolariciresinol diglucoside (Rabetafika *et al.,* 2011).

Today, approximately 70% of the global production of flaxseed oil is intended to technical applications in the field of paints, varnishes, linoleum and PVC plastics (Nykter & Kyma¨ la¨ inen, 2006), while another small amount is used in inks and personal care products.

The flaxseed fibre has a wide range of applications in various areas such as textile, paper, and biocomposite manufacturing while flaxseed mucilage is applied in cosmetic field (Alix *et al.*, 2008, Majlessi & Marcheggiani, 2004; Altunkaya, 2006).

Flaxseed has now new prospects in food because of the growing consumers' interest for functional food with health benefits. Indeed, flaxseed is a raw material rich in biologically active compounds such as polyunsaturated fatty acids (omega-3) protective of the cardiovascular system and lignans with antioxidant and anti-cancer properties (Tarpila *et al.,* 2005). Products from flaxseed including oil, mucilage, and lignans are above all designed for the functional foods and nutraceutical markets (Oomah, 2003).

Last researchs on food applications of flaxseed oil, the largest flaxseed fraction, reported their use in salad dressings, as food additives (Nykter & Kyma¨ la¨ inen, 2006), or as a partial substitute for milk fat (Goh *et al.*, 2006; *Lim et al.*, 2010). Soluble flax mucilage is used as a food ingredient to improve texture and to prevent syneresis in dairy products, and can play the role of the stabiliser in vegetable and fruit juices (Lan *et al.,* 2005; Anttila *et al.,* 2008).

Concerning the protein fraction, flax is not actually used as a source of food protein but used in animal feed as a cheaper material where available.

Nevertheless, proteins represent 35– 45% on dry oil-free matter basis, and are concentrated in aleuronic grains in cotyledons in the range of 56–70% of total proteins. Their solubility in various solvents reveals two major fractions namely globulin (linin) and albumin (conlinin) (Madhusudhan & Singh, 1985; Chung *et al.,* 2005).

Oomah & Mazza (1993, 1995), in review papers, described their physico-chemical characteristics, their functional properties and uses. On the other hand, Wanasundara & Shahidi (2003) gave information on the influence of processing on flaxseed protein functional properties and potential food applications of protein-rich flaxseed meals.

Also if, the potential health effect of flaxseed proteins was not well identified before, recently flaxseed proteins have received attention for their health benefits (Marambe *et al.,* 2008; Udenigwe & Aluko, 2010).

Among the functional properties of these proteins we find: emulsifying and foaming ability and stability, which are comparable to those of other oilseed proteins. Besides, they exhibit physiological properties like hypotriglyceridemic and hypocholesterolemic effects superior to those of commonly known soy proteins (Rabetafika *et al.,* 2011). Flaxseed proteins can also play the role of food preservative.

Linseed proteins definitely have real potential uses as techno-'functional' ingredient in several manufactured food products (e.g. bread and sauce).

4.3 CASE STUDY III

THE APPLICATION OF A NEW FORMULATION FOR AN EGG-FREE MAYONNAISE

A BRIEF INTRODUCTION

Mayonnaise is a typical oil in water emulsion prepared from vegetable oil, egg yolk, vinegar, sugar, salt, mustard and a variety of food additives (Juszczak *et al.* 2003). Among its ingredients, egg yolk is most critical in term of stability of the mayonnaise. Egg is considered a high profile ingredient because of its high nutritional value and multifunctional properties, including emulsification, coagulation, foaming, and flavour product (Narsimhan and Wang, 2008). The desire to replace eggs in food systems was brought about by a multitude of concerns from consumers, and processors desired to have low cholesterol foods (Liu *et al.* 2007).

In the last years, this process has also been accelerated by another emerging factor: the veganism's spread. Although past studies have already shown that several commercially available ingredients can successfully replace 100% of the egg yolk in a mayonnaise formulation (Herald *et al.*, 2009), there are few vegan products on the European market and existing ones do not seem to meet the consumer expectations.

In order to meet consumer demand, the application of protein, such as whey protein isolate along with some thickeners, to prepare low cholesterol mayonnaise has been reported in literature (Rahbari *et al.*, 2015). However, there is no published data on application of linseed proteins with beta glucan and starch to replace egg yolk in mayonnaise formulation.

Linseed proteins are potent multi-functional ingredients for food formulation owing to their techno-functionalities, food preservation capacity, and health benefits. The functional characteristics include solubility, rheological behaviour, emulsifying capacity, and foaming and whipping ability, while the factors that mainly affect their solubility are the pH and ionic strength.

Generally, there is the need for improving the functional performance of flaxseed since its viscosity and cohesion are low for food applications.

On other hand products containing β-glucan have numerous functional food applications to reduce fat content and calories in a variety of foods (Lee *et al.*, 2004); control the rheology and texture of food products (Rosell *et al.*, 2001); modify starch gelatinization and retrogradation (Rojas *et al.*, 1999, Lee *et al.*, 2005); and also provide freezing/thawing stability (Lee *et al.*, 2002). Besides β-glucan provides an excellent source of soluble dietary fiber for attenuating blood glucose, and reducing low-density lipoprotein cholesterols (LDL) (Cui & Wang, 2009), with beneficial health effects on coronary heart disease prevention (Inglett *et al.*, 2013).

Finally, the starch contributes to the textural properties of these foods as a thickeners, colloidal stabilizers, as well as gelling, bulking, and water retention agents (Singh *et al.*, 2007).

Therefore, the present study was conducted to prepare an egg-free vegan mayonnaise by combination of the ingredients mentioned above and of others described in the text, with good water holding capacities, texture, useful viscoelastic qualities, and comparable properties those of the conventional mayonnaise.

EXPERIMENTAL PART

Thirty formulations were prepared, using different combinations of Rapeseed Oil, Water, Linseed Proteins (provided by TH. Geyer), beta glucan (OatWell® 28 distributed by *DSM Nutritional Products, Inc.*), vinegar, mustard, salt, sugar, starch (Avanté 1 by *Ulrick&Short*) and lemon juice, and progressively varying their concentrations in order to obtain a more stable emulsion. Except for linseed proteins, beta glucan and starch purchased directly from the respective manufacturers, the other ingredients were bought from local supermarkets.
Three different types of mayonnaise on the market (Hellmann's real full fat mayonnaise, Asda light mayonnaise and Plamil egg free mayo) were used in rheological analysis and panel tests as control samples (Figure 20).

Full fat mayonnaise ***Light mayonnaise*** ***Vegan egg free mayo***

Fig. 20 Mayonnaise control samples

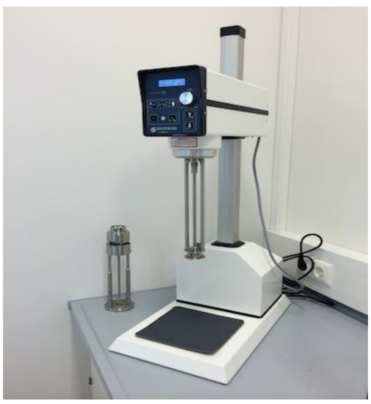


Fig. 21 Silverson L5M-A Lab Mixer

Preparation of the emulsion

Once the ingredients were combined, the preparation was heated by microwave at 57°C for 25 seconds, and mixed by the Silverson L5M-A Lab Mixer (Figure 21) equipped with the emulsifying attachment, at 4000 rpm (revolutions per minute) for four minutes.

Optimization of the preparation process

Several experiments to identify the best preparation method and optimize the parameters involved in the process were carried out.

Preparation method

Three different methods were tested to optimize the emulsion preparation process.

- 1st Method (**27a.**): Two mix have been prepared, one formed by linseed protein, β-glucan, water, sugar, mustard and salt, while the other one consisting in oil, starch, vinegar and lemon juice. The two preparations were then allowed to stand for one hour at room temperature. At this point, the first *solution* was heated for 25 seconds in a microwave oven at 57°C, then the two preparations were combined and amalgamated by a mixer equipped with the emulsifying attachment at 4000 rpm for three minutes.
- 2nd Method (**27b.**): The two mix (prepared as described above) were combined, and the mix obtained was allowed to stand for one hour at room temperature. In this case the formulation was not heated but it was directly mixed at 4000 rpm for three minutes.
- 3rd Method (**27c.**): The two mix (prepared as described above) were combined, and the resulting mix was allowed to stand for one hour at room temperature. The preparation was first heated for 25 seconds in a microwave oven and then "homogenized" by the mixer (under the same conditions previously reported).

Parameter optimization

After tested the three preparation methods (described in the previous section) and identified the most appropriate, we tried to improve the process modifying the parameters involved. First of all, several measures of the frequency of rotation (which by definition corresponds to the number of rotations around a fixed axis in one minute) have been tested: 1) 4000 rpm for 2 min; 2) 3000 rpm for 6 min; 3) 2000 rpm for 10 min.

Taking into account the importance of industrial process heating in the food sector to guarantee the long term food storage, we tried to increase the heating time of preparation and also to change the heating mode. Relatively to the first point, three solutions were experimented, heating the preparation by microwave at 57°C for: - 20 seconds; - 40 seconds; - 1 minute and finally comparing the differences of the resulting emulsions in their sensory characteristics, especially in relation to the vinegary taste and flavour.

In the second part, the Burton Sous Vide (Figure 22) was used to replace the microwave, and the experiments performed can be summarized in the following three procedures:

Fig. 22 Burton Sous Vide Water

1. All the ingredients, with the exception of lemon juice and vinegar, were combined and placed in the specific container left in hot water at 50°C for 15 minutes;

2. All the ingredients were combined and placed in the specific container left in hot water at 75°C for 30 minutes.

3. Liquid ingredients were combined and placed in the specific container left in hot water at 90°C for 15 minutes. Only once the heating process was terminated the dry ingredients were added to prepare the emulsion.

Rheological behaviour

Once the best preparation method and the parameters to be used were identified, rheological analyses have been performed both on the new formulation and on the mayonnaise controls (three replicates for each sample) in order to highlight any differences.
The rheological measurements were performed in the Bohlin C-VOR digital rheomoeter (Figure 23), that can perform strain, shear rate, and stress rheometer measurements in a single modular system.
Specifically, The C-VOR is a high resolution modular system with triple-mode control which allows strain, shear rate, or stress rheometer measurements to be performed using a single test station.

Fig. 23 Bohlin C-VOR digital rheometer

A built-in normal force sensor is used for a range of measurement, gap control, and sample loading protocols. The strain controlled tests are torque rebalanced which ensures that both the target strain is achieved and that the data is compliance free.
Gelation, Isothermal gelation and Viscosity were tested and flow properties of our samples and the mayonnaise controls were determined at 25 °C using a parallel stainless steel plate having a diameter of 20 mm, in the shear rate range of 0.1-100 s^{-1}. The apparent viscosity was determined as a function of shear rate. The differences in rheological properties of our sample were also analysed over time (t0, t1 = 12 days from t0; t2 = 20 days; t3 = 36 days).

Droplet size distribution

Mean droplet size and droplet size distribution of mayonnaise samples were determined by static light scattering using a Malvern Mastersizer 2000 (Malver Instruments Ltd., Malvern, Worcester, UK) according to the procedure described by Quintana *et al.* (2002). Sauter average diameter D[3,2], was calculated for each emulsion as follows:

$$d_{32} = \frac{\sum_{i=1}^{N} n_i d_i^{\,3}}{\sum_{i=1}^{N} n_i d_i^{\,2}}$$

Where d_i=droplet diameter, N=total number of droplets, n_i=number of droplets having d_i diameter. Aliquots of fresh samples were observed after a 120 dilution with distilled water on a microscope (Leica DC100 microscope equipped with a digital camera).

Sensory evaluation tests

Sensory evaluations were conducted on sample 27c after one-day storage at room temperature and on three types of mayonnaises currently on the market: full fat mayonnaise, light mayonnaise, vegan mayo (as showed in figure 20), in the sensory laboratory of Queen Margaret University, in two sessions, conducted in different days but with the same experimental design, and the panels consisted of untrained panelists, eight in the first session and nine in the second.
Sensory characteristics including colour, consistency, fatty and vinegar aroma, fatty and acidic flavour, saltiness, creaminess, overall quality were evaluated by untrained panelists based on a 9-point Hedonic scale (1 as the lowest and 9 as the highest score); the panelists were requested to assess the samples according to the parameters stated in the sensory evaluation score sheet, and also to write their comments about off-odours, off-flavours and aftertastes of the samples.
During the sensory evaluation, mayonnaises of each type were served at room temperature in plastic plates, that were labelled randomly with three-digit codes. According to Venkatachalam & Nagarajan (2017), prior to each tasting a sample, lemon water was served to the panelists to neutralize their mouth feel, in order to maintain comparable testing over a sequence of samples.
Finally, the panelists had to express their preference, selecting just one of the four samples tested.

Statistical analysis

Sensory characteristics were analysed by Two way ANOVA analysis and also pairwise comparisons performed using Tukey's HSD control for maximum experiment-wise error rate (regardless of the sample p-value) were carried out.

Results

Although past studies have already shown that several commercially available ingredients can successfully replace 100% of the egg yolk in a mayonnaise formulation (Herald *et al.,* 2009), there are few vegan products on the European market and existing ones do not seem to meet the consumer expectations; that sounds strange, especially if we take into account that, over the past few years, the number of veggie and vegan foods available in western countries has soared. According to Mintel Global New Products Database (2017), between 2014 and 2016 there was a 92% increase in the number of food products launched in Australia carrying a vegan claim, and an 8% increase in the number of products launched carrying a vegetarian claim.
In this research, we prepared an egg free vegan mayo by combination of the various ingredients, among which linseed proteins and beta glucan that, together, allow the formation of a viscous mix with extremely interesting properties. The sample 27 has been

identified as the best formulation especially for its taste and consistency, and its recipe is showed in the following table (Table 8).

In some experiments, lower concentrations of rapeseed oil were also used, but with texture results that are anything but satisfactory.

Furthermore, in definite tests that involved the use of cold pressed rapeseed oil was instead of the normal one, profound negative changes in the taste, flavour, texture and colour of the samples were registered. As well we used different ratios of vinegar and lemon juice, and the best ratio resulted 3:7, instead of the proportion used initially (1:1) linked to the onset of pungent odours and to an unpleasant taste.

Table 8. Recipe of the sample 27 with the percentage of each ingredient

RECIPE SAMPLE 27c		
Ingredient	**Amount**	**%**
Rapeseed Oil	200	68,14
Water	55	18,74
Linseed Protein	6	2,04
Beta-Glucan	1	0,34
Vinegar	6	2,04
Mustard	2,5	0,85
Salt	3	1,02
Sugar	3	1,02
Starch	3	1,02
Lemon juice	14	4,77
Total	293,5	100,00

Using specific spreadsheets, the nutritional values (energy kilocalories and contents of fats, carbohydrates, fibers, proteins and salt) were estimated per 100g of the sample 27, in order to compare them with those shown on the labels of commercial mayonnaise controls (Table 9).

According to data showed in the table 9, our emulsion presents a level of protein comparable with that found in full fat and vegan mayonnaises, a medium energetic value and the lowest salt content; besides it contains fibers unlike the other samples, its lipid profile is more similar to that of the vegan mayo for the total fats content, while for the percentage of saturates to that of full fat mayonnaise. It has the highest rate of sugars among the carbohydrates which, however, are present in lower percentage than those contained in light and vegan samples.

Table 9. Nutritional values of sample 27c and of the mayonnaise controls per 100 g of product.

	ALL VALUES PER 100g			
	Sample 27c	**Hellman's Real Mayonnaise***	**Asda Light Mayonnaise****	**Plamil Egg Free Mayo**
ENERGY KCALORIES	**585**	721	246	509
FAT (g)	**63,4**	79	26	54
of which SATURATES (g)	**5**	6,2	2,6	6,5
CARBOHYDRATE (g)	**2,4**	1,3	6	4,5
of which SUGARS (g)	**1,4**	1,3	2,3	0,5
FIBRE (g)	**1,1**	-	-	0,5
PROTEIN (g)	**0,9**	1,1	0,5	1
SALT (g)	**1,04**	1,5	1,7	1,25
		*Omega 3 claim - 7.0g/100g	**Omega 3 claim - 2.3g/100g	

Taking a look at the table 10 and specifically at the ingredients list of the products analysed in our study, you can immediately note the limited number of ingredients contained in sample 27 in comparison with the other ones, and the presence of just one allergen: the mustard, added to stabilize the mixture (Becher, 2001), unlike the vegan mayonnaise that presents also soybeans. This is a rather interesting factor considering that soybean allergy is one of the more common food allergies, especially in babies and children (Yang *et al.*, 2011; Zeiger, 2003).

Table 10. Ingredients list of sample 27 and mayonnaises on the market, used like control samples. The allergens are highlighted in bold

INGREDIENTS	
Recipe 27	Rapeseed Oil (68%), Water, Lemon Juice, Linseed Protein, Vinegar, Starch, Salt, Sugar, **Mustard** Powder, OatWell TM Beta-Glucan Powder.
Hellman's Real Mayonnaise	Rapeseed Oil (78%), Water, Pasteurised Free Range **Egg** & **Egg** Yolk (7.9%), Spirit Vinegar, Salt, Sugar, Lemon Juice Concentrate, Antioxidant (Calcium Disodium EDTA), Flavourings, Paprika Extract .
Asda Light Mayonnaise	Water, Rapeseed Oil (25%), Spirit Vinegar, Modified Corn Starch, Sugar, Salt, Pasteurised Free Range **Egg** Yolk (1.5%), Cream Powder (contains **Milk**), Citrus Fibre, Thickener (Guar Gum, Xanthan Gum), **Mustard** Flour, Lemon Juice Concentrate, Antioxidant (Calcium Disodium EDTA), Natural **Mustard** Flavouring, Paprika Extract.
Plamil Egg Free Mayo	Sunflower oil (53%), water (33%), cider vinegar, sea salt, dehulled **soya** beans (1%), **mustard** flour, stabilisers: guar & xanthan gums, lemon juice.

After identifying the best formulation, three different methods were tested to optimize the emulsion preparation process. The main differences found between them are summarized in the table below (Table 11).

Table 11. The main differences in the methods used to prepare the sample 27.

<table>
<tr><th colspan="2"></th><th colspan="5">STAGES OF THE PROCESS</th></tr>
<tr><th colspan="2" rowspan="2"></th><th rowspan="2">Combine the 2 preparations</th><th rowspan="2">Leaving 1h/rt</th><th colspan="2">Heating</th><th rowspan="2">Emulsion</th></tr>
<tr><th>Just one of the two preparations</th><th>The mix of the two preparations</th></tr>
<tr><td rowspan="3">METHODS</td><td>27 a.</td><td>✗</td><td>✓</td><td>✓*</td><td>✗</td><td>✓</td></tr>
<tr><td>27 b.</td><td>✓</td><td>✓</td><td>✗</td><td>✗</td><td>✓</td></tr>
<tr><td>27 c.</td><td>✓</td><td>✓</td><td>✗</td><td>✓</td><td>✓</td></tr>
</table>

*After being heated, it has been combined with the other preparation before emulsifying.

In all three cases a more than satisfactory result was obtained. Nevertheless the best result was achieved in the third case, in fact the corresponding emulsion showed the best structure, homogeneity and sensory features. Specifically the colour was slightly darker than a traditional full fat mayonnaise, the taste and the flavour less acidic than the other samples but very similar to typical those of commercially mayonnaises, and it was more "pleasant to the touch".

Regarding the rotation frequency, the best emulsion was obtained at the highest value of revolutions per minute (4000 rpm). This result is in line with previous works, for example in their study Silva *et al.*, (2016) found that the effect of high shear homogenization on the physical properties of the oil in water emulsion was dependent on the speed applied to the homogenizer pump, where 3600rpm speed was confirmed to be effective in improving the physical aspects of size emulsion, drop shape, and eliminating separation of phases when submitted to stress conditions.

As regards the temperature experiments, instead, between the microwave-heated preparations, only that heated for 20 seconds has allowed the attainment of an emulsion with pleasant and convincing sensory properties. In order to obtain a more stable emulsion we tried to heat the samples by using other system, like the Burton Sous Vide. Unfortunately, in none of the three cases tested, good results were achieved.

However, in order to make the product marketable, it is necessary to identify an effective method to sterilize the ingredients mix and obtain a long-keeping emulsion and, in case, introduce a food preservative to extend its shelf life. According to Amit *et al.*, (2017) to ensure food safety and long shelf life of foods, it is important to understand food spoilage mechanisms and food preservation techniques. Increasing shelf lives of food items without compromising original food properties is still critical and challenging. Our future efforts must focus in this direction.

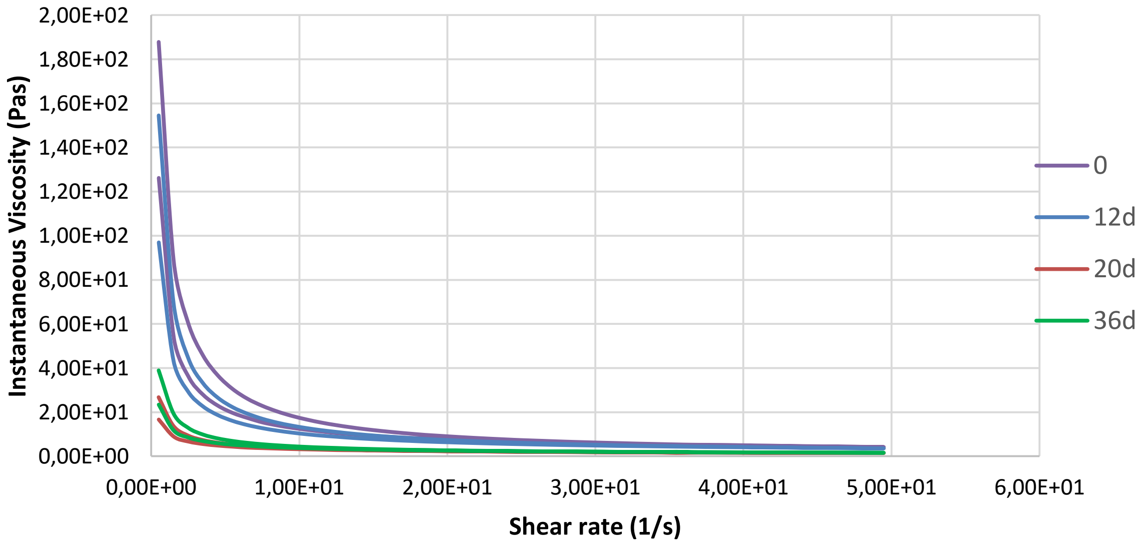
With time and growing demands, preservation techniques have been improved and modernized. Irradiation, high-pressure food preservation, and pulsed electric field effect are the latest innovations used to increase shelf life of foods (Amit *et al.*, 2017). Different natural compounds and chemical reagents have also been introduced as food additives and preservatives. The rosemary extract is a natural preservatives used as antioxidant for mayonnaise and in general for margarine, oils and fats (Meyer *et al.*, 2002). Relatively to artificial preservatives, sorbic acid (2,4-hexadienoic acid), potassium sorbet, benzoic acid and sodium benzoate have often been added in the industrial preparation of mayonnaise and other food products for their preservation (Rahman, 2007). However, there are growing concerns of using chemical additives and preservatives in food items because of possible health hazards.

Furthermore we analysed the rheological behaviour of our formulation at different times (t_0=0, t_1= 12 days from t_0, t_2= 20 days, t_3=36 days) to see if there were any differences over time.

With regard to the viscosity test, in figure 24 we can see that after 12 days no significant changes were recorded in the instantaneous viscosity values as a function of the shear rate.

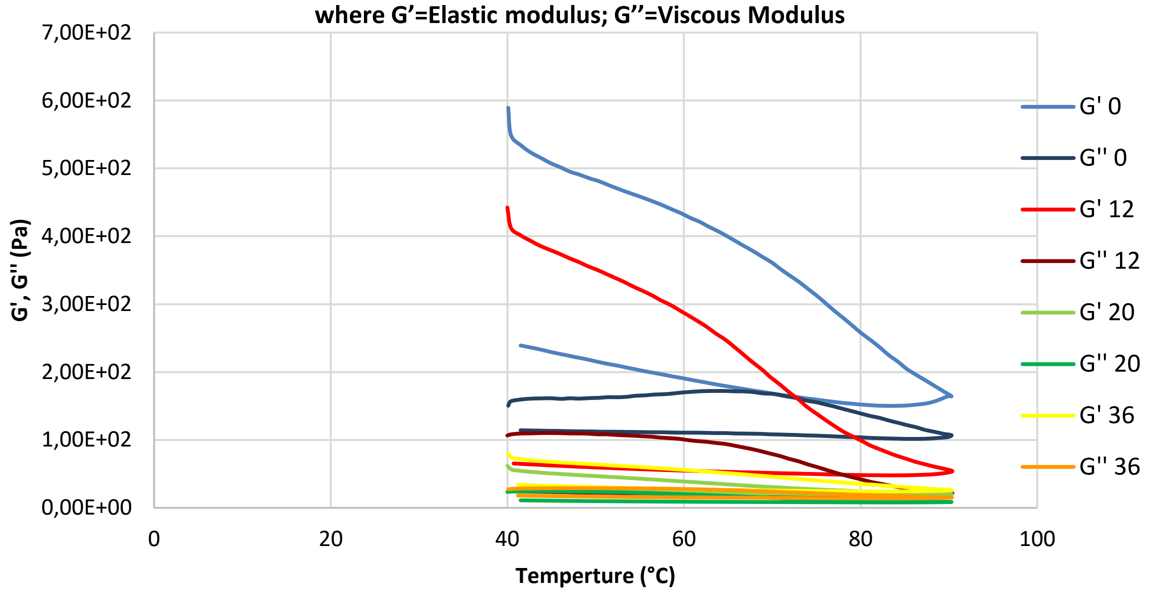
In both cases, we noted a drastic reduction of the instantaneous viscosity with increasing shear rate and its progressive restoration (although the initial values were no longer achieved) with decreasing of shear rate values. Contrariwise after twenty days there were significant decreases in values, while a slight increase was surprisingly observed in t_3 in comparison with t_2.

Fig. 24 Viscosity test during the time



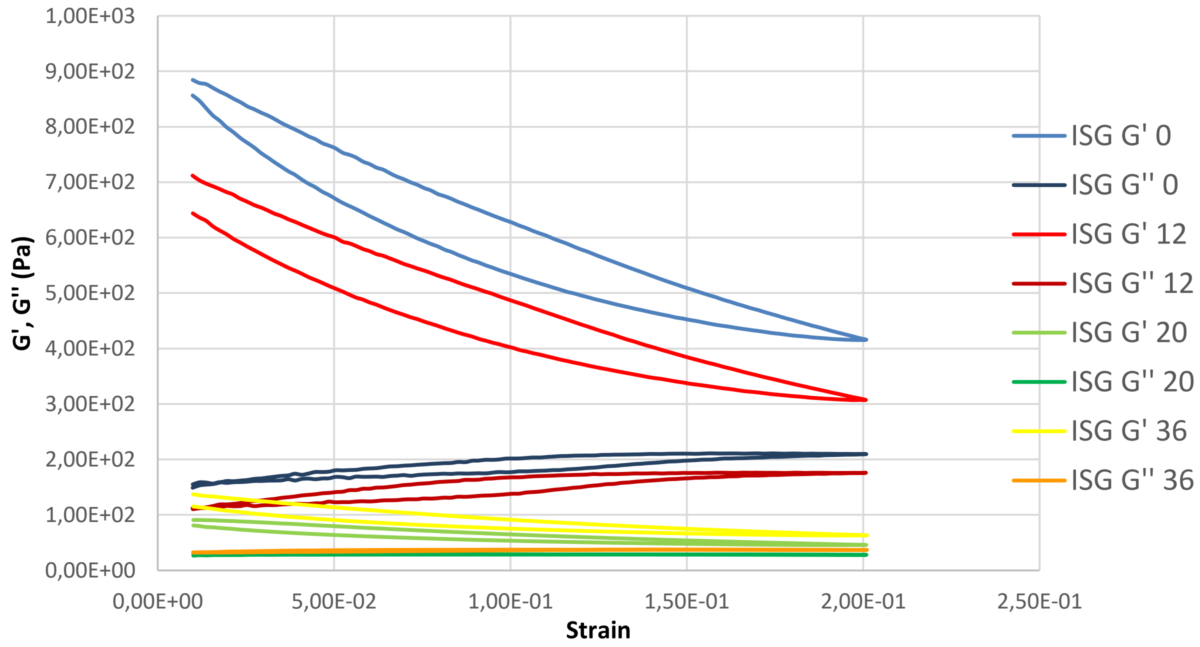
From the following figure (Figure 25) showing the results of the Gelation tests, it is possible to notice that both G’ and G’’ of t_1 present the same trend of G’ and G’’ of t_0, while on the contrary G’ and G’’ of t_2 and t_3 are different from the first two, especially in the first trait of the graphic. Also in this case the values of G’ and G’’ of t_3 would seem to be higher than those of t_2.

Fig. 25 Gelation Tests of sample 27c during the time
where G'=Elastic modulus; G''=Viscous Modulus



The aspects, we just underlined, are even more evident in the graphs related to the isothermal gelation tests (Figure 26).

Fig. 26 Iso-thermal Gelation Tests
G' = Elastic Modulus, G''= Viscous Modulus, expressed in Pa
as a function of the Strain.



Generally a correct interpretation of the rheological results is not so immediate and easy, but from our data we can deduce that over time, all viscosity, gelation and isothermal gelation values significantly decreased. The rheological characteristics, and the loss of viscosity and elasticity certainly reflect the deterioration of our emulsion over time. In fact, also the consistency, the colour and taste changed considerably with the passing of the days. Regarding the consistency, after twenty days, the emulsion becomes like a pudding, and after another week we assist to a phase separation. Unfortunately, the taste changes more quickly. At this point, we decided to compare the rheological properties of our emulsion with those of the mayonnaise controls purchased from local markets (Figure 27 a,b,c).

Fig. 27 (a) Viscosity tests.
Sample 27 c), Full fat Mayonnaise, Light Mayo and Vegan Mayo in comparison

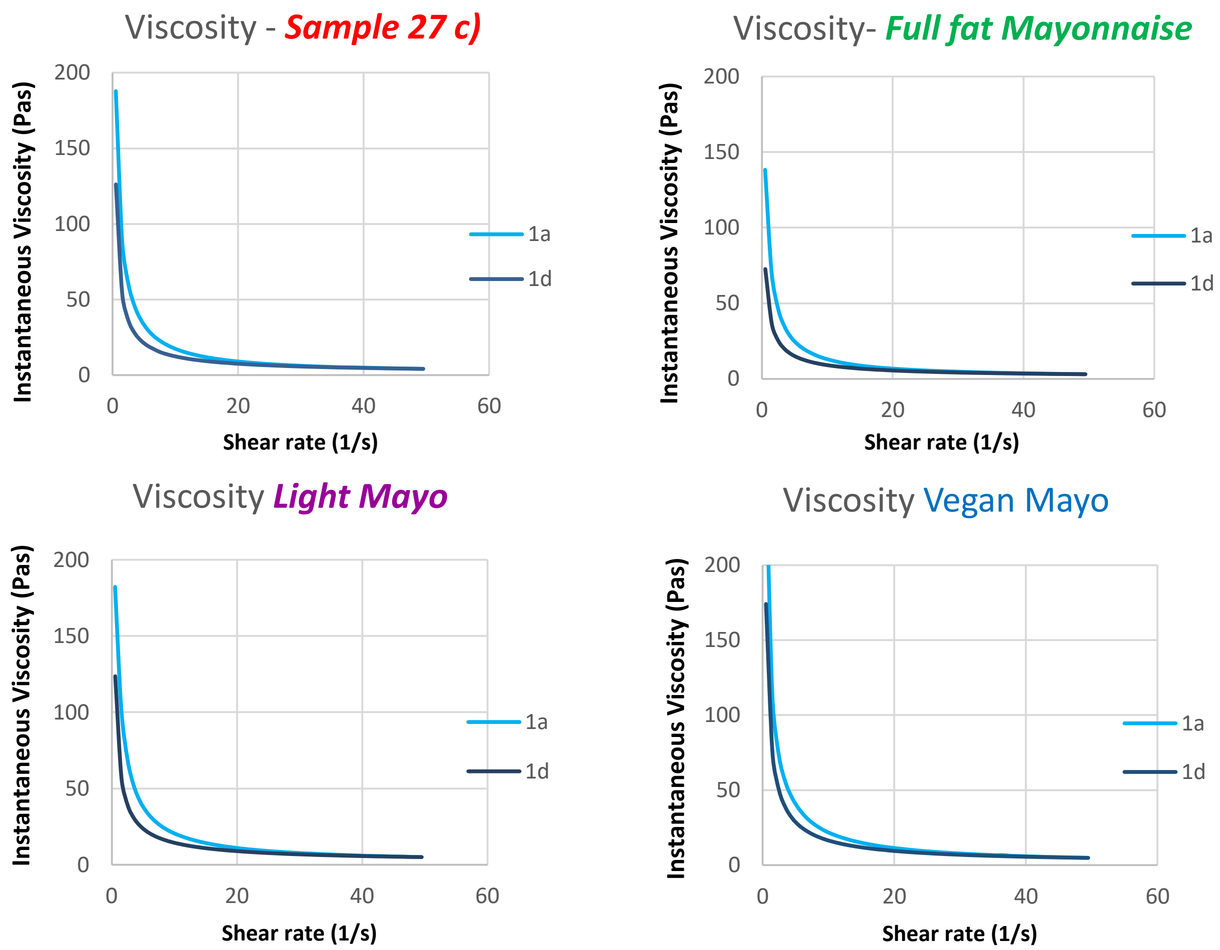
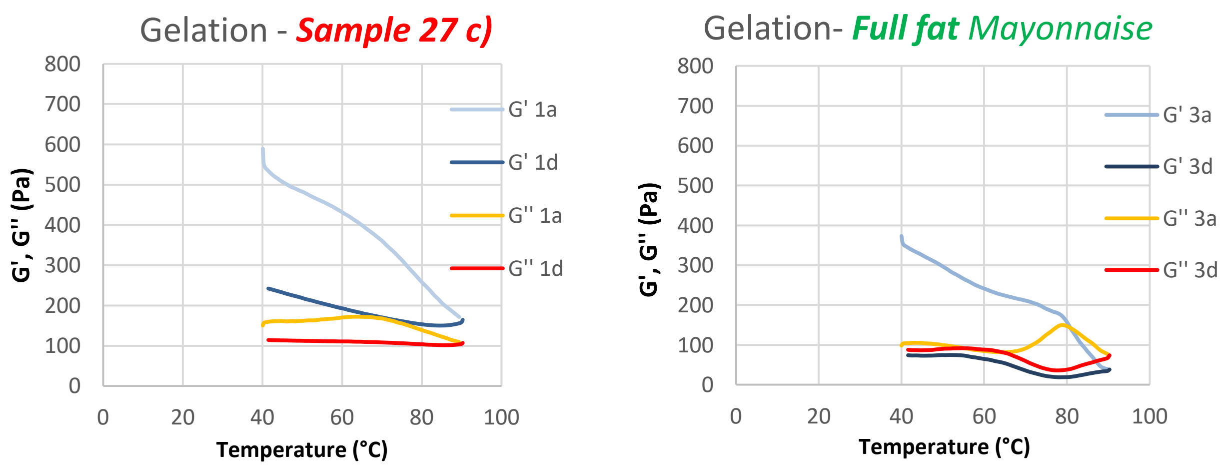


Fig. 27 (b) Gelation tests.
Sample 27 c), Full fat Mayonnaise, Light Mayo and Vegan Mayo in comparison
The number next to G' and G'' corresponds to the replica used to construct the graph.
The letter ***a*** is related to the ascending strain values, while **d** to the descending ones.



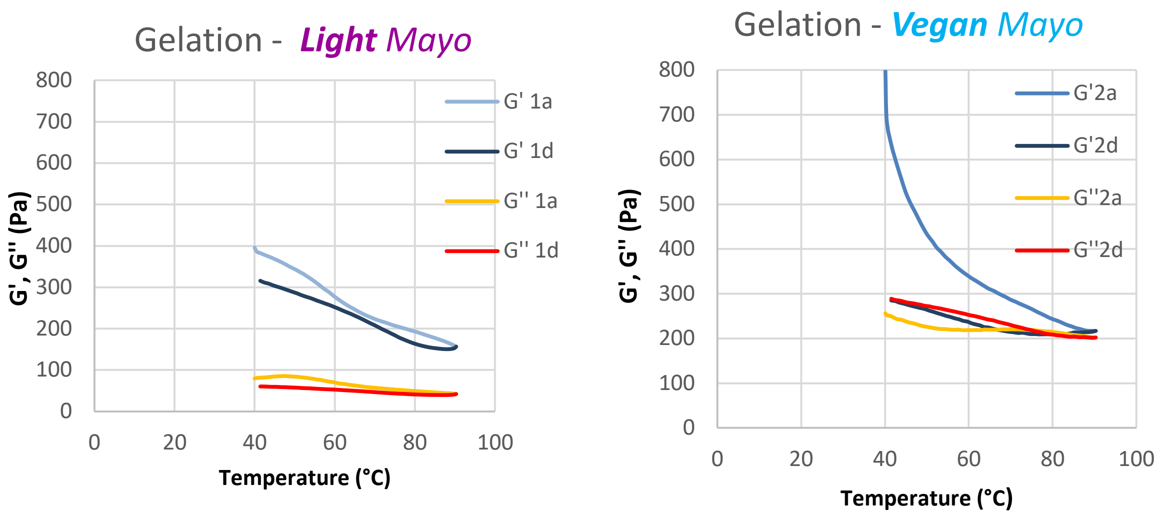
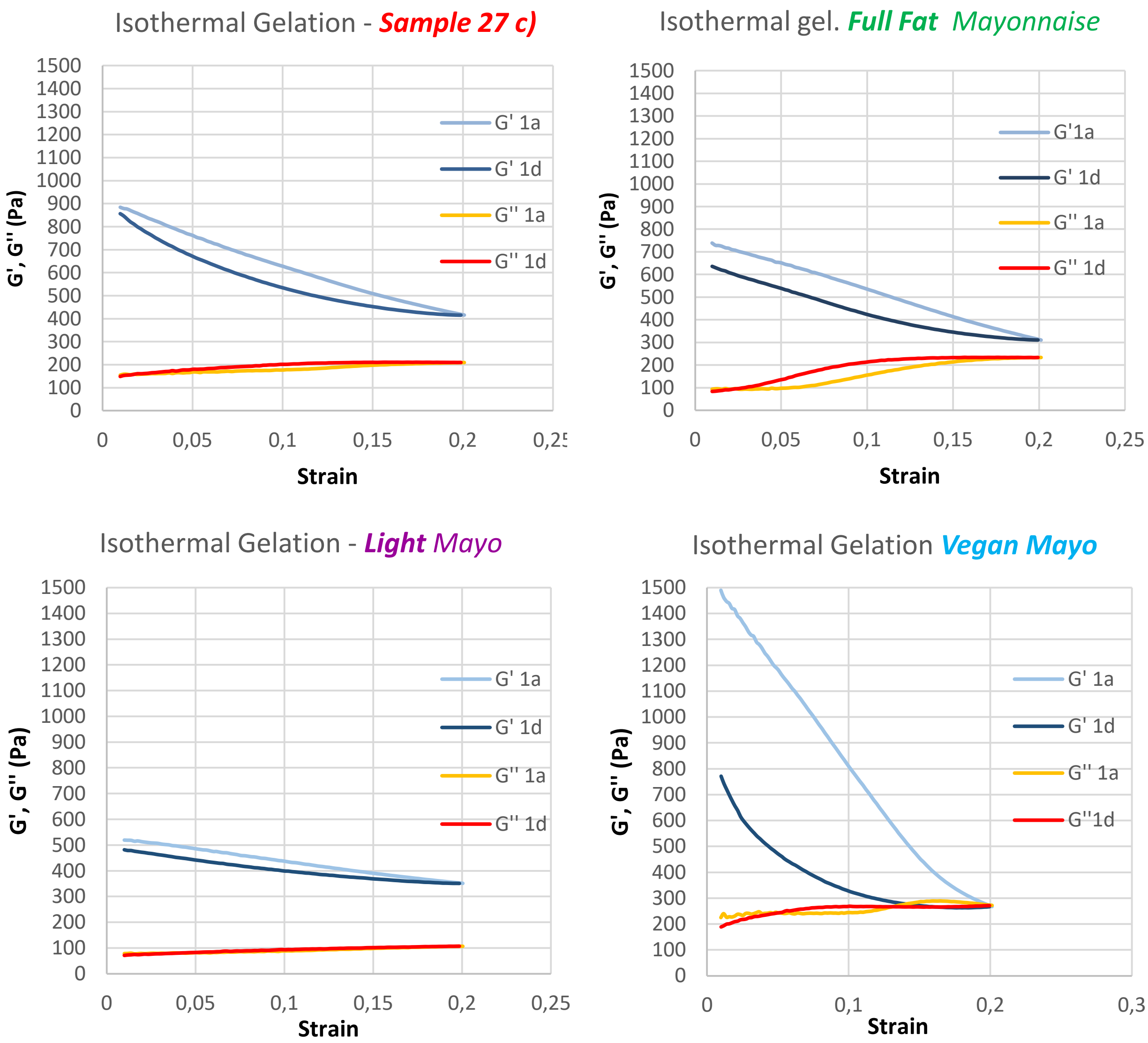


Fig. 27 (c) Iso-thermal Gelation tests.
Sample 27 c), Full fat Mayonnaise, Light Mayo and Vegan Mayo in comparison
The letter ***a*** is related to the ascending strain values, while **d** to the descending ones.



As shown in the graphs, the vegan mayonnaise on the market has rheological properties completely different from the other samples. Relatively to viscosity tests, our formulation showed properties more similar to those of light mayonnaise while full fat mayonnaise recorded the lowest values of Instantaneous Viscosity. The linear viscoelastic behaviour range for mayonnaise-like systems may be indicative of the nature of intermolecular forces between the lipoproteins adsorbed around oil droplets (Quintana *et al.*, 2002).
From the image 27 (b) we can note that the G'1a of our formulation is similar to that of full fat mayonnaise, whilst its G'1d presents intermediate values between full fat and light mayonnaises in all replicates. G''1a and G'' 1d have trends similar to those of light mayonnaise. Also in this case, the differences presented by the vegan mayo are prominent.
In the isothermal gelation test, the sample 27c presented values of G'1a in a similar way as full fat mayonnaise, instead G'1d values are intermediate between full fat and light controls. No evident differences have been recorded between G''1a and d values between the samples. However results obtained from test on our formulation showed higher values than the other controls, with the exception of the vegan mayo, which displayed completely different values.
Even though from the rheological point of view the results obtained were more than satisfying, one of the main purposes that we intend to achieve in future studies is to optimize the homogeneity of the emulsion. In fact, also if the emulsion has a good taste and consistency, the oil droplets were still visible to the naked eye. Generally microstructure of mayonnaise affects rheological properties (Rahbari *et al.*, 2015) and also if the homogenization process is expensive, it's necessary to improve the quality and the consistency of emulsions, and on other hand the determination of particle size during formulation and manufacturing is important to ensure the acceptance of the new product.
Microstructure of mayonnaise is affected by different factors such as type and concentration of emulsifying and stabilizing agents, size of droplets, viscosity of the water phase, and oil content (Laca *et al.,* 2010).

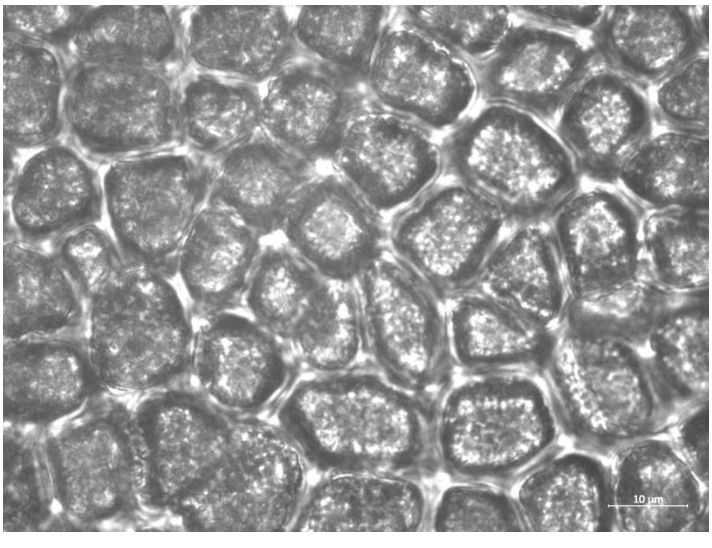


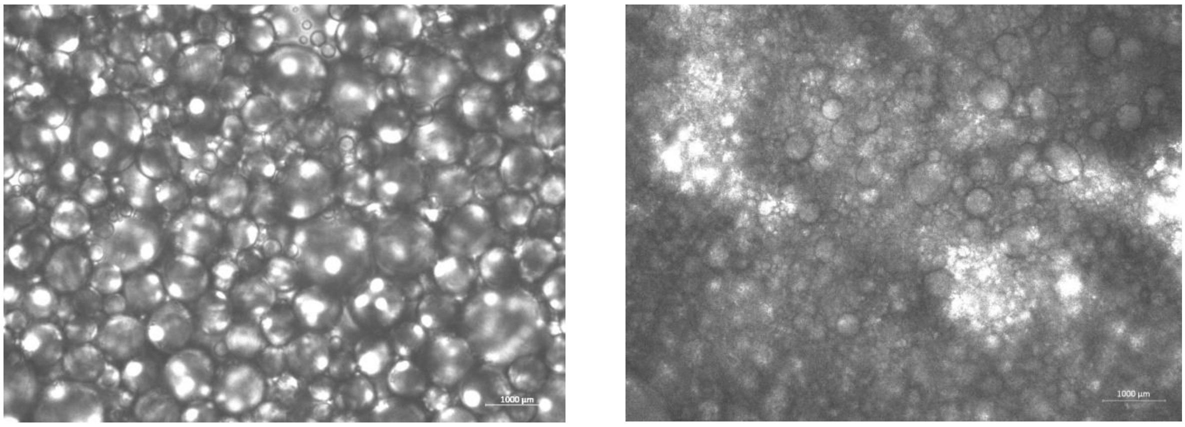
Fig. 28 Microstructure of the 27c premix

The figure 28 shows the microstructure of the 27c premix (determined after 24 hours of preparation) composed by linseed protein, β-glucan, water, sugar, mustard and salt droplets; from the image, it is possible to notice large structures: probably they are conjugates of linseed proteins and beta-glucan molecules, formed during the processing, that are linked to the high viscosity and viscoelasticity observed in rheological tests.

Subsequently, the microstructure of sample 27c was determined (after 24 hours of preparation) and compared with that of full fat mayonnaise (Figure 29).

Fig. 29 Optical microscope of different mayonnaise formulations

a. Sample 27c. (x100) **b. Full fat mayonnaise (x40)**



In spite of the poor quality of the photographs, it is possible to see the difference in the droplets dimensions of the two emulsions. In the first case (Figure $29_{(a)}$) the emulsion droplets are quite large, but their size could be reduced through the optimization of the homogenization process, as discussed above.

In mayonnaise, the large contact surface area between oil droplets leads to important friction forces which oppose the free flowing of the emulsion in a shear field, hence increasing its viscosity (Phillips & Williams, 2011). A decrease in oil droplet diameter leads to a greater contact surface area between droplets, and therefore to an increased viscosity (Langton *et al.*, 1999; Liu *et al.* 2007).

Despite these considerations, more than satisfactory results have been recorded in the two sessions of the sensory evaluation. In the next section, the results obtained by the two panel tests will be compared and discussed (see Table 12). Mayonnaise samples were labelled randomly with three-digit codes.

Initially, panelists were asked to judge the colour of the product. In the first trial only the vegan mayo resulted significantly different than the other samples, but this outcome was not confirmed in the second trial. However, in both cases the colour of the sample 27c reached scores similar to those of full fat mayonnaise. The vegan mayo obtained the lowest values and some of the judges who participated in the focus groups, which held at the end of the two sessions, expressed similar perplexities for its colour, sustaining that it is too white for a mayonnaise.

The second parameter analysed was the consistency. According to the Tukey's test results emerged in the first trial, vegan mayo presented values significantly different, while full fat mayonnaise showed intermediate characteristics with respect to the other samples. Similarly in the second session, 27c has been considered more similar to light mayonnaise also if, in this case, high p-values and no significant differences were recorded. In both cases, vegan mayo presented the lowest consistency scores.

Regarding fatty aroma, similar averages and medians were observed with no significant statistical differences, although the vegan product obtained lower scores in the first session.
About vinegar aroma, vegan sample collected the highest scores also if only in the second session a significant difference has been recorded from a statistical point of view. In the first evaluation 27c registered the lowest score. With reference to fatty flavour, results reflected our expectations and there is nothing interesting to report.
Apropos of the acidic flavour, vegan sample was found significantly different with respect to light and full fat mayonnaises. The acidic flavour of 27c is a middle ground: in the first session it was found more similar to that of full fat mayonnaise, while in the second one to that of light product.
In the matter of saltiness, no significant differences were noted in the first evaluation, while they were observed between vegan mayo and 27c in the second trial. Also with regard to creaminess, in the first session there weren't registered significant differences despite lower values of the vegan mayo, in the subsequent trial they were found between full fat mayonnaise and the vegan product.
At this point, panelists had to judge the overall quality. In both cases vegan mayo resulted significantly different than the other samples, with the lowest scores. In the first trial our sample was evaluated similar to full fat mayonnaise despite the high p-value for the judges, while in the second case it was associated to the light mayonnaise.

Table 12. Sensory evaluations and statistical analysis of the results, for both sessions.

			1st Trial				2nd Trial			
		Mayo	Mean	p-value		Tukey's HSD	Mean	p-value		Tukey's HSD
1.	Colour	*Light*	7,19	*Samples*	0	a	6,4	*Samples*	0,39	a
		Vegan	3,61			**b**	4,99			a
		27)c.	**5,56**	*Judge*	0,28	a	**5,82**	*Judge*	0,74	a
		Full Fat	6,02			a	5,7			a
2.	Consistency	*Light*	6,86	*Samples*	0	**a**	6,08	*Samples*	0,65	a
		Vegan	4,54			**b**	5,3			a
		27)c.	**6,11**	*Judge*	0,02	**a**	**5,97**	*Judge*	0,55	a
		Full Fat	5,99			**ab**	5,68			a
3.	Fatty Aroma	*Light*	4,82	*Samples*	0,09	a	4,72	*Samples*	0,83	a
		Vegan	3,94			a	4,88			a
		27)c.	**5,24**	*Judge*	0	a	**4,99**	*Judge*	0,02	a
		Full Fat	5,2			a	5,21			a
4.	Vinegar Aroma	*Light*	5,45	*Samples*	0,08	a	5,28	*Samples*	0	**b**
		Vegan	6,71			a	7,32			a
		27)c.	**4,78**	*Judge*	0,79	a	**6,07**	*Judge*	0,01	**b**
		Full Fat	5,65			a	5,24			**b**
6.	Fatty Flavour	*Light*	5,41	*Samples*	0,44	a	5,60	*Samples*	0,16	a
		Vegan	5,19			a	5,49			a
		27)c.	**6,21**	*Judge*	0,46	a	**6,24**	*Judge*	0,01	a
		Full Fat	6,27			a	6,61			a
7.	Acidic Flavour	*Light*	5,39	*Samples*	0	**b**	6,01	*Samples*	0	**b**
		Vegan	7,98			**a**	7,98			**a**
		27)c.	**6,69**	*Judge*	0,41	**ab**	**6,93**	*Judge*	0,4	**ab**
		Full Fat	6,13			**b**	5,98			**b**
8.	Saltiness	*Light*	4,55	*Samples*	0,10	a	5,28	*Samples*	0,06	**ab**
		Vegan	5,96			a	6,29			**a**
		27)c.	**5,17**	*Judge*	0,01	a	**4,98**	*Judge*	0	**b**
		Full Fat	5,69			a	5,48			**ab**
10.	Creaminess	*Light*	6,70	*Samples*	0,07	a	6,33	*Samples*	0,06	**ab**
		Vegan	**5,43**			a	**5,68**			**b**
		27)c.	6,66	*Judge*	0,18	a	6,12	*Judge*	0,42	**ab**
		Full Fat	6,76			a	7,18			**a**
12.	Overall Quality	*Light*	7,03	*Samples*	0	**a**	6	*Samples*	0,03	**ab**
		Vegan	3,19			**b**	4,4			**b**
		27)c.	**6,3**	*Judge*	0,73	**a**	**5,93**	*Judge*	0,03	**ab**
		Full Fat	6,5			**a**	6,37			**a**

Despite some small discrepancy in the results emerged from the comparison of samples between the two sessions, our emulsion obtained similar averages for each parameter in both tests, except perhaps for the "vinegar aroma" in which the values difference resulted slightly greater. This showed that, although the two emulsions were prepared on different days, their characteristics were comparable and the preparation method is repeatable.

However, also if the scores detected in the two sessions were comparable, some of the comments expressed on the overall quality of our sample were a bit divergent highlighting that there was a slight difference in the two preparations. Here below, the most recurring opinions expressed by the panelists on the overall quality of the samples tested have been reported. In the case of our sample they have been also divided by session (Table 13).

Table 13. Comments expressed by panelists on overall quality of the products tested.

Type		Positive attributes	Negative attributes
Light		- Best balance between fatty and acidic flavour - Quite bland and fine taste - Nice creaminess and colour	- Boring - Too salty - No enough fatty flavour - Bitter Aftertaste
Vegan		//	- Too salty and overbearing acidic - No nice aftertaste - No good consistency - Unpleasant, disgusting - Poor texture
27)c	**1st session**	- Best overall balance between flavour attributes - Best balance in terms of acidity, salty, creaminess - Best taste and texture - Lovely consistency and aftertaste	- Too yellow - Little bit grainy - Acidic aftertaste
	2nd session	- Fine flavour and nice consistency - Good visual appearance and colour - Good fatty flavour and creaminess	- Little bit matt - Bitter taste and acidic aftertaste - Synthetic sensations
Full fat		- Best balance of flavour - Amazing taste, balanced between vinegar and fat - Nice colour and consistency - Natural, smooth and the most appealing	- Too much salted - Strange and tangy aftertaste - Too yellow - Strong egg flavour - Lacked aroma - Shiny and wet appearance

Panelists were asked also to express any comments and add personal considerations on the following aspects: off odours, off flavours and after taste. The results obtained have been summarized and shown in the table below (Table 14).

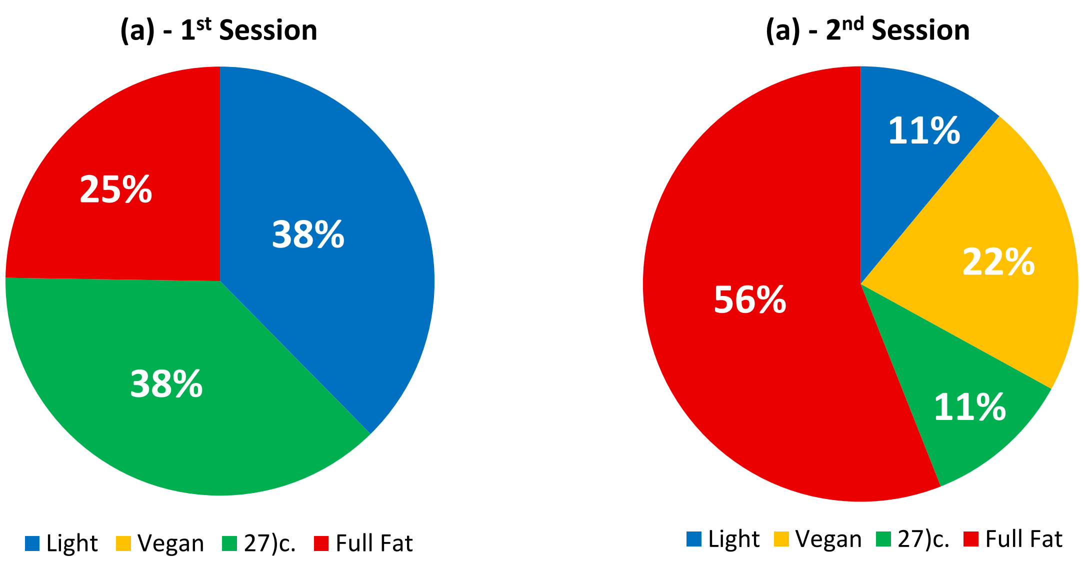
Table 14. Summary of comments collected on off-odours, off-flavours and aftertaste for each sample in both sections.

Samples	Off Odours	Off Flavours	After-Taste
Light	- No off odour - Bland - Typical - Slightly too acidic/vinegar smelling	- No off flavour - Strong acidic flavour - Fatty flavour	- No unpleasant and lingering aftertaste. - Pleasant vinegar aftertaste - Slightly sharp and acidic aftertaste - Spice/mild cheese-like aftertaste
Vegan	- Too strong smell, like vinegar. - Too acidic - No typical - Salty	- Too vinegary, - acidic and salty	- Too salty and acidic - Very bitter aftertaste in mouth - Capers - Stingy/tangy aftertaste associated with the strong vinegar taste. - Slightly bitter and unpleasant
27c	- Slight acidic aroma (as would be expected) - No 'off-odour'. - Fatty aroma - Slight metallic odour.	- No off flavours - No salty - Slightly acidic - Mustard	- slightly tangy taste - Acidic - oily and slightly bitter - No standard 'dairy' aftertaste, typical of mayo.
Full fat	- No obvious off odours, or lingering smells - No unpleasant odours - Slight vinegar aroma - Strong fatty aroma - Slightly salty	- No off flavours - Bit acidic/like vinegar. - Too salty - Rancid	- Egg-y taste - Slight fatty and acid after-taste (pleasant) - No unpleasant aftertaste. - Standard mayonnaise aftertaste - Garlic aftertaste

From an analysis of all the comments expressed on the issues shown in the table above, it is not possible to draw conclusions.

About the preferences expressed, in the first session the light mayonnaise and our formulation achieved more votes, with a percentage of 38%, while vegan mayo did not obtain any votes (Figure 30 (a)). In the second case the full fat mayonnaise “ousted” the rivals, ranking first with more than fifty per cent of the total votes. (Figure 30(b)).

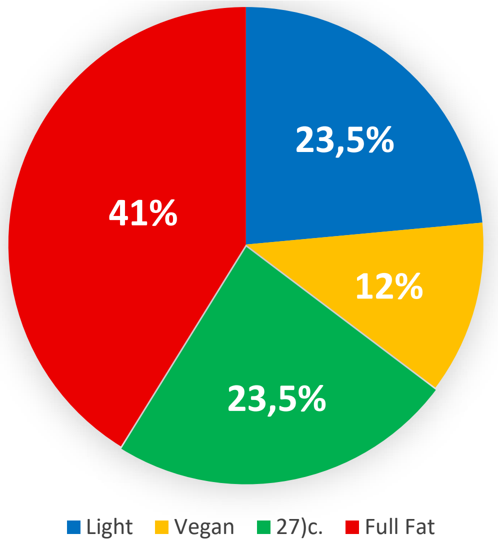
Fig. 30 Consumer preferences



Finally, the preferences achieved from each trial were summed to have a general overview. Our formulation 27c reached the second place,obtained the same percentual score of the light mayo (Fig. 31).

Fig. 31 Preference summary

	1st Trial	2nd Trial	Sum	%
Light	3	1	**4**	***23,5***
Vegan	0	2	**2**	***12***
27)c.	3	1	**4**	***23,5***
Full Fat	2	5	**7**	***41***
	8	9	17	***100***



Summing up the outcome of sensorial tests, our compound resulted similar to the full fat mayonnaise for some parameters (i.e. colour, consistency, fatty flavour) and, for other ones, to the light mayonnaise, contrary to vegan mayo that differs considerably from all other samples.

During the focus group held at the end of the tasting, the panelists (among which there were some vegetarians and a vegan person) expressed negative opinions on the vegan mayo, specifying that they would never buy it. If we consider that in the last few years the veganism is going mainstream, involving also many non-vegan people, several strategies should be put in place in order to attract more all those who are only following the trend of the moment.

The product used as control in our experiments is one of the few vegan mayo currently sold in all major English supermarkets, and according to the results emerged from the sensory tests, it does not meet consumer demands. Taking into account also that UK Plant-based foods sales are up 1,500 per cent in the last year, we think it's necessary introduce new products, that can satisfy consumers, especially creating flavors that are more similar to the most widely marketed products.

Given these premises, future efforts should be aimed in this direction; our sample could be an interesting starting point to work on, whose processing obviously needs to be optimized.

In spite of the good starting rheological properties of our formulation, it's necessary to optimize the homogenization process and identify a suitable sterilization method in order to obtain a long-life food product. Also if in the manufacture of mayonnaise and salad dressings a heat-treatment stage usually is not included. The acidity of the final product isn't sufficiently low to inhibit the proliferation of microorganisms.

It could be interesting also analyse in which way the rheological behaviour of our sample changes by varying the parameters involved in measurement phases.

As regards instead sensory characteristics, our formulation obtained good results despite having a limited ingredient number compared to controls (Table 10), without additives, preservatives and food colouring. Moreover it contains just one allergen (the mustard) and it's free of soybeans. It provides kilocalories as a vegan mayo, it has less fats than a full fat mayonnaise and less sugars than light and vegan controls but, at the same time, its taste lives up to the others.

No panelist realized that the sensory test had been organized to test a new product, that currently doesn't exist on the market. When we told them what was going on and asked them to identify which was the experimental mayonnaise, almost no one has been able to identify it, mistakenly believing that it was the vegan control (already on the market), because of its very clear colour and its very acidic taste.

Considering that consumers are beginning to show preference to low-fat food products, our experimentation should continue with a new purpose: try to decrease the fat contents using fat replacers, that will not only improve processing functionalities but also contribute to nutritional benefits (Su *et al.,* 2010). We have already begun to take this new direction, using beta glucan to create our compound, which offers several health benefits (for example, it reduces intestinal absorption of cholesterol) and can be used due to its high water-binding and viscosity-enhancing properties (Kalinga & Mishra, 2009).

CONCLUSION

Today, there is a growing consumer demand for low fat, low cholesterol, healthier, vegan and allergens-free food products, but possibly with the same taste of the traditional ones.

The present study was conducted to prepare an egg-free vegan mayonnaise by combination of several ingredients among which linseed proteins and beta glucan. These ingredients allowed to achieve good results and to create a final product with properties comparable to those of the conventional mayonnaise, based on an interesting mix with high water-binding and viscosity-enhancing properties.

Despite our formulation has shown to have good rheological properties, microscopic analysis highlighted the need to better homogenize our compound. Since larger oil droplet sizes formed in the final emulsified product may have negative influenced its texture and storability, the application of higher shear force or the use of more surface-active emulsifiers may be considered in future studies. Furthermore, it is necessary to identify an appropriate method for the sterilization of our formulation in order to remove any contaminants and obtain a long-life product, responding to the essential requests for a process of scaling up. Unfortunately, tests conducted using the microwave and Burton Sous Vide have shown that at high temperatures there is a decline in the overall quality of our product.

As regards instead sensory characteristics, our formulation obtained good results despite having a limited ingredient number compared to controls, without the addition of additives, preservatives and food colouring. Moreover it contains just mustard as allergen and it's free of soybeans. It provides kilocalories as a vegan mayo, it has less fats than a full fat

mayonnaise and less sugars than light and vegan controls but, at the same time, its taste lives up to the others.
Also regarding preferences, sensory tests showed that our formulation can compete with other mayonnaises on the market and some consumers have even declared to prefer it to the other samples.
Definitely, these results demonstrate the ability of our mix to successfully replace egg yolk in a mayonnaise formulation. Our formulation can be a good starting point, from which to get a new product able to better meet consumer demand.

4.4 CASE STUDY IV

BAKING WITH MAYONNAISE: ***An application of our new formulation***

A BRIEF INTRODUCTION

In the last decades fresh eggs constituted one of the primary ingredients in the bakery sector, especially for home-made cakes and retail bakeries in western countries (Pyler, 1988), thanks to their nutritional value and multi-functional properties, including emulsification, coagulation, foaming, and flavor (Yang and Baldwin, 1995).

Briefly, to emulsify means to combine two liquids that normally do not combine easily, such as oil and vinegar; foaming properties of a protein refer instead to its ability to form a thin tenacious film at gas-liquid interface so that large quantities of gas bubbles can be incorporated and stabilized. Finally, coagulation is defined as the transformation of proteins from a liquid state to a solid form. Once proteins are coagulated, they cannot be returned to their liquid state.

Nevertheless, the production of cakes moved, with the course of the time, from home and small retail bakeries to the widespread use of box mixes and large production facilities. During these decades, despite the advantages provided by egg's unique properties, several factors have led have led the baking industry to search for ingredients to replace it (Hard *et al*., 1963; Lin *et al*., 2003; Swaran *et al*., 2003). Among these factors we can find: the market volatility, the need to reduce production costs and meet consumer demands, increasingly oriented towards environmentally-frienldy products against the intensive livestock farming. Their concerns also include a desire for low-cholesterol foods, reduced allergens, less expensive ingredients, increased shelf life, no refrigeration requirements (Romanoff, 1949; Lin *et al*., 2003; Swaran *et al*., 2003).

With the advent of new technologies, many new food ingredients are being advertised (Kohrs *et al.,* 2010). However, there is very little literature that compares these ingredients to eggs in a scientific study (Abu-Ghoush *et al*., 2010; Kohrs *et al*., 2010). Unfortunately, much of the research completed is old and it do not include any sensory evaluation which is very essential in product development (Lawless and Heymann, 1999).

In this study we prepared several mayonnaise cakes, but not all of them contained eggs. Mayonnaise cakes became popular during World War II when common baking ingredients, such as eggs, butter, oil and sugar were rationed and families needed to be more resourceful. The egg yolks and oil in mayonnaise provided fat for a moist cake, but also created a recipe high in fat. Today you can find lower fat mayonnaise, but there are alternatives to this condiment that provide more health benefits.

Bakery goods are an incredibly diverse category of products, which are generally made with a significant proportion of wheat flour. A specific component of this wheat flour, gluten, once hydrated and worked conveys on uncooked doughs a viscoelastic structure that traps air bubbles, which in turn are inflated by the gases produced during leavening (O'sullivan, 2016). In both biological and chemical leavening, the carbon dioxide (CO2) is essential for the formation of the breads light cellular foam like network, which forms a sponge structure on baking (O'Sullivan, 2016).

However, confectionary sponges, muffins and cakes are produced quite differently from breads, with minimal gluten action, where the foam-stabilising ingredients include egg protein, fat, and emulsifiers. For sponges, leavening is typically achieved physically through the incorporation of air during mixing, e.g., mixing air in to egg whites, which is then folded in to a sponge batter and baked to produce a light airy texture. Chemical leavening can be also used for some cakes and cookies where baking powder produces CO2 in the presence of water.

Unlike baking soda, baking powder contains sodium bicarbonate and at least one acid salt to produce gas. However also other acidic ingredients can be added to influence the leavening process.

Acidic ingredients play an important role in cake baking. They add and enhance flavors as well as contribute to leavening and tenderization of cakes (Stone, 2017). Batter acidity can come from a wide variety of sources including vinegar and lemon juice, that you will find in all the recipe of the mayonnaises used to prepare the chocolate cakes. Indeed, the vinegar and the lemon juice are often included in cake and cookie batters to react with baking soda and start the chemical reaction needed to produce carbon dioxide and give those batters a lift as they bake (Leach, 2003). However, both in the case of chemical and in biological leavening, the carbon dioxide (CO2) is essential for the formation of the breads light cellular foam like network, which forms a sponge structure on baking (O'sullivan, 2016).

Essentially cakes, muffins and sponges are fat-in water emulsions. The high level of liquid from water and the egg components produces a low-viscosity cake batter that is easy to pour and the resulting steam produced in the oven during baking helps produce a light texture in the end product.

One of the most important difference between breads and confectionary baked goods such as cakes, muffins, pastry and biscuits are their very different sensory properties. The latter are sweet, high-calorie baked products, particularly appreciated by consumers all over the world, where sucrose contributes to the sweet taste but also acts as a bulking agent in the batter, aids in moisture retention and air entrapment and creates a fine crumb grain in the products (Manisha *et al*., 2012; Nip, 2014).

On the other hand, the fat contributes to the texture, mouthfeel, flavour and aroma of food (Drewnowski and Almiron-Roig, 2010). Initially, the fat globules entrap air in the batter during mixing, thus aerating the product (Martı´nez-Cervera *et al*., 2012). The fat has also important emulsifying properties and it is able to hold a large amount of liquid, which increases and extends the softness of the cake (Barcenilla *et al*., 2016, Cauvain, 2011). Finally, the presence of fat has a 'shortening' effect on the crumb texture of cake and muffins, in that it interferes with the protein matrix, resulting in a 'short', more tender crumb (O'sullivan, 2016). Therefore, it would be expected that a fat reduction process would induce changes such as lower overall volume (less aeration), increased crumb firmness (because of a greater degree of starch swelling) and a reduced palatability. Fat reduction will result in reduced volume and increased crumb firmness (Rodríguez-García *et al*., 2014).

In the following work, we tested the new vegan mayonnaise formulation (described in the paragraph 4.3) in a bakery application.

One of the main aim was to understand if the mix of the ingredients contained in the new vegan mayonnaise, could replace the egg in the preparation of a chocolate cake, emulating some of the functional characteristics of the whole-egg.

Another purpose was to test if the resulting cake could compete, with reference to all physical and sensory attributes, with the other cakes, especially with those obtained by using full fat mayonnaise, despite having a lower content of sugars and fats.

EXPERIMENTAL PART

Chocolate-mayonnaises cakes were prepared using the emulsion obtained as described in the previous chapter and three mayonnaise products on the market, which were used as controls.
In addition to colour measurements, firmness and springiness analysis were carried out. These tests were repeated on two consecutive days to monitor any change. Finally, two sensory evaluations were conducted to verify the acceptability of the products, compare their characteristics, and finally to analyse the repeatability of the method.

Recipe

The chocolate-mayonnaise cake recipe includes 285g self-raising flour, 225g sugar, 1½ teaspoon of baking powder, 200g mayonnaise, 4 tablespoons of cocoa powder, 237ml boiling water, 1 teaspoon of vanilla essence. The new vegan mayonnaise was prepared using Rapeseed Oil, Water, Linseed Proteins (provided by TH. Geyer), beta glucan (OatWell® 28 distributed by *DSM Nutritional Products, Inc.*), vinegar, mustard, salt, sugar, starch (Avanté 1 by *Ulrick&Short*) and lemon juice (Chapter 4).
Three different types of mayonnaise on the market (Hellmann's real full fat mayonnaise, Asda light mayonnaise and Plamil egg free mayo) were used as control samples.

Directions

Fig. 32 Paddle attachment

After all the ingredients were weighted, we mixed flour, sugar, baking powder and mayonnaise in a mixer with paddle attachment (Fig. 32). We dissolved the cocoa in the boiling water & striated it into the mixture along with the essence until thoroughly blended (3 min). The mixture was placed into a 7 inch greased & lined cake tin, and baked at gas mark 180°C for 30 minutes. After testing the cake with a skewer, we let it cool in the tin before removing. Percent weight loss due to moisture loss was calculated by weighing the cakes before and after baking.

Colour measurements

In order to measure the colour of cakes, we used L*a*b* or CIELab colour space, which is an international standard accepted by the Commission Internationale d'Eclairage (CIE) in 1976 (Leon *et al*., 2006), commonly employed by food industry (Tarlak *et al*., 2016), according to which the coordinate *L** defines a lightness axis, *a** a redness-greenness axis, and *b** a yellowness-blueness axis.

Specifically in this *method*, values of L* (the lightness component or luminance) range between 0 for black and 100 for a perfectly diffusing white (and possibly >100 for specular reflecting surfaces) (Foster, 2008); a* is a chromatic component ranging between -120 and +120, from green (negative a*) to red (positive a*); and b* is a chromatic component ranging between -120 and +120, from blue (negative b*) to pure yellow (positive b*). Color-appearance attributes can be obtained from *L**, *a**, *b** by defining relative colorfulness or chroma C^*ab as $(a^{*2} + b^{*2})^{1/2}$ and hue *hab* as an angle $\tan^{-1}(b^*/a^*)$, in degrees, to form a cylindrical coordinate system (Fairchild, M. D., 2013). The hue angle hab is calculated from a* and b* values as: where hab lies between 0° and 90° if b* and a* are both positive, between 90° and 180° if b* is positive and a* is negative, between 180° and 270° if b* and a* are both negative, and between 270° and 360° if b* is negative and a* is positive (Galaffu *et al*., 2015).

Conventionally, the colour of foods is analysed with a colorimeter that measures small and non-representative areas of the food and the measurements usually vary depending on the point where the measurement is taken. For this reason, in order to minimize the error, in our experimental design

For this reason, in order to minimize the error, in our experimental design, have been cut four pieces from each cake, and for every one were carried out four inner measurements and four measurements on the top (for a total of 32 measurements for cake). The color measurements were carried out also the day after on other cakes pieces to check any colour changes.

The same experiment was repeated on a different day with fresh cakes, in order to verify if the results obtained in the first trial were similar.

Texture analysis

Before to analyse the texture of the cakes, we have cut the samples in more pieces and measured the height of each one. Finally we calculated the average height of each cake.

A measurement of both firmness (according to the AACC method 74-09) and springiness (elastic recovery) was made by adaptation of the AACC method into a 'Hold Until Time' test, where a compression distance is held for a chosen period of time, over which the product's recovery is recorded (Fig. 33). While the Firmness is defined as the force (in g, Kg o N) required to compress the product by a pre-set distance e.g. 25%., the springiness (originally called “elasticity”) is instead the rate at which a deformed sample returns to its original size and shape.

In order to evaluate the springiness of the sample, the force is recorded after 60 seconds and its value is divided by the maximum force and then multiply by 100%.

$$\frac{F_{60}}{Fmax}\, x\; 100\% \; = \%\, recovery$$

Fig. 33 Stable Micro System with probe

The closer the resulting value is to 100%, the more like a " spring" the product is.

Before carrying out the test using "25% strain" measurement, we calibrated the probe so that it could acknowledge the texture analyser test surface (which is recorded as "100% strain"). To do this, we lowered the probe, so that it was close to the test surface, and we specified the distance that the probe had to return to, after sample compression. The samples were placed centrally under the cylinder probe, avoiding any irregular or non-representative areas of the crumb.

This test assumes that the surface of the sample is larger than the probe diameter. The probe compressed the samples until the 25% of their height, holding this distance for 60 seconds and then it returned to its starting position.

Sensory evaluation tests

Sensory evaluations were conducted on four types of cakes, prepared using the fresh formulation described in the previous chapter, full fat mayonnaise, light mayonnaise or vegan mayo (the same controls used in the mayonnaise sensory evaluations). They were carried out in two sessions at the sensory laboratory of Queen Margaret University, in different days but with the same experimental design, and the panels consisted of untrained panelists, eight in the first session and nine in the second.

Sensory characteristics including appearance, colour, cocoa aroma, cocoa flavour, sweetness, softness, chewiness, overall quality were evaluated by untrained panelists based on a 9-point Hedonic scale (1 as the lowest and 9 as the highest score); the panelists were requested to assess the samples according to the parameters stated in the sensory evaluation score sheet, and also to write their comments about off-odours, off-flavours and aftertastes of the samples.

The cakes were served at room temperature in plastic plates, that were labelled randomly with three-digit codes. According to Venkatachalam & Nagarajan (2017), prior to each tasting a sample, lemon water was served to the panelists to neutralize their mouth feel, in order to maintain comparable testing over a sequence of samples.

Finally, the panelists had to express their opinion on all the samples and their preference, selecting just one of the four cakes tested. Their answers were collected and analysed using the Compusense Cloud sensory software.

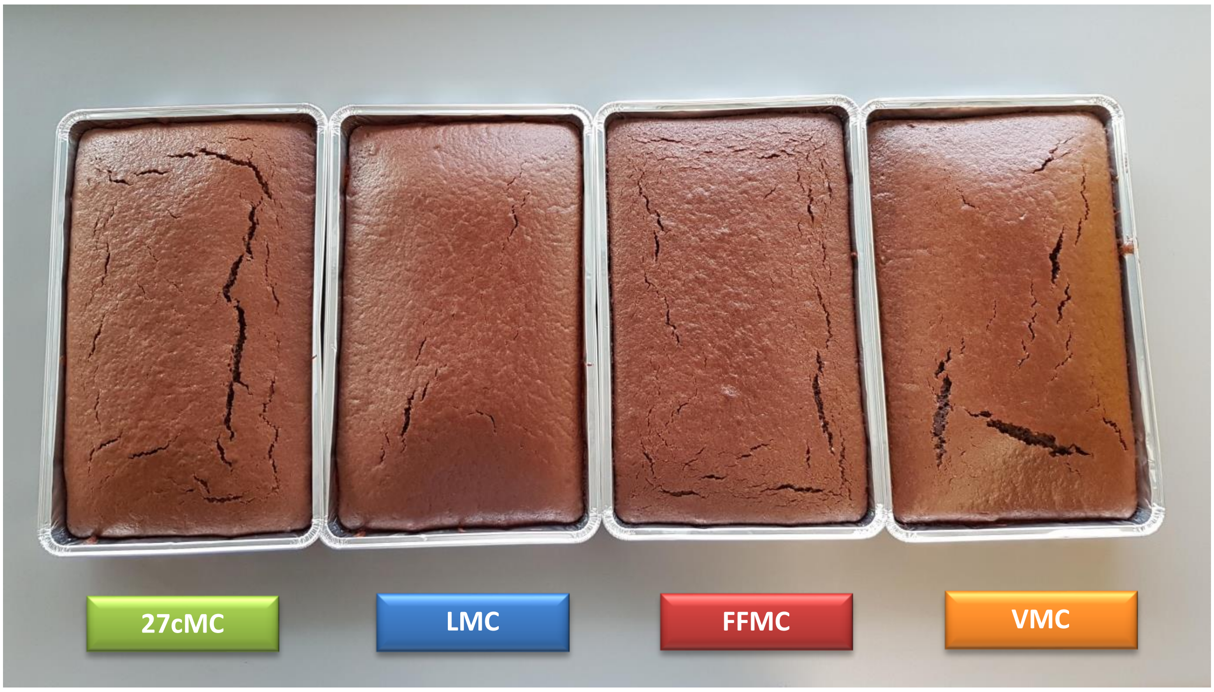
Statistical analysis

Sensory characteristics were analysed by Two way ANOVA analysis and also pairwise comparisons performed using Tukey's HSD control for maximum experiment-wise error rate (regardless of the sample p-value) were carried out.

RESULTS

As described in the previous chapter, a mix of linseed proteins, beta glucan and starch were used to prepare a new kind of vegan mayo, obtaining excellent results both from a rheological and sensory point of view. It was interesting to understand if this mix could replace the egg in the preparation of a cake, emulating some of the functional characteristics of whole-egg and with reference to all physical and sensory attributes of the final product. Chocolate-mayonnaises cakes were prepared using our emulsion (obtained as described in the previous chapter) and three mayonnaise products as controls (Fig.34).

Fig. 34 27c mayonnaise chocolate cake (27cMC), light mayonnaise chocolate cake (LMC), full fat mayonnaise chocolate cake (FFMC), vegan mayo cake (VMC) represented, respectively, from left to right).

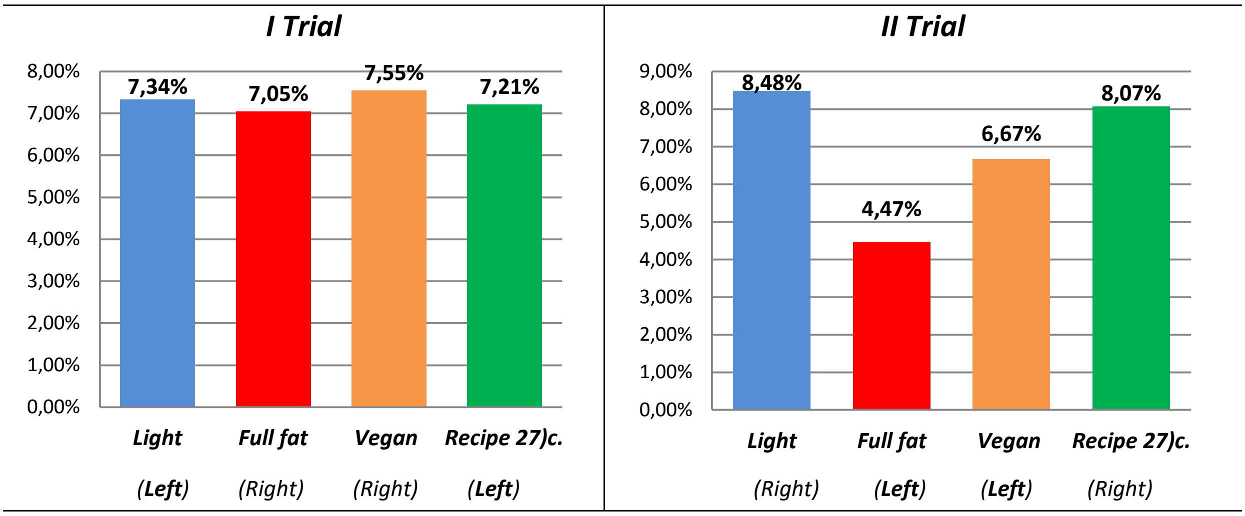


Percent weight loss due to moisture loss was calculated by weighing the cakes before and after baking and the results obtained are showed in the figure 35.
In the first trial, similar results were recorded, "full fat mayonnaise cake" (FFMC) showed the lowest percentage loss, while the "vegan mayonnaise cake" (VMC) the highest loss; the cake prepared with our formulation presented intermediate values (27cMC).
In the same oven, were baked two cakes at a time. The cakes baked on the right side of the oven in the first trial, were placed on the left in the second trial, and vice versa.
In this way it was possible to understand if the position in the oven influenced the weight loss of the cakes.
In the second trial, the cake made with full fat mayonnaise again showed the least loss, while the "light mayonnaise cake" (LMC) recorded the highest value.

In general all the cakes that had previously been baked on the left and in the second experiment on the right have reported an increase in weight loss; vice versa the cakes cooked on the right and then on the left reduced the weight loss. This could be explained by the fact that the heat diffusion in the furnace is not homogeneous.

Figure 35.-Percent weight loss of the cakes after baking.

% Weight loss



The colour of foods is one of the most important selection criteria of how we purchase our food (Kays, 1991; Mendoza & Aguilera, 2004). If the colour of the food is not what is expected, the consumers generally have a negative perception of quality (Francis, 1995) with inevitable repercussions on their purchasing decisions (Costa *et al.*, 2011; Meléndez-Martínez *et al.*, 2005).

Therefore, nowadays, it is necessary to measure the color of the food with extreme accuracy. The colour of an object can be determined either by human (visual) inspection or by using a colour measuring device, but it is generally recommended to determine the colour via a colour measuring instrument (Leon *et al.*, 2006), especially if we consider that the determination of colour could be subjective and variable from observer to observer.

As shown in the Table 15, regarding measurements of the cakes top, LMC and FFMC presented similar values, VMC had slightly higher values than the other two and 27cMC recorded the highest values for all the parameters. On the other hand, regarding the inner measures LMC and VMC appeared similar, FFMC showed the lowest values while 27cMC the highest ones.

We repeated the test the following day by measuring the same parameters on other four pieces of each cake. In this case we noticed that with regard to the surface measurements, the 27cMC presented slightly higher values than the other samples (which were rather similar to each other), for all parameters with the exception of the hue value, that resulted lower than that of VMC. As for the inside, LMC and VMC were similar, FFMC showed lower values, while 27cMC reported also in this case the highest values.

Table 15. Determination of the cakes' colors using L*a*b* or CIELab colour space, by calculating the averages of internal and surface measurements (I Trial).

	I Experiment			
	Top (Average)		***Inner (Average)***	
	1st day	*2nd day*	*1st day*	*2nd day*
Light Mayonnaise Cake (LMC)	L* = 42,46 (± 0,42)	L* = 37,65 (± 2,50)	L* = 34,77 (± 1,02)	L* = 34,21 (± 0,49)
	a* = 12,80 (± 0,39)	a* = 13,61 (± 0,86)	a* = 12,84 (± 0,10)	a* = 12,72 (± 0,31)
	b* = 10,70 (± 0,55)	b* = 11,70 (± 1,34)	b* = 13,16 (± 0,23)	b* = 12,94 (± 0,38)
	C* = 16,68 (± 0,61)	C* = 17,96 (± 1,42)	C* = 18,38 (± 0,21)	C* = 18,15 (± 0,45)
	h* = 39,86 (± 1,06)	h* = 40,58 (± 1,53)	h* = 45,66 (± 0,40)	h* = 45,43 (± 0,64)
27)c Mayo Cake (27cMC)	L* = 46,15 (± 0,15)	L* = 44,86 (± 0,75)	L* = 38,03 (± 0,54)	L* = 39,79 (± 1,75)
	a* = 15,15 (± 0,12)	a* = 14,51 (± 0,30)	a* = 15,73 (± 0,39)	a* = 15,62 (± 0,33)
	b* = 14,09 (± 0,43)	b* = 13,09 (± 0,55)	b* = 17,42 (± 0,39)	b* = 17,71 (± 0,76)
	C* = 20,68 (± 0,38)	C* = 19,55 (± 0,58)	C* = 23,48 (± 0,54)	C* = 23,63 (± 0,78)
	h* = 42,91 (± 0,65)	h* = 42,02 (± 0,64)	h* = 47,88 (± 0,16)	h* = 48,56 (± 0,65)
Full Fat Mayonnayse Cake (FFMC)	L* = 42,16 (± 0,56)	L* = 40,01 (± 1,81)	L* = 30,50 (± 0,74)	L* = 31,24 (± 0,96)
	a* = 12,76 (± 0,34)	a* = 12,54(± 0,07)	a* = 12,36 (± 0,34)	a* = 12,37 (± 0,33)
	b* = 10,97 (± 0,40)	b* = 10,73 (± 0,17)	b* = 11,49 (± 0,38)	b* = 11,61 (± 0,69)
	C* = 16,83 (± 0,50)	C* = 16,51 (± 0,14)	C* = 16,90 (± 0,31)	C* = 16,99 (± 0,66)
	h* = 40,65 (± 0,53)	h* = 40,52 (± 0,41)	h* = 42,73 (± 1,54)	h* = 42,99 (± 1,30)
Vegan Mayo Cake (VMC)	L* = 43,82 (± 0,42)	L* = 38,96 (± 3,41)	L* = 34,42 (± 0,84)	L* = 34,30 (± 1,17)
	a* = 13,10 (± 0,12)	a* = 13,92 (± 0,18)	a* = 13,42 (± 0,48)	a* = 13,00 (± 0,34)
	b* = 11,36 (± 0,43)	b* = 12,63 (± 0,08)	b* = 13,74 (± 0,71)	b* = 12,93 (± 0,80)
	C* = 17,35 (± 0,37)	C* = 18,80 (± 0,18)	C* = 19,21 (± 0,84)	C* = 18,34 (± 0,80
	h* = 40,92 (±0,82)	h* = 42,18 (± 0,20)	h* = 45,55 (± 0,58)	h* = 44,78 (± 1,15)

In the second experiment it was found that, with reference to the top of the cakes, 27cMC presented the lowest values whilst LMC the highest ones (for all parameters) contrary to what was recorded in the first trial (Table 16). FFMC and VMC measurements were in line with those of LMC. As regards the measurements of the inside of the cakes, LMC and VMC showed similar values, as well as the FFMC and 27cMC.

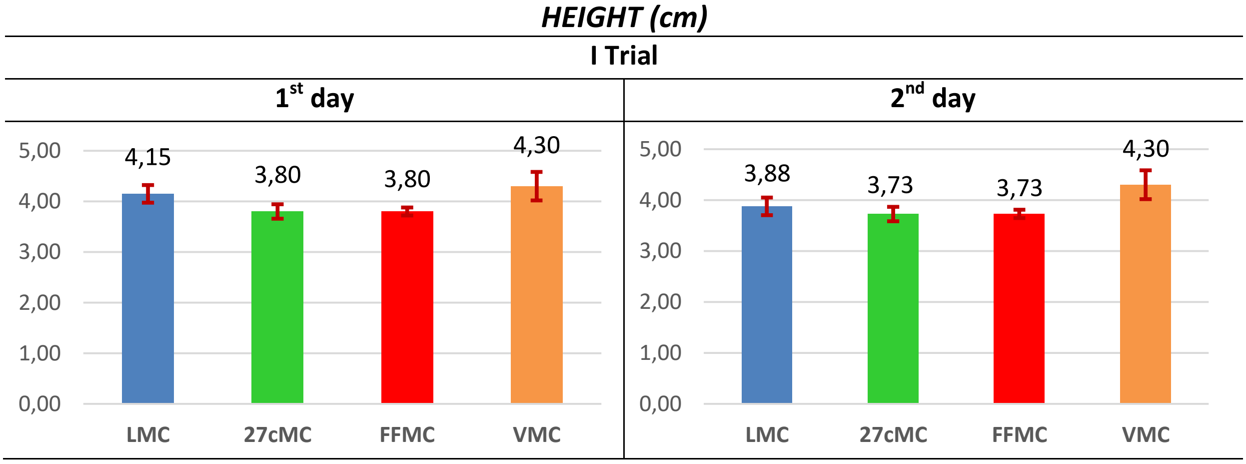
The most interesting consideration is that among the four samples, with reference to the comparison of the data obtained in both tests, the 27cM cakes are those that have shown greater consistency between the results. The other types of cakes have presented much higher values of luminance, of the chromatic component "a" (tending to red), and of "b" (tending to yellow),than those recorded in the first test.

Table 16. Determination of the cakes' colors using L*a*b* or CIELab colour space, by calculating the averages of internal and surface measurements (II Trial).

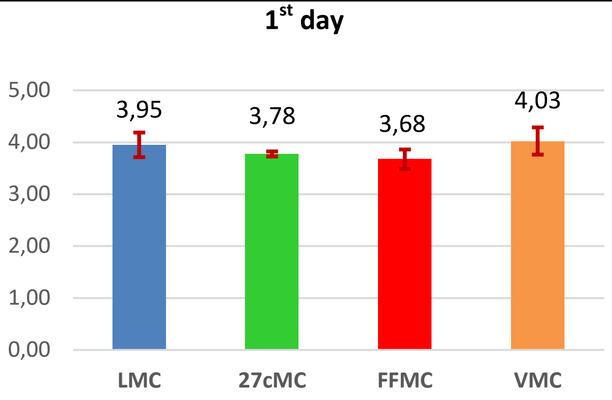
	II Experiment	
	Top (Average)	***Inner (Average)***
	1st day	***1st day***
Light Mayonnaise Cake (LMC)	**L*** = 48,13 (± 0,80)	**L*** = 40,04 (± 1,50)
	a* = 15,88 (± 0,20)	**a*** = 14,77 (± 0,13)
	b* = 15,38 (± 0,25)	**b*** = 17,68 (± 0,37)
	C* = 22,11 (± 0,21)	**C*** = 23,04 (± 0,36)
	h* = 44,07 (± 0,61)	**h*** = 50,02 (± 0,50)
27)c Mayo Cake (27cMC)	**L*** = 44,87 (± 0,78)	**L*** = 36,91 (± 0,58)
	a* = 14,60 (± 0,28)	**a*** = 15,03 (± 0,25)
	b* = 12,92 (± 0,86)	**b*** = 16,88 (± 0,38)
	C* = 19,50 (± 0,78)	**C*** = 22,63 (± 0,50)
	h* = 41,47 (± 1,35)	**h*** = 48,31 (± 0,19)
Full Fat Mayonnayse Cake (FFMC)	**L*** = 45,77 (± 0,29)	**L*** = 37,22 (± 0,76)
	a* = 15,19 (± 0,10)	**a*** = 14,64 (± 0,20)
	b* = 14,66 (± 0,21)	**b*** = 16,58 (± 0,14)
	C* = 21,11 (± 0,08)	**C*** = 22,12 (± 0,23)
	h* = 43,96 (± 0,57)	**h*** = 48,49 (± 0,20)
Vegan Mayo Cake (VMC)	**L*** = 46,34 (± 0,73)	**L*** = 40,30 (± 1,30)
	a* = 15,75 (± 0,38)	**a*** = 15,55 (± 0,36)
	b* = 14,50 (± 0,70)	**b*** = 18,53 (± 0,52)
	C* = 21,41 (± 0,73)	**C*** = 24,19 (± 0,62)
	h* = 42,59 (± 0,86)	**h*** = 49,95 (± 0,30)

We determined the height of each cake, calculating the average of measurements carried out on the same pieces used for the colour determination, as showed in figure 36.

Figure 36. Height measurement of the cakes

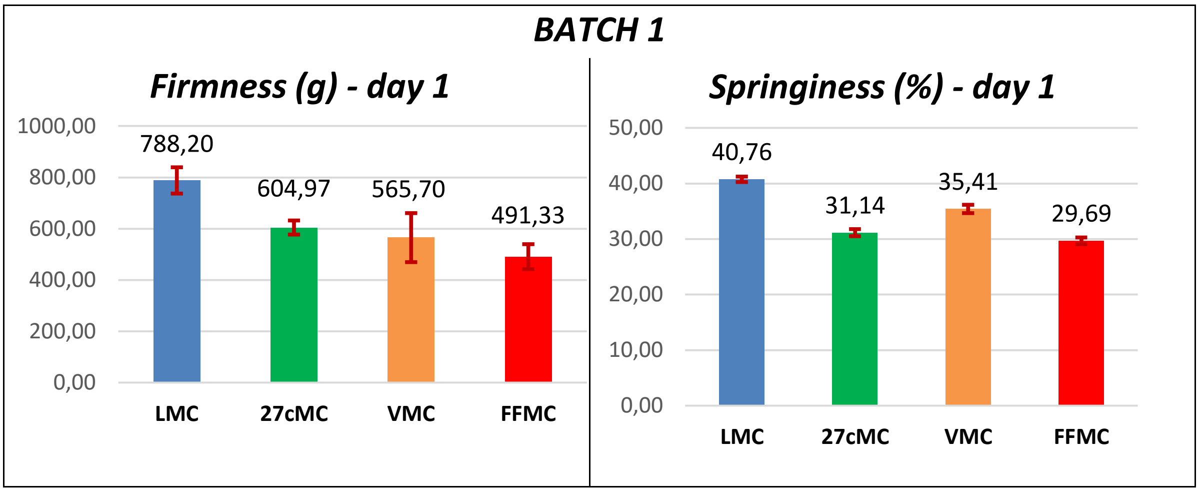


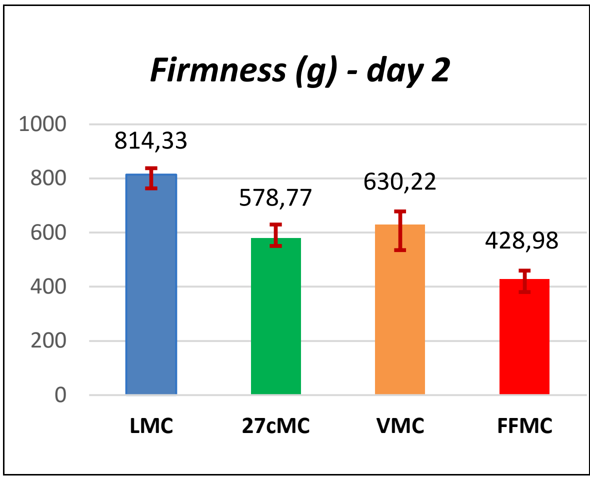
II Trial

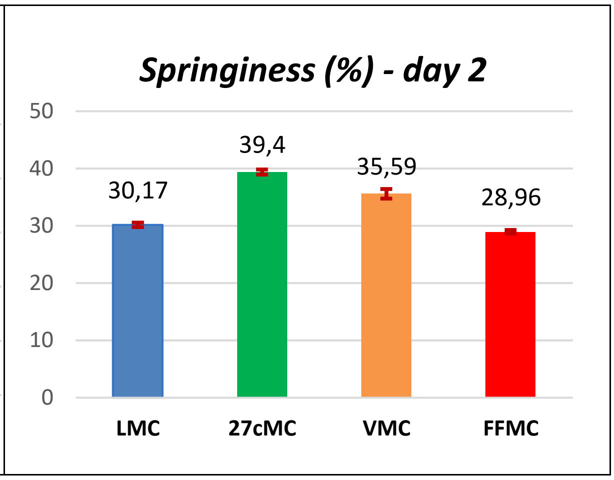


No significant result emerges from the comparison of the data. The cake height can be influenced by many factors: first of all by the ingredients contained in the dough (as for example baking powder, sugar), and then from the oven temperature, the homogeneity of the cooking, the effect of humidity, the temperature of the air around the ingredients and the mixing area. At this point, firmness and springiness were calculated following the same experimental design, as showed in figure 37

Figure 37. Determination of Firmness and Springiness in Cake Samples by adaption of AACC method into a 'Hold Until Time' test - Batch 1 (for two consecutive days)







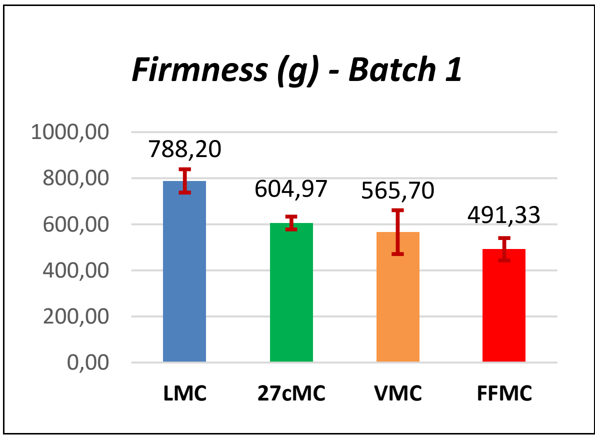
In the first day of the *Batch 1*, LMC recorded the highest firmness value while FFMC the lowest one, and 27cMC has showed an intermediate behaviour. It was the same with regard to the springiness, but in this case 27cMC has resulted more similar to FFMC, while VMC showed an intermediate value.

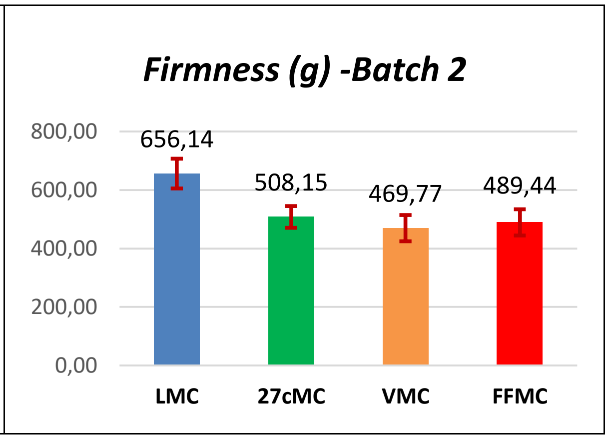
From the comparison with the data recorded the second day (Batch 1), with other four pieces of the same cakes, we note that VMC and LMC increased their firmness the following day, while 27cMC and FFMC have become more friable. The springiness decreased in all the samples.

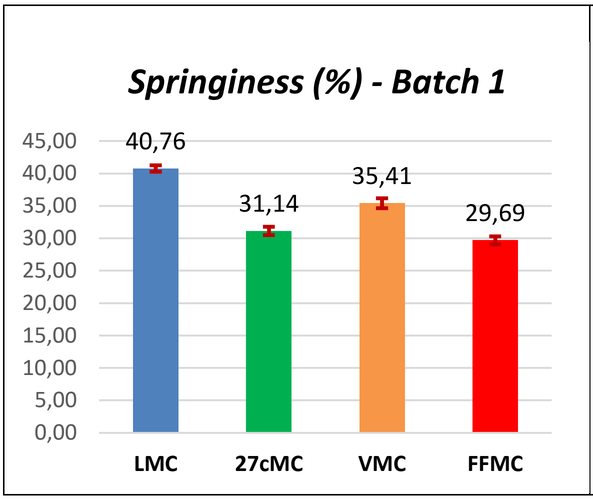
In the second trial *(Batch 2)*, the firmness and springiness measurements were carried out in a single day on eight pieces of each fresh cake. With reference to the first parameter, we find values slightly different from the first test but with a similar trend: LMC recorded the highest value and FFMC the lowest one. In this case, however, 27cMC and VMC appeared similar to FFMC. All samples had an *elasticity* slightly higher than that recorded in the first experiment.

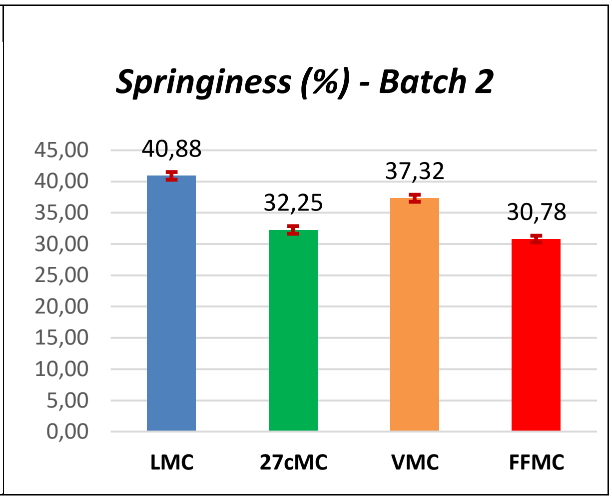
We can summarize the results obtained in the following graphs (Fig. 38)

Fig. 38 A brief visual summary of the firmness and springiness values detected.









The texture of the cakes and their sensory characteristics were evaluated by panelists in two sessions, whose results are presented hereafter.

As shown in the Table 17, no significant differences were found for the first two parameters considered. With regard to cocoa aroma no significant differences have been detected among the samples also if 27cMC obtained higher results, especially in the first trial, and many panelists who participated in the focus group held at the end of the session told us that they preferred the cake prepared with our formulation principally for its cocoa aroma and flavour.

Significant differences were recorded for the cocoa flavour in both sensory evaluations. According to Tukey’s test results in the first trial LMC was found significantly different with respect to FFMC, 27cMC showed an intermediate behaviour but its value was much more similar to that of FFMC. In the second trial 27cMC and FFMC recorded similar results, and were found significantly different to VMC; LMC in this case had an intermediate value.

With reference to the sweetness and chewiness, the cakes didn't shown significant differences from the statistical point of view.

With reference to the softness, in the first evaluation FFMC presented significant differences with respect to LMC. Similarly in the second trial, FFMC showed the highest value of softness and LMC the lowest one, but in this case applying Tukey’s test there weren't found statistical significant differences between the samples.. These results are in line with those found in the firmness analysis, according to which FFMC resulted the most friable among the cakes in both tests. Besides, in the focus groups at the end of the two sessions many of the panelists reported that the FFMC was too crumbly.

Finally, regarding the overall quality, despite the fact that no significant differences were found, 27cMC obtained the highest scores, of about one unit higher than those of the other samples.

Table 17. Results of Chocolate Cake Panel Tests.

		Cake	1st Trial Mean	1st Trial p-value		1st Trial Tukey's HSD	2nd Trial Mean	2nd Trial p-value		2nd Trial Tukey's HSD
1.	**Appearance**	*LMC*	6,30	*Samples*	0,62	a	6,26	*Samples*	0,99	a
		VMC	6,70			a	6,10			a
		27cMC	**6,74**	*Judge*	0,43	a	**6,14**	*Judge*	0,44	a
		FFMC	6,06			a	6,20			a
2.	**Colour**	*LMC*	7,38	*Samples*	0,34	a	6,68	*Samples*	0,6	a
		VMC	6,86			a	6,4			a
		27cMC	**6,75**	*Judge*	0	a	**6,24**	*Judge*	0,01	a
		FFMC	7,00			a	6,26			a
3.	**Cocoa aroma**	*LMC*	4,81	*Samples*	0,14	a	5,26	*Samples*	0,28	a
		VMC	4,69			a	5,41			a
		27cMC	**6,29**	*Judge*	0,25	a	**6,04**	*Judge*	0,01	a
		FFMC	5,96			a	6,09			a
4.	**Cocoa flavour**	*LMC*	5,15	*Samples*	0,03	**b**	4,99	*Samples*	0	**ab**
		VMC	4,05			**ab**	4,42			**b**
		27cMC	**5,97**	*Judge*	0,5	**ab**	**6,18**	*Judge*	0	**a**
		FFMC	6,10			**a**	6,26			**a**
5.	**Sweetness**	*LMC*	4,72	*Samples*	0,14	a	5,53	*Samples*	0,47	a
		VMC	5,26			a	5,17			a
		27cMC	**5,21**	*Judge*	0,01	a	**5,82**	*Judge*	0	a
		FFMC	5,76			a	5,69			a
6.	**Softness**	*LMC*	5,56	*Samples*	0,06	**b**	5,97	*Samples*	0,18	a
		VMC	6,23			**ab**	6,41			a
		27cMC	**6,38**	*Judge*	0,83	**ab**	**6,31**	*Judge*	0,55	a
		FFMC	7,55			**a**	7,11			a
7.	**Chewiness**	*LMC*	5,55	*Samples*	0,57	a	5,5	*Samples*	0,68	a
		VMC	4,59			a	6,26			a
		27cMC	**4,23**	*Judge*	0,36	a	**5,32**	*Judge*	0,56	**a**
		FFMC	5,30			a	5,64			a
8.	**Overall quality**	*LMC*	5,30	*Samples*	0,59	a	5,29	*Samples*	0,03	**a**
		VMC	5,06			a	5,39			a
		27cMC	**6,34**	*Judge*	0,81	a	**6,74**	*Judge*	0,11	**a**
		FFMC	5,38			a	**6,74**			a

In the following graphs (Figures 39,40), the cake 27cMC showed a sensory profile very similar to that of FFMC, but with lower scores of chewiness and softness, characteristics that beyond a certain threshold are no longer appreciated by the panelists, as they themselves stated in their comments, which will be shown in the next section.

Fig. 39 Spider chart for displaying results obtained from LMC, VMC, 27cMC, FFMC in the first sensory evaluation with regard to the parameters considered.

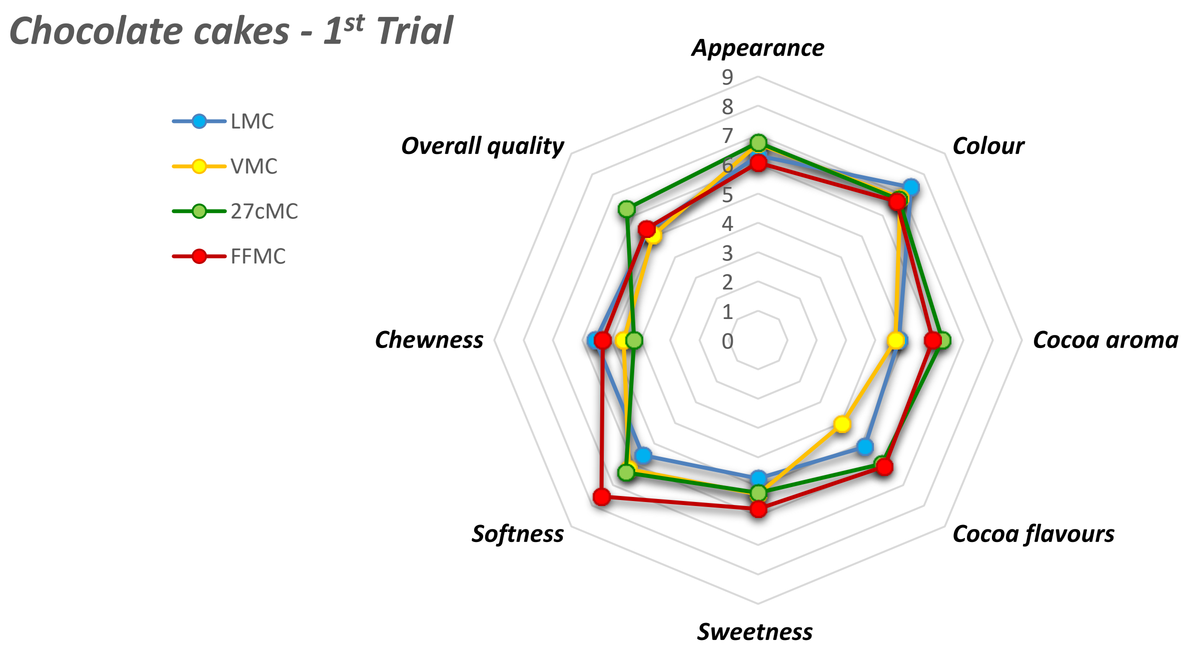
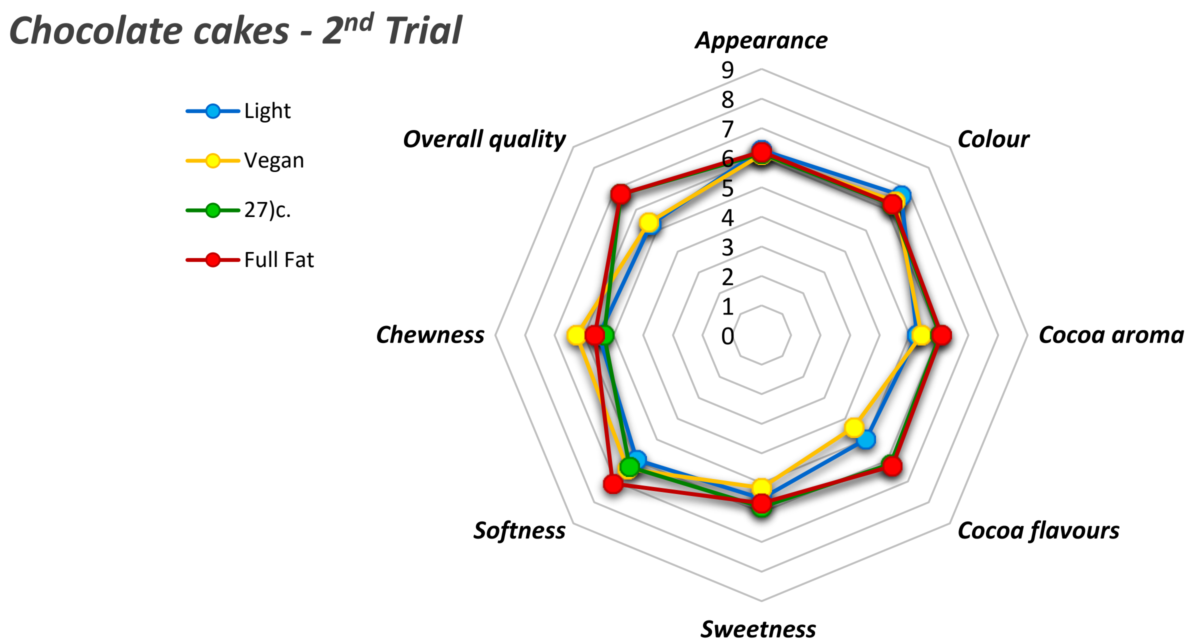


Fig. 40 Spider chart for displaying results obtained from LMC, VMC, 27cMC, FFMC in the second sensory evaluation with regard to the parameters considered.



Despite a small difference in chewiness values, 27cMC obtained similar averages for each parameter in both tests. In this experiment, the mix contained in the new formulation of the vegan mayonnaise (27c) has shown to be able to replace eggs; in fact, also if the new formulation does not contain eggs unlike the other mayonnaises employed, it has shown similar coagulant, foaming and emulsifying properties in the cake bakery process.
Panelists were also asked to judge the cakes with reference to off-odours, off-flavours and after-taste. The most repeated comments have been selected and included in the table below (Table 18).

Table 18. Summary of comment inheriting off-odours, off-flavours and aftertaste for each mayonnaise chocolate cake in both sensory evaluations.

Chocolate cake	OFF-ODOURS	OFF-FLAVOURS	AFTER TASTE
LMC	- None - Lacks cocoa aroma	- No off flavour - Not very sweet - Not enough cocoa flavour	- Pleasant – n/a - Blind - Strange, unpleasant aftertaste, sweet aftertaste (artificially?) - Vinegary - Buttery
VMC	- No off odour - Slight bitterness - Lacks cocoa aroma (only smell bread)	- No off flavour - Quite bland (a bit nondescript) - Salty/Bitter/Strange	- n/a - Unpleasant: Bitter and odd - Processed taste - no cocoa - Pleasant aftertaste of cocoa - Weird fatty sensations
27cMC	- No off odour - Most chocolaty	- No off flavour - Nice balance btw the cocoa flavour and the sweetness	- Nothing unpleasant/The best aftertaste - Nice lingering cocoa aftertaste - Slight bitterness/savoury (fish-like after-taste?)/Very sweety
FFMC	- None - Chocolate aroma not strong enough	- No off flavour - Little weird - Too sweety - Slightly acidic flavour/mayo	- Pleasant - Slight sweet/bitter aftertaste - Chocolaty/Mayo aftertaste - Sticky and chewy

As previously mentioned, two of the parameters that have been evaluated as most important by the panelists are the cocoa aroma and the cocoa flavor. In fact, even if no particular off-odours and off-flavors have been reported, the most frequent expressions used to describe the cakes are: "lacking aroma", "tasteless", etc. especially with reference to those made with light mayonnaise and vegan mayo. In the case of the off-flavours and of the aftertaste, many of the comments focused on the sweetness and few on the fatty perception. this can be explained by the fact that multiple factors are linked to consumer perceptions of sweetness and fat. Sweetness is certainly due to the sugar content (Drewnowski *et al*., 1998), but it also depends on the fat content and moisture. On the other hand, fat perception is more complex than sweetness and it depends on several factors as the fat content, the sugar content, texture, moisture, flavour, the nature of the food (liquid or solid) and mouthfeel (Abdallah *et al*., 1998, Biguzzi *et al*., 2014). Fat plays vital sensory and functional roles in baked products and its presence contributes to flavour or the combined perception of mouthfeel, taste and aroma. Besides it contributes to the appearance and lubricity of baked goods and increases the feeling of satiety (Nikoofar *et al.*, 2013). For products that are fat-in-water emulsions, like cakes, air bubbles form the discontinuous phase, and the egg, sugar, fat and water mixture forms the continuous phase, where the flour is also dispersed. Due to the complex structural, textural and sensory functionality of sugar in baked products, obtaining good quality low-sucrose products is a difficult task (O'Sullivan, 2016). With reference to the cakes aftertaste, many panelists reported perceptions of bitterness, more or less marked according to the sample tasted. Since this has been found for all four samples, it may be related to the presence of vinegar and lemon juice in the mayonnaises used to prepare the cakes.

Judges were invited to express personal comments on the overall quality of the cakes tasted. Their opinions are summarized in the table below (Table 19).

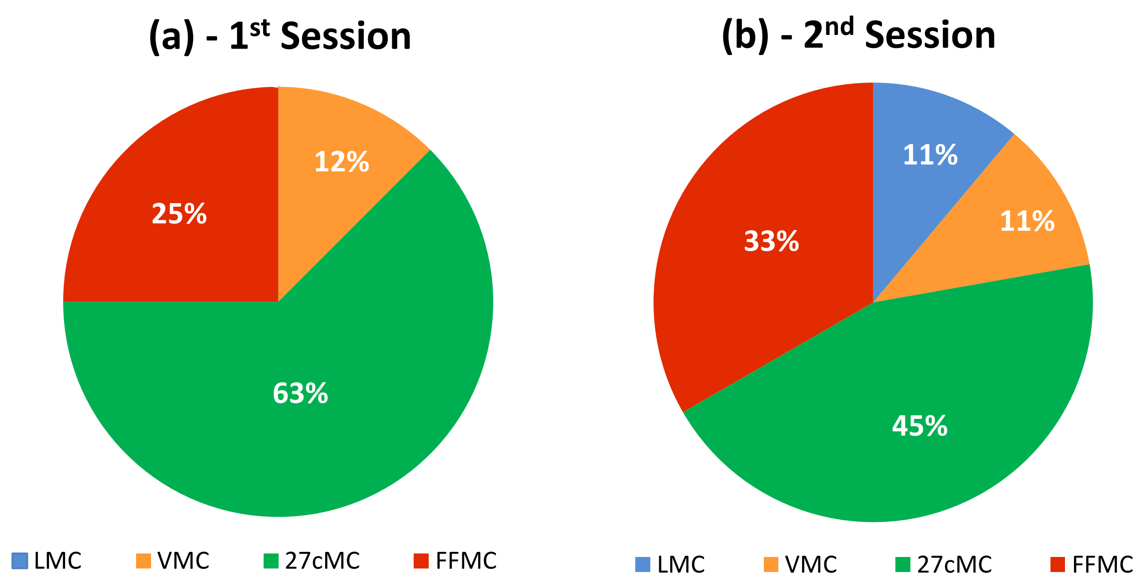
Table 19. Comments expressed by panelists on the overall quality of the cakes tested

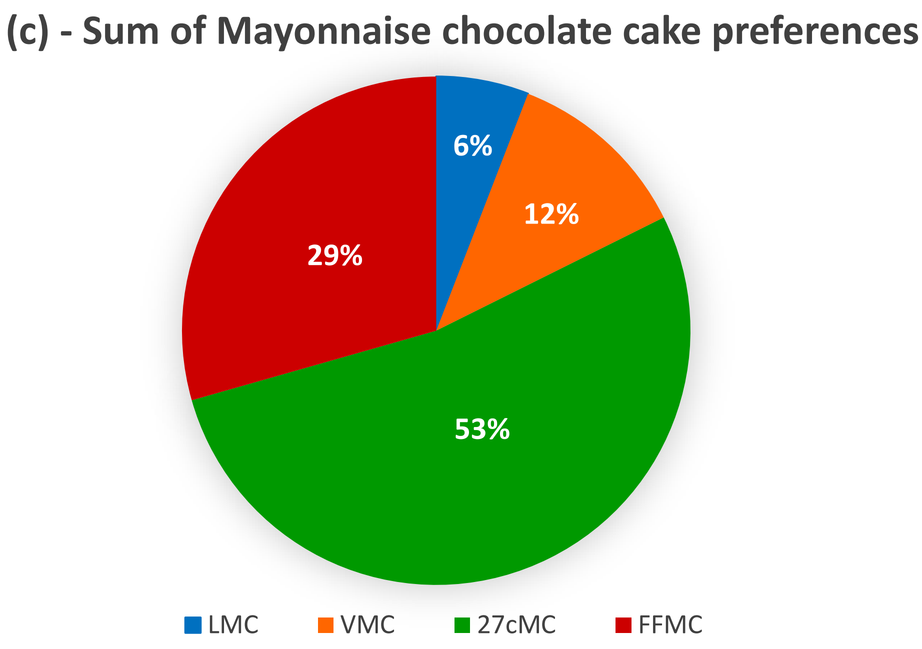
Chocolate cake	COMMENTS RESUME
LMC	Bit shiny on top - The colour is slightly paler as well; great chewing moist texture but lacked flavour; doughy texture (slightly unpleasant) - dry texture; too strong flour/egg flavour too sweet feels sticky to touch.
VMC	Dense and dry texture but quite crumbly on top too; bland; lacked a proper chocolate-y taste, bland; good balance between chewy texture and moisture.
27cMC	Best texture, aroma, flavour; crumbly; nice overall appearance; good taste/most chocolate-y; best balance between cocoa and sweetness.
FFMC	Too soft and crumbly; poor texture; lovely flavour; chocolate aftertaste; strong aroma/lacked aroma; good balance between cocoa flavour and sweetness; egg smell.

The most frequent comments on LMC and VMC were related to their dry texture and to the lack of flavor. Almost all the comments concerning the texture, the flavor and the taste of

27cMC were positive, while those related to FFMC turned out to be sometimes contradictory. In the case of the cakes prepared with light mayonnaise and full fat mayonnaise, the panelists reported the unpleasant persistence of the egg taste.
About the preferences expressed, in the first session the chocolate cake made with our formulation (27cMC) achieved more votes, with a percentage of 62,5% while LMC did not obtain any votes (Fig. 41a). Similar results were obtained in the second trial, and in this case 27cMC scored the 44,4% of the preferences (Fig. 41b). The chocolate cake prepared with our vegan mayo reached the first place with a total percentage score of 53% (Fig. 41c).

Fig. 41 Mayonnaise chocolate cake preferences





Good results were obtained both with reference to the texture, the appearance, and above all to the sensorial characteristics of the cake.
This research was carried out to test the formulation of a new vegan mayonnaise in a bakery application, and compare the physico-chemical and the sensorial profile of the resulting cake with that of the other cakes made by using other types of mayonnaise.
The firmness and springiness values of 27cMC have been found intermediate in comparison with those of the other cakes, besides the 27cMC resulted quite crumbly but no so much as the FMC, that has crumbled.
In both sensory evaluations, the panelists positively judged the appearance, the texture, but especially the flavor and the taste of "our cake". Judges didn't like the cakes made using light mayonnaise or vegan mayo for the lack of cocoa aroma and flavor. On the other hand, they found a pleasant flavor in FMC and 27cMC but in the first case they also highlighted the presence of some defect.
Definitely, they preferred the cake 27cM although it is devoid of eggs and it contains less fats than that made with full fat mayonnaise.
In this last decade, consumers expressed several concerns, which include the desire for low-cholesterol foods, reduced allergens, (Lin *et al*., 2003; Swaran *et al*., 2003).
One of the main future objectives is to succeed in obtaining a gluten-free chocolate cake, especially because the gluten can produce symptoms in people that suffer from coeliac disease, an intestinal intolerance to the storage proteins of wheat, rye and barley (Masure *et al*., 2016).
In this experiment, only wheat flour was used to make the cakes, but in future other types of flours could be tested (sorghum flour or quinoa flour, for example) in order to totally eliminate gluten in the cakes, adding also, if necessary, other ingredients to avoid an

excessive loss of flavor. It could be interesting try to understand if the premix contained in our mayo formulation can totally replace the functionality of gluten (present in the wheat flour) and at the same time the emulsifying properties of eggs.

In the future, other experiments could be conducted to understand what influence baking powder, wheat flour and vinegar exert in the leavining process, varying their concentrations and analysing any differences in the properties of the resulting cakes. On the other hand, food science efforts must be addressed also to reduce the sugar and fat contents in order to obtain healthier products.

Cake is a food that is relished by consumers all over the world and it incorporates sugar as one of the basic ingredients (Manisha *et al*., 2012).

The sucrose contributes to the sweet taste but also acts as a bulking agent in the batter, aids in moisture retention and air entrapment and creates a fine crumb grain in the products and helps to inhibit microbial spoilage (Drewnowski and Almiron-Roig, 2010; Nip, 2014).

Sucrose therefore performs different functions and it seems rather difficult to find another ingredient that can replace it without affecting the final product quality.

However in a recent study, some researchers found that maltitol, sorbitol and xylitol can be used to prepare low-calorie, gluten-free rice chiffon cake, in place of sucrose without affecting its quality (Kim *et al*., 2014). With regard to the ability of sucrose to inhibit the growth of microorganisms, it is not possible to define the role that it played in this experiment, since no microbiological tests have been conducted. Furthermore, no tests have be conducted to assess changes in the physical and sensorial profiles of chocolate cakes over time.

Nevertheless no evident sign in the overall appearance has emerged for more of two weeks.

Usually, cake shelf life is between 1 and 4 weeks, but industrial cakes usually have longer shelf lives due to the use of certain additives such as preservatives (propionic, sorbic and benzoic acids) and correct packaging conditions (optimal heat sealing and modified atmosphere) (Barcenilla *et al*., 2016). In this case, good results were achieved although no preservative and additives have been added during the preparation phase. Besides the sugars, fat also plays an important role in cake manufacture contributing to the texture, mouthfeel, flavour and aroma of food (Drewnowski and Almiron-Roig, 2010). The fat in cake batter not only helps the incorporation of air but it also produces emulsifying properties and holds considerable amounts of liquid to increase and extend cake softness and 'shortens', that is, it interrupts the protein particles to break gluten continuity to tenderise the crumbs (Bennion and Bamford, 1973). Therefore, it would be expected that a fat reduction process would induce changes such as lower overall volume (less aeration), increased crumb firmness (because of a greater degree of starch swelling) and a reduced palatability. Fat reduction will result in reduced volume and increased crumb firmness (Rodríguez-García *et al*., 2014). It will be an interesting challenge to succeed in replacing fats in mayonnaise chocolate cake and reducing the associated health risks.

CONCLUSION

In the last decades fresh eggs have constituted one of the primary ingredients in the bakery sector, especially for home-made cakes and retail bakeries around the country, thanks to its nutritional value and multi-functional properties, including emulsification, coagulation, foaming, and flavor.

Today many concerns from consumers and processors have led the baking industry to search for ingredients to replace eggs. In particular, these concerns include a desire for low-cholesterol foods, reduced allergens, less expensive ingredients, increased shelf life, no refrigeration requirements.

In this study, chocolate-mayonnaises cakes were prepared using 27c formulation (described in the previous chapter) and other three types of mayonnaise products on the market as controls. The new vegan mayo contains an interesting mix of linseed protein, beta glucan, starch and other ingredients but no eggs. This research was carried out to understand if this mix can replace the egg in the preparation of a chocolate cake, emulating some of the functional characteristics of whole-egg and if the resulting cake could compete, with reference to all physical and sensory attributes, with the other cakes.

Measurements of colour, height, firmness, springiness and two sensory evaluations were carried out.

Regarding colour and height, the cakes didn't show significant differences between them. With reference to the firmness and springiness measurements, the cakes made with our formulation (27cMC) recorded intermediate values with respect to those of the chocolate cakes prepared with light mayonnaise (LMC) and full fat mayonnaise (FFMC).

In both sensory evaluations, the panelists preferred the texture of the sample 27cMC, whilst they didn't particularly appreciated neither the texture of the LMC nor that of FFMC, sustaining that this latter crumbled too much.

Moreover, they particularly liked the cocoa aroma and the cocoa flavor of 27cMC, as well as its right softness and chewiness. Panelists have stated in both tests to prefer this sample to the others.

In the future, firstly we will vary the concentrations of baking powder, wheat flour and vinegar and analyse any difference in the physical and sensory properties of the cakes, trying to understand in which way these ingredients influence the leavening process.

Besides, taking into account that in this last decade, consumers expressed several concerns, which include the desire for low-cholesterol foods and reduced allergens product, one of the main future objectives is to succeed in obtaining a gluten-free chocolate cake reducing, at the same time, the content of sugar and fat.

CHAPTER V

Safety, quality and consumers

We can define the food safety in a broad or in a more narrow way; in the second case, the concept of food safety can be defined as the opposite of food risk, or more simply, as the probability of not contracting a disease after consuming a specific product (Grunert, 2005).
Referring to a broad definition, we can instead view the food safety as also encompassing nutritional qualities of food and more wide-ranging concerns about the properties of unfamiliar foods, such as consumers' uneasiness about OGM.
Besides, as with food quality, it's possible define the food safety according to an objective or subjective point of view. In the first case, the concept of food safety is based on the assessment of the risk of consuming a certain food and corresponds to the definition provided by food experts and researchers (Grunert, 2005).
Subjective food safety is in the mind of the consumer. It is widely acknowledged that objective and subjective safety (or risk) deviate in many cases.
Until a few decades ago, it was thought possible to tack this deviation, considered as a nuisance, through consumer information. However, after the failure of attempts to educate consumer to become amateur food scientists, this attitude has given way to a recognition of the necessity to deal with consumers' perceptions of risk and safety as they are (Frewer *et al.*, 2005).
According to Grunert (2005), the safety is just another dimension of quality, in fact the food quality could be defined food can be said of quality when it reflects everything a consumer would find desirable in a food product, and safety is certainly a desirable quality of food.
However, it seems that safety perceptions play a role predominantly in two ways. In situations where major safety problems are perceived ("food scares" as BSE, or the dioxin problem for example) risk perceptions can come to dominate all other considerations in food choice and lead consumers to avoid certain categories or brands for some time, until the situation has returned to normal. In metaphorical key, safety perceptions act as a 'sleeping giant' that does not enter quality perceptions under normal circumstances, but can have sweeping effects at times of crisis.
With reference to second way, consumers apply safety considerations to certain production technologies (food irradiation, GMOs, etc.), whose use is perceived as unsafe; this involves that they develop negative attitudes towards the use of these technologies, and these standpoints can be revealed powerful forces in the marketplace. For this reason, both industry and regulators take seriously these attitudes, and they have resulted in the non-use of irradiation and a considerable delay of the adoption of GMOs in Europe.
Nevertheless, qualitative studies of food quality perception suggest that when we ask to consumers to describe their own view of food quality, safety is not uppermost in their mind

(Brunsø *et al.*, 2002). On the basis of this consideration, the food safety perception could affect consumer food choice in ways that are different from perceptions of the other dimensions of quality, described in the first chapter.
Through studies on consumer risk perception, in more general terms, some regularities have been observed (Fischhoff *et al.*, 1978; Slovic, 1987, Frewer *et al.*, 2005).
Specifically, some phenomena seem to be rather robust: - self-imposed risk is more acceptable to consumers than technology-based risk; thus, although meal preparation at home is, by objective standards, much riskier than meal production in a factory, consumers tend to perceive ready-made meals as more dangerous than meals they have cooked themselves; - although consumers can usually appreciate the risk associated with their own handling of food in general terms, they believe that the probability of being hit themselves by a problem is lower than the probability of the average consumer being hit by the same problem, a phenomenon also known as 'optimistic bias'; - the importance of the dimensions of dread and familiarity in risk perception has been amply demonstrated, implying that familiar risks are perceived as less severe than unfamiliar ones.
Food safety is a major topic for public policy and we can divide the regulatory responses in two categories: the enforcement of common standards for food safety (without immediate impact on consumer food choice), attempts to provide transparency and encourage consumers to form their own judgements on food safety, supported by mechanisms of public participation, consumer education and information tools.

5.1 Food safety hazards

Safe food supplies support national economies, trade and tourism, contribute to food and nutrition security, and underpin sustainable development.
Urbanization and changes in consumer habits, including travel, have increased the number of people buying and eating food prepared in public places. Globalization has triggered growing consumer demand for a wider variety of foods, resulting in an increasingly complex and longer global food chain.
As the world's population grows, the intensification and industrialization of agriculture and animal production to meet increasing demand for food creates both opportunities and challenges for food safety. Climate change is also predicted to impact food safety, where temperature changes modify food safety risks associated with food production, storage and distribution.
These challenges put greater responsibility on food producers and handlers to ensure food safety. Local incidents can quickly evolve into international emergencies due to the speed and range of product distribution. Serious foodborne disease outbreaks have occurred on every continent in the past decade, often amplified by globalized trade.
Food safety is threatened by numerous contaminants, which can originate from environmental pollution, such as toxic metals and organic halogenated compounds; chemicals used in the production of food, such as pesticides and veterinary drugs;

contaminants formed during food production and cooking; contaminants arising from food packaging, or natural toxins in food (Oskarsson, 2012).

More specifically, it is possible to classify food safety risks into three categories:

Physical hazards include any potentially harmful extraneous matter not normally found in food: glass, metal, stones, wood, plastic, rubber or pests, commonly result from accidental contamination and poor food handling practices that can occur at various points in the food chain from harvest to consumer (McSwane and Linton, 2000)

Microbiological contamination (eg bacteria, fungi, mycotoxins, viruses or parasites). This category causes, in most cases, acute symptoms.

Chemical contaminants, including chemicals present in the environment (air, soil, water), veterinary drugs residues, pesticides, heavy metals, food additives and other residues incidentally introduced into the food chain.

Chemical hazards can also be separated into three categories (Dearfield & Rigby, 2009):

- Naturally occurring chemicals. They are derived from a variety of plants, animals or microorganisms, and include also allergens.
- Intentionally added chemicals. These chemicals are intentionally added to food at some point during the food's growth and distribution. Intentionally added chemicals are safe when used at established safe levels but can be dangerous when those levels are exceeded.
- Unintenionally or incidentially added chemicals

5.2 Contaminants in the food chain

Environmental contaminants are chemicals that accidentally or deliberately enter the environment, often, but not always, as a result of human activities. Some of these contaminants may have been manufactured for industrial use and because they are very stable, they do not break down easily. If released to the environment, these contaminants may enter the food chain. Other environmental contaminants are naturally-occurring chemicals, but industrial activity may increase their mobility or increase the amount available to circulate in the environment, allowing them to enter the food chain at higher levels than would otherwise occur.

Some examples of environmental contaminants include lead, arsenic, bromates, dioxins, furans, mercury and polychlorinated biphenols (PCBs).

A number of persistent environmental contaminants tend to accumulate in all types of animals, and are frequently found in meat, poultry, fish, and dairy products. Other chemicals, such as perchlorate and a variety of pesticides, are often found in fruits, vegetables, and other agricultural commodities. Some chemicals in food, such as mercury and perchlorate, have naturally occurring as well as man-made sources. The health risks from chemicals in food are dependent on both the actual level of a chemical in the food as well as the amount of the food consumed by individuals.

Many factors must be considered in order to determine whether a contaminant represent a health risk: for example its absorption capacity, its toxicity, its content in the food, the amount of contaminated food consumed and the exposure duration. Besides, people have different sensitivity to the contaminants and other factors in the diet can affect the toxic consequences of the contaminant ingestion/absorption.

A further complication with regard to chemical contaminants is that many of the data on their toxicity need to be extrapolated from experiments carried out on animals and there is no certainty that these substances have the same effects on humans as well.

Measurable health effects depend on the toxicity of the substance, the level at which it is present in food, the quantity of food consumed, and the vulnerability of the individual or population.

In the following study we have calculated the concentrations of 6 marker PCBS congeners and 4 trace elements, all having a maximum tolerable level of intake set by EU regulation, in samples of European hake.

This species sustains a relevant fishery in the Mediterranean Sea and is one of the most consumed fish species in the Mediterranean diet. Since it occupies a high trophic position and may be subject to bioaccumulation, an analysis of contaminant concentrations is advisable to assess the risk for human health associated to the consumption rate. In the successive studies: - the heavy metals concentration and Health Risk Index (HRI) have been calculated for herbal products based on *Malva sylvestrisis*, an herbaceous species used in infusions, decoctions and liniments for its emollient and laxative properties; - arsenic content and HRI were calculated for lettuce and radish samples from Lazio (Italy). In both cases the accumulation of heavy metals is due to their presence in the areas where these vegetables grow, to anthropogenic processes involving the application of synthetic fertilizers, organic fertilizers and industrial wastes and to inadequate hygienic transport and storage conditions (Dual *et al.*, 2009).

5.3 CASE STUDY V

A PERSPECTIVE ON THE POTENTIAL HEALTH RISKS FROM PCBS AND HEAVY METALS CONTAMINATION OF *M. merluccius* FROM MEDITERRANEAN SEA.

A BRIEF INTRODUCTION

Polychlorinated biphenyls (PCBs) are persistent organic contaminants with chemical-physical properties, such as flammability and low volatility at room temperature, which made them widely used in industry (dielectrics for transformers and capacitors, fluids heat transport, printing inks and papers copiers "carbonless", paints, plasticizers, cutting oils, etc.). Growing evidences highlighted their impact on both human health and environment, so that PCBs use is now restricted or banned in most countries (U.S. EPA; 2015). However, they are still present in the environment because of their high persistence and ease of dispersion allowing them entering the food chain. Since PCBs are classified as dangerous contaminants, they should be constantly monitored in marine waters and sediments, together with other inorganic pollutants such as heavy metals. Indeed, their bioaccumulation capacity through the food chain is known to cause adverse effects to both humans and animals (Rasmussen Crisp *et al.*, 1999; Angeletti *et al.*, 2013; Randhawa *et al.*, 2015; Robertson and Hansen, 2015), including increased cancer risk, diabetes, immune suppression, developmental disorders and even reduced birth weights of children born from mothers who ate contaminated fish (Koopman-Esseboom *et al.*, 1994; Brouwer *et al.*, 1995). Along marine food webs, PCBs and heavy metals bioaccumulate in predator species including commonly consumed fish such as hake, walleye, bass, and salmon so making seafood consumption potentially risky (Streets *et al.*, 2006; Gewurtz *et al.*, 2011; Mosesso *et al.*, 2012; Angeletti and Carere, 2014). Indeed, after the PCBs ban at Stockholm Convention in 2001 (UNEP, 2001), direct environmental exposure of humans decreased (Hu *et al.*, 2002; Knobeloch *et al.*, 2011) but exposure via dietary intake still remains a concern, when fish comes from contaminated waters. This potential risk is in contrast with the health benefits from fish consumption, since it provides an important source of protein, polyunsaturated fatty acids (PUFA), liposoluble vitamins and essential minerals (Daviglus *et al.*, 2002; Wim *et al.*, 2007). According to FAO statistics, fish accounted for about 16% of the global population's intake of animal protein and 6% of all protein consumed (FAO, 2010b). People in the Mediterranean basin consume a considerable amount of fish, as reported by FAO statistics showing high fish per capita consumption in the Mediterranean countries with Spain (64 g/capita/day), Israel (50), and France (48) having the higher intakes (Preedy and Watson, 2015). Since fish constitute an important part of the Mediterranean diet, it is not surprising that the quality and safety aspects of fish food are of particular interest, so that the concentrations of PCBs in fish have been extensively studied over the past decade in the Mediterranean, as in the rest of the world (Bocio *et al.*, 2007; Storelli *et al.*, 2011; Costopoulou *et al*, 2016). Since the diet is the main route of human exposure to trace elements too, PCBs assessment has been

frequently coupled with the study of inorganic toxic elements in the edible commercial fish species (Castro-González and Méndez-Armenta, 2008; Rodríguez-Hernández *et al.*, 2016).
Along the Mediterranean basin, the consumption of blue fish is particularly high, and among the various species included under this name, the European Hake (Merluccius merluccius) is one of the most relevant in terms of both landings and market value (FAO, 2013). In addition, this species is extremely important in dietary terms, providing an important source of protein and polyunsaturated fatty acids (Daviglus *et al.*, 2002; Wim *et al.*, 2007).
The aim of this work was to determine persistent organic contaminants, specifically PCBs, and trace elements in the flesh of the European hake from the Mediterranean Sea, to assess the risk for human health due to the consumption of this relevant component of the Mediterranean diet. The detection and quantification of PCBs was performed by GC/MS, analysing 6 of the PCBs defined as indicators by both International Council for the Exploration of the Sea (ICES) and European Food Safety Authority (EFSA) Scientific Panel concerning Contaminants in the Food Chain (CONTAM Panel). The presence and levels of 4 trace elements abundantly recovered in fish (Pb, Cd, As, Hg) were analyzed by Atomic Absorption. The results obtained have been used to verify if different areas of the Mediterranean Sea may host fish of different quality. Moreover we estimated the dietary intake of such contaminants, to assess if the present consumption of European hake may constitute a risk source of PCBs and inorganic toxic elements.

EXPERIMENTAL PART

In order to determine the concentration of PCBs and heavy metals in *M. merluccius* caught in relevant fishery zones of Mediterranean basin, the following areas were selected (Fig.42): Adriatic Sea (10–40 miles off the coastline of Mestre, Termoli and San Salvo); Balearic Sea (10–40 miles off the coastline of Barcelona and Valencia); Crete Sea (10–40 miles off the coastline of Crete); Ligurian Sea (10–40 miles off the coastline of Genova and La Spezia); Tyrrhenian Sea (10–40 miles off the coastline of Gaeta, Ponza, Olbia and Fiumicino).
These areas are all part of FAO Zone 37 (www.fao.org) and correspond to 37.2.1, 37.1.1, 37.3.1, 37.1.3, 37.1.3 geographical sub-areas, respectively. The sampling design covered these areas because they correspond to the sampling sites most representative for the origin of the fish available on the Italian market (ISMEA, 2007).
A total of fifty hakes from 25 to 30 cm of total length (TL) were analysed (Fig. 42; Table 20).

Fig. 42 Sampling area

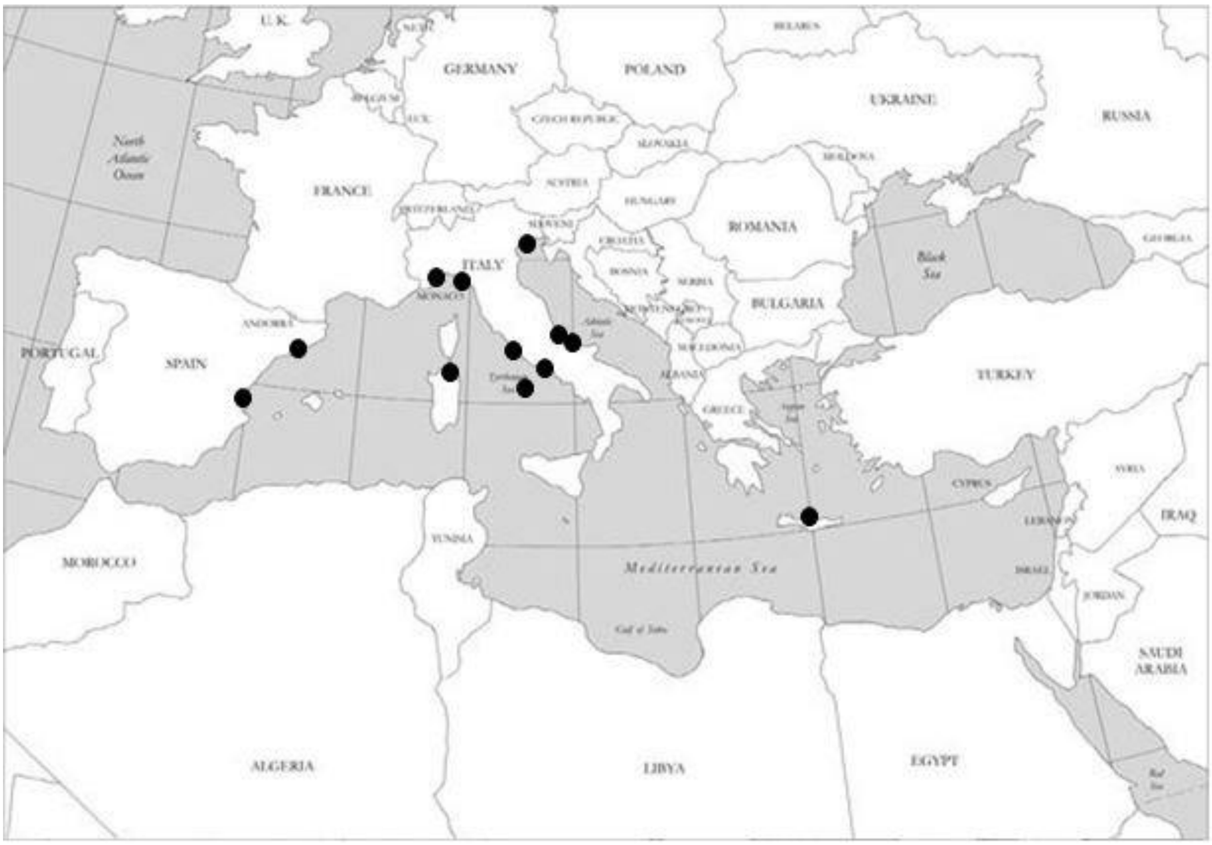


Table 20. Sampling areas of *M.merluccius* in the Mediterranean Sea with fishing localities and number of studied specimens (N).

Sampling area	Fishing locality	Number
Balearic Sea	Barcelona	3
	Valencia	4
Ligurian Sea	Genova	3
	La Spezia	3
Tyrrhenian Sea	Gaeta	3
	Ponza	2
	Olbia	3
	Fiumicino	6
Adriatic Sea	Mestre	6
	Termoli	3
	San Salvo	2
Sea of Crete	Crete	12
	TOT	**50**

Wild samples of hake were caught during 2016 using bottom trawls, bottom trammel nets, and seine nets placed on the surface and at mid-water (Crete, Fiumicino, Ligurian Sea). Sampled fish were stored in tanks with sea water ice. Some samples were purchased from local fish markets, whose geographical origin was checked by label and warranted by suppliers. All the samples were transported to the laboratory, where they were measured and their weight recorded. Each sample was individually wrapped in aluminium foil and stored a -20°C until analysed. Analyses were conducted on the edible portions of the samples after removal of the head, skin, tail, and visceral package.

The six PCB congeners 28, 52, 101, 138, 153 and 180 were chosen since they are considered markers of exposition according to the EFSA Scientific Panel concerning Contaminants in the Food Chain (CONTAM Panel). Indeed, these congeners are considered appropriate indicators of different PCBs patterns in various sample matrices and are most suitable for a risk assessment of non-dioxin-like (NDL)-PCBs. In its scientific opinion on the presence of NDL-PCBs in feed and food, the CONTAM Panel highlighted that the sum of the six indicator PCBs represents about half the total PCBs in food (EFSA, 2005). To determine polychlorinated biphenyl concentrations we adapted the analytical procedures described in Storelli *et al.* (2007a). Specimens were defrosted, dissected and dried overnight at 105°C, before analysis. Even if PCBs tends to accumulate mainly in liver (Bodiguel *et al.*, 2008), we analyzed muscle tissue since it is the main edible part of the fish, directly involved in the dietary intake, being the skin very rarely consumed in Italy.

Extraction procedure was performed by a Soxtec System HT2 tecator (Fig 43).

Fig. 43 Soxtec System HT2 tecator

Each aliquot (2g) was mixed with sodium sulfate, and the homogenate was transferred to thimbles and was Soxhlet-extracted with 60mL of petroleum-ether (40-60°C) at 135°C using the following Extraction Times: Boiling Time - 40 minutes; Rinsing Time - 20 minutes; Recovery Times - 10 minutes. The extract was then recovered with 5 ml of hexane, purified by florisil columns, and concentrated by rotovapor to a final volume of 0.5 ml. The final obtained PCBs extracts were injected and analysed separately on a Thermo Trace GC connected with a Thermo PolarisQ MS (Fig 44). The chromatographic separation was achieved by splitless injection on a capillary column with length of 30 m, i.d. 0.25 mm and 0.25 lm thickness stationary phase film (RTX-5, Restek, US, Bellefonte, PA, USA).

The MS was used in the SIM mode with the two most intensive ions of the molecular ion cluster monitored in specific windows. PCB congeners (tetra-, penta-, hexa-, hepta-CB) were included in the analysis. Individuals PCBs standards were purchased from Chem Service, Inc – 660 Tower Lane- West Chester, PA 19380 United States. QA/QC was performed through the analysis of procedural blanks, a duplicate sample and standard reference material CRM

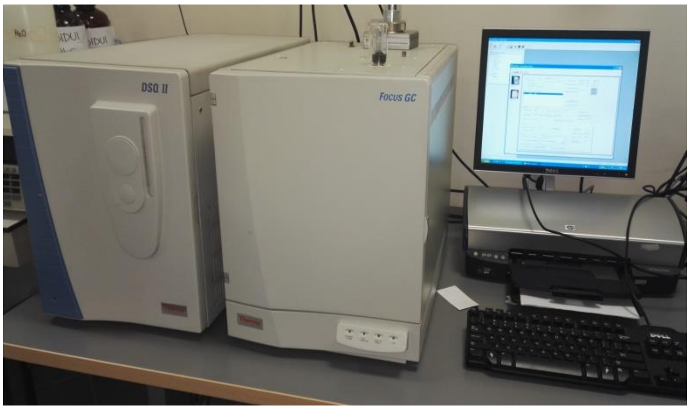


Fig. 44 ISQ™ Series Single Quadrupole GC-MS Systems (MS - Agilent 5973N)

349 for PCBs (cod liver oils). For the replicate and standard reference materials, the relative standard deviations (RSD) were <10% for all the detected compounds. Finally, 10 replicates of a reference material, CRM349 (Cod Liver Oil), were analysed in order to determine the accuracy of the method. PCB mean recovery = 93,5± 17%.

The presence and levels of 4 trace elements (TEs) in fish (Pb, Cd, As, Hg) was measured. To determinate concentrations, dried muscle tissues were weighed (0,3-0,5 g) and mineralized with 3 ml of HNO_3 and 0,5 ml of H_2O_2 for 6-8 hours (Papetti and Rossi, 2009). After mineralization, the mixture was brought to a final volume of 10 ml with deionized water. TEs levels were determined by Atomic Absorption Spectrometer AAnalyst 600 PerkinElmer (Fig.45).

Fig. 45 Atomic Absorption Spectrometer AAnalyst 600 PerkinElmer

Cd was analyzed at the wavelenght 228.8 nm, Pb at 283.3 nm, As at 193.7 nm, Hg at 253.7 nm. Total Hg has been determined through Hydride Generation Atomic Absorption Spectrometry (HGAAS). Instrument detection limits on the GFAA were 0.2 ppb for arsenic, 0.1 ppb for cadmium, 2.0 ppb for lead.
The TE concentrations were expressed in milligrams per kilo of wet weight (mg/kg ww).

The TEs calibration was made using appropriate dilutions of stock solutions of Pb, Cd, As, Hg (1000 ppm). The relative standard deviation (SD) and percent standard deviation (%RSD) calculations were based upon five replicate samples. To ensure the accuracy of the method, the same analyses were conducted on certified control samples (IEAE-407) with mean recovery =95±9%.
Analysis of variance (ANOVA) tests were conducted for the concentrations of metals, PCBs and the sum of PCBs by site, using as independent variables the metals considered, the PCBs and their sum respectively, the sampling sites, and the interactions. According to what resulted as significant, Tukey's HSD post-hoc tests were performed.
After conducting statistical analysis, the estimated dietary intake (EDI) of the six congeners of NDL-PCBs was calculated as:

$$EDI = C_P \times F_{IR} / W_{AB}$$

according to this formula, the Italian chronic and acute fish consumption ($C_{P\ chronic}$ = 55 g/day; $C_{P\ acute}$ =159 g/day) (EFSA, 2015) was multiplied by mean NDL-PCBs contamination of hake expressed in mg/kg of fresh weight (F_{IR}) and then divided by the average adult body weight (W_{AB}), that was considered to be 75 kg (Arnich *et al.,* 2009; Storelli *et al.,* 2011). According to a recent survey of Codacons (Coordinamento delle associazioni per la difesa dell'ambiente e dei diritti degli utenti e dei consumatori), the average weight of an Italian adult is 74.1 kg, so the authors decided to approximate this value to 75 kg (https://codacons.it/crisi-codacons-italiani-mangiano-meno-cala-peso-medio/).
In the same way, we calculated the estimated daily intake of trace elements, multiplying C_p my mean TE concentration in fish (mg/Kg ww), (Ali and Hau, 2001; Akoto *et al.*, 2014; EFSA, 2015).
Health risk of consumers due to intake of TE contaminated fish was assessed by using HRI (Health Risk Index). A HRI lower than 1 means the exposed population is unlikely to experience obvious adverse effects; whereas a HRI above 1 means that there is a chance of non-carcinogenic effects, with an increasing probability as the value increases (Akoto *et al.*, 2014). The HRI was calculated by using the equation of Wang *et al.* (2005):

$$HRI = EDI/R_fD$$

where R_fD is the reference oral dose expressed in mg/kg/day and it represents an estimation of the daily exposure of a contaminant to which the human population may be continually exposed over a lifetime without an appreciable risk of harmful effects. The R_fD is $1.0x10^{-3}$ for Cd, $3,5x10^{-3}$ for Pb, $3.0x10^{-4}$ for As and $4.0x10^{-4}$ for total Hg (Akoto *et al.*, 2014; USEPA, 2009).
It has been reported that exposure to two or more pollutants may result in additive and/or interactive effects. The total HRI of heavy metals for individual foodstuff was treated as the arithmetical sum of the individual metal HRI (Zheng *et al.,* 2007 (a), (b)): Total HRI (individual foodstuff) = HRI (toxicant 1) + HRI (toxicant 2) + HRI (toxicant n).

Results

Fifty specimens of European hake have been analyzed for the determination of both PCBs and TEs. PCBs were quantified in more than 95% of the analysed samples. The mean concentrations (ng g^{-1}) of the sum of the six PCBs and of each congeners for all sampling sites are reported in table 21.

Table 21. Mean and total concentrations (ng g^{-1} Fw) of individual congeners and total PCBs in fish muscle.

	Balearic Sea	Ligurian Sea	Tyrrhenian Sea	Adriatic Sea	Sea of Crete
PCB 28	1.37±0.21	1.33±0.86	1.53±0.21	2.60 ±0.82	1.22 ±0.57
PCB 52	2.11±0.54	4.66±2.70	2.51±0.12	8.63±5.91	1.27 ±0.12
PCB 101	2.31±0.12	7.07±2.23	2.67±1.01	3.4±1.45	1.22 ±0.24
PCB 138	5.34±1.23	9.6±4.0	3.47±0.89	14.7±3.3	6.97 ±1.32
PCB 153	7.52±1.12	15.5±6.0	8.01±1.11	29.5±4.8	8.57 ±2.34
PCB 180	5.20±2.0	11.3±2.9	2.11±0.15	10.5±1.7	5.3 ±1.12
∑PCB	**23.85±4.51**	**49.46±10.5**	**20.30±4.51**	**69.42± 9.9**	**24.55 ±4.50**

PCBs congeners distribution showed that hexachlorobiphenyls PCB 153 (31.0–42.0%) and PCB 138 (17.9–28.39%) were the most abundant, followed by PCB 180 (10.4–21.8%). These three congeners represented nearly 80% of the Σ6PCBs contamination (Table 21).

The predominance of these congeners is consistent with data reported in literature for other marine inhabitants, including either fish (Kannan *et al.*, 2002; Storelli *et al.*, 2007a), reptiles (Lazar *et al.,* 2011) and mammals (Fair *et al.*, 2010; Storelli *et al.,* 2010). PCBs abundance in such different taxa is due both to their high stability and persistence, facilitating the accumulation in the aquatic ecosystem, and to their molecular structure, which enhances their lipophilicity and makes them refractory to metabolic attack by mono-oxygenases.

The Σ6PCBs concentrations showed similar values in the samples from the Balearic, Tyrrhenian and Crete Sea, ranging between 20.3±4.51 and 24.55±4.50 and comparable to those recorded for other fish species from the central Mediterranean Sea, including hake (Storelli and Marcotrigiano, 2001; Ferrante, 2007; Moraleda-Cibriàn, 2015). Both Ligurian and Adriatic Sea showed higher values of Σ6PCBs contents in fish respect to the other localities (49.46±10.5 and 69.42± 9.9, respectively). Our data are however in agreement with those reported in other in which PCBs contamination in commercial fish and invertebrates from the Adriatic Sea showed values up to 80.57 ng g^{-1} in mackerel and the comparison of polluted vs. industrial free central-eastern Adriatic areas provided values between 59 and 287 ng g^{-1} studies (Kipcic and Vukusic, 1991; Bayarri *et al.*, 2001). Also in the research of Miniero and coworkers (2014) in 38 out of 40 samples of wild species (including European hake), fished in FAO Zone 37, Σ6NDL-PCBs was found in the range of 0.768–48.3 ng/g ww, with an average of 7.32 ng/g ww, that falls between one half and one third of the European mean contamination levels reported for "Muscle meat fish" and for the sub-category "Other wild caught fish" (Miniero *et al.,* 2014).

In our study, the overall mean contribution of the individual PCBs to the sum of the six indicators followed the pattern PCB-153 > PCB-138 and PCB-180 > other PCBs in all the samples except Tyrrhenian Sea, where PCB-153 > PCB-138 > PCB-101 is observed. Accordingly, PCBs 153, 138 and 180 were analytically predominant in all fish specimens. PCB-101, 52 and 28 showed the lower concentrations in all the samples (Table 21). These results are in line with findings reported in the studies of EFSA, 2005; BFR, 2006; Jursa *et al.*, 2006; Squadrone *et al.*, 2012, 2013; Miniero *et al.*, 2014 which have demonstrated that PCB-153 has an average contribution of roughly one third to the sum of the six indicator PCBs. Although the occurrence of pollutants is widespread in the samples, their concentration is below recommended legal limits for fish or other foodstuffs and never exceeded the limit for human consumption (75 ng/g ww in muscle of fish) established by Commission Regulation (EU 1259/2011).

According to ANOVA analysis results related to PCBs concentrations, both the "Site" variable, the "PCB" variable, and the interaction between them, were found significant. Hence, Tukey's HSD post-hoc tests were performed and results were showed in figure 46a (for the variable "Site") and 46b (for the variable "PCB").

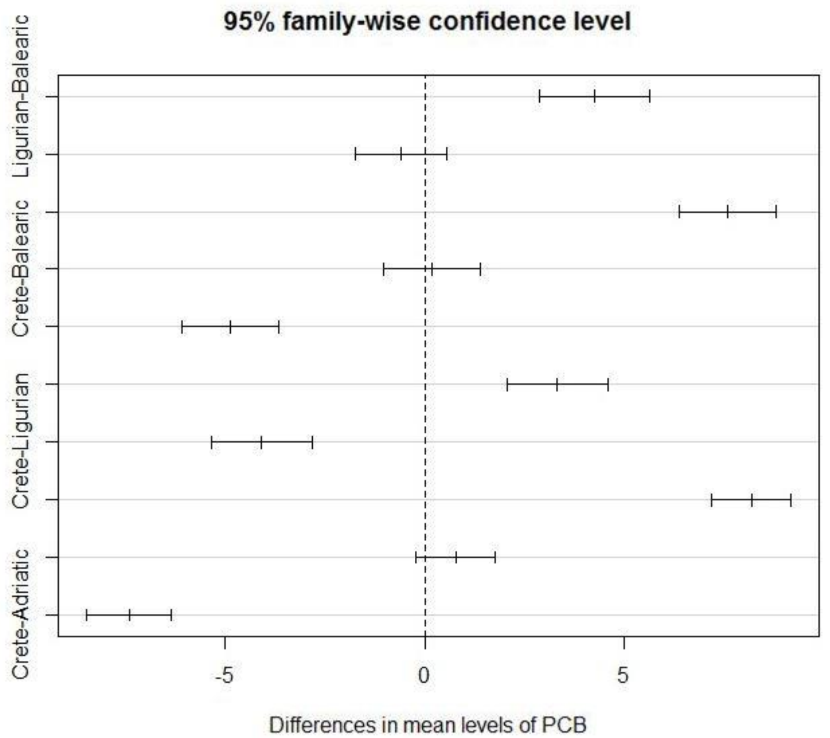


Fig. 46a Tukey's Test Plot (95% family wise confidence level) for the variable "Site". Differences in mean levels of Site

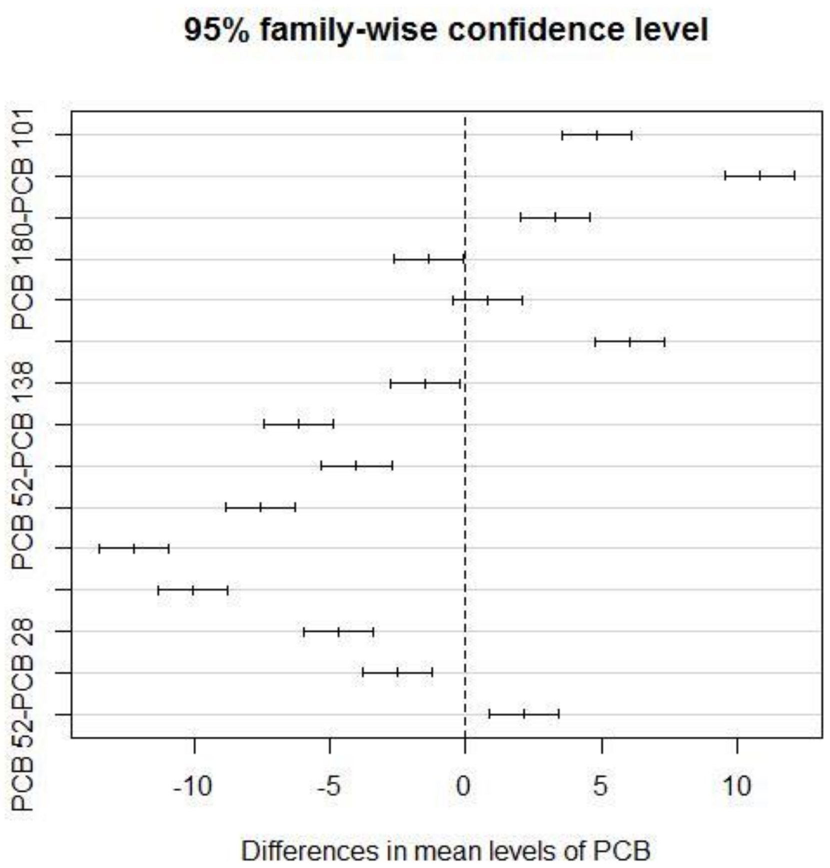


Fig. 46b Tukey's Test Plot (95% family-wise confidence level) for the variable "PCB"- Differences in mean levels of PCBs

As showed in figure 46a, only three pairs did not show any significant difference: Tyrrhenian-Balearic, Crete-Balearic, Crete-Tyrrhenian, for which the corresponding 95% family-wise confidence intervals include zero. In figure 46b almost all pairs were found significant except for PCB 28 – PCB 101 and PCB 52 – PCB 101.
The Tukey’s test plot (not shown) with the interaction between the two variables showed that almost all couples were found significant, with high overlapping between segments.
By performing an ANOVA analysis with the sum of Σ6PCBs (for each site), the site variable was found significant. Hence, the concentration of PCBs differs in mean among most sampling sites. In particular, Tukey's test was performed, which suggested that the only pairs not showing any significant difference were: Crete-Balearic Sea, Tyrrhenian-Balearic Sea, Tyrrhenian-Crete Sea as shown in figure 47, where the corresponding 95% family-wise confidence intervals include zero.

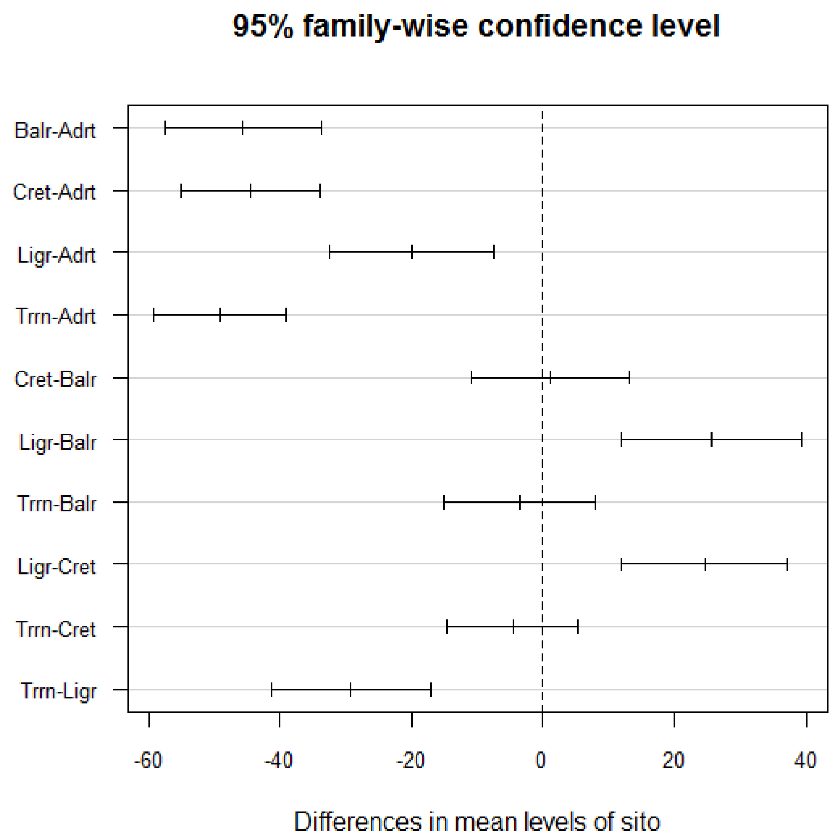


Fig. 47 Tukey’s Test Plot (95% family-wise confidence level) – Differences in mean levels of the sampling sites.

Regard to the TEs contamination, the levels of lead (Pb), cadmium (Cd), arsenic (As), total mercury (Hg) and the total concentrations of heavy metals are reported in Table 22.

Table 22. Average and total concentrations (mg/kg wet weight) and standard deviations of Cd, Pb, As and Hg

Metal	Balearic Sea	Ligurian Sea	Tyrrhenian Sea	Adriatic Sea	Sea of Crete
As	0.047 ± 0.017	0.045 ± 0.020	0.077 ± 0.067	0.037 ± 0.020	0.031 ± 0.009
Cd	0.002 ± 0.001	0.001 ± 0.001	0.020 ± 0.021	0.001 ± 0.001	0.005 ± 0.002
Hg	0.420 ± 0.240	0.405 ± 0.150	0.505 ± 0.436	0.375 ± 0.355	0.375 ± 0.195
Pb	0.125 ± 0.064	0.109 ± 0.052	0.156 ± 0.164	0.125 ± 0.064	0.139 ± 0.165

The average concentrations obtained showed the same pattern in all the geographic samples analysed: Hg>Pb>As>Cd. Overall, the samples from the Tyrrhenian Sea showed the highest values of TEs.

Arsenic concentrations were quite low if compared with previous studies (Juresa *et al.*, 2003; Perugini and *al*., 2004 , Falcó *et al*, 2006; Sebbio *et al.*, 2014), but in the same order of magnitude of recent surveys of the European Scientific Cooperation Report for Mediterranean countries as Greece (Olmedo *et al*, 2013; EC, 2004).

Total mercury accumulation was sea-specific, with the samples from the Tyrrhenian Sea and Balearic Sea showing the higher content of this element. The mean concentrations of total Hg resulted quite high in all the samples with a range from 0.375 to 0.505 mg/kg ww, but of the same magnitude of other values reported in literature. For example, Storelli *et al.* (2005) found concentrations up to 0.480 and 0.300 mg/kg ww for hake muscle from the Adriatic and Ionian Sea, respectively. Moreover, a recent review evidenced that the Italian Seas have high levels of mean Hg values, frequently due to both anthropogenic burden and the geologic nature of the inland district (Renieri *et al.*, 2014; Scanu *et al.*, 2016)**.**

Cadmium concentrations showed values comparable to those recorded in hakes from western and central Mediterranean Sea by many Authors (Juresa *et al.*,2003; Ersoy *et al.*, 2006; Falcó *et al.*, 2006; Olmedo *et al.*, 2013), despite its concentration may be affected by local environmental variables such as salinity and temperature (Renieri *et al.*, 2014).

Lead levels are quite homogeneous across the studied samples, with values comparable to those found in literature for this species (Gaspic *et al.*, 2002; Juresa *et al.*, 2003; Perugini *et al.*, 2004; Ersoy *et al.*, 2006; Falcó *et al.*, 2006; Olmedo *et al.*, 2013). Interestingly, Pb concentrations in hake are confirmed as higher than in many other demersal species such as blue whiting, sea bass or gilt-head sea bream or even pelagic top predators as blue shark, tuna and swordfish (Damiano *et al.*, 2011; Olmedo *et al.*, 2013). Food is the main pathway of contaminants uptake in the marine environment and contaminant concentrations are known to increase with trophic level. However, it has been demonstrated that bioaccumulation rates in the European hake differ between contaminants, according to their properties and to their pathways of metabolization and elimination (Harmelin-Vivien *et al.*, 2012), thus suggesting that the biomagnification of Pb in this species may follow an exponential increase.

Overall, TEs concentrations were within the prescribed limits set by European Community regulation in all samples (Commission Regulation, 2006; EFSA, 2009). Analysis of variance

(ANOVA) test was performed using as independent variables the metals and the sampling sites, and the interaction between them. Only the variable "Metal" was found significant. Also in this case Tukey's test was performed, which suggested that the only pairs not showing any significant difference were Cd-As, Pb-As as shown in figure 48, where the corresponding 95% family-wise confidence intervals include zero.

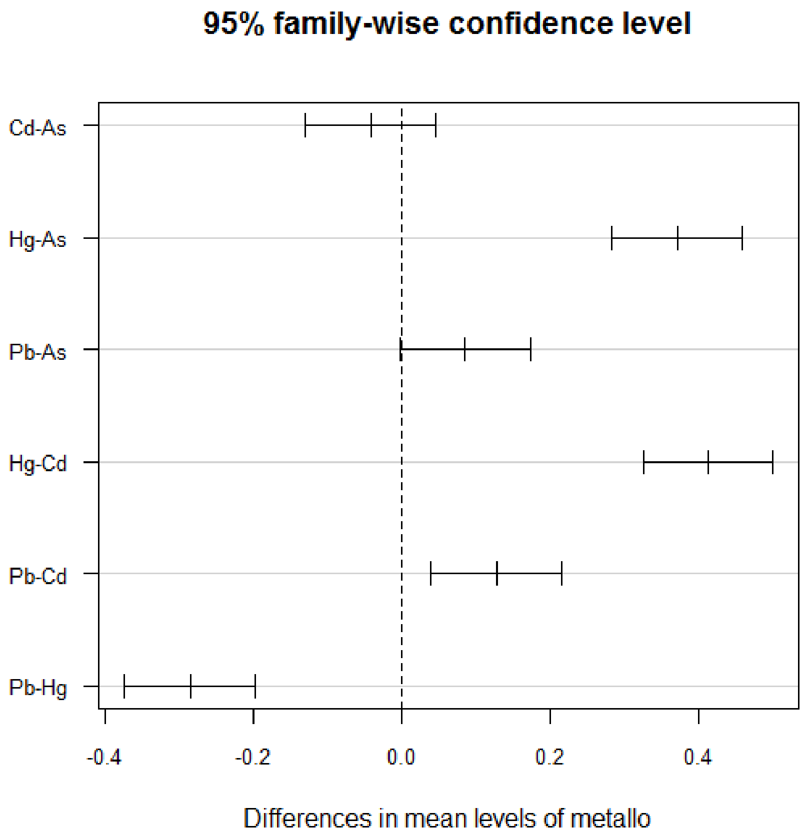


Fig. 48 Tukey's Test Plot (95% family-wise confidence level) – Differences in mean levels of metals.

The exposure pathway of humans to organic and inorganic contaminants through ingestion of contaminated food has been studied by many researchers (Chary *et al.*, 2008; Copat *et al.*, 2012; Xue *et al.,* 2012; Akoto *et al.*, 2014). In this study, health risk of consumers due to intake of contaminated fish was assessed by using the estimated dietary intake and HRI index (Table 23, Table 24). Specifically, authors proposed two scenarios considering the Italian chronic and acute hake consumption data (Tables 23, 24) (EFSA, National Food Consumption Database, 2015).

Table 23. Range, mean concentration with standard deviation, EDIs of PCBs, EDIs and HRIs of trace elements in the flesh of European hake from five areas of the Mediterranean Sea, considering Italian chronic consumption data available for adults.

Chronic Consumption

Pollutant	**Range** (mg/Kg ww)	**Mean ± Std. Dev.** (mg/Kg ww)	**MTL (Max.Tolerable Limit)** (mg/Kg ww)	**EDI** (mg/kg b.w./day)	**Reference oral doses** (mg/kg b.w./day)	**HRI**
Cd	0.001-0.020	**0.006** ± 0.008	**0.050**	0,0000044	0,001	0,0044
Pb	0.109-0.156	**0,131** ± 0,018	**0.300**	0,0000961	0,0035	0,0274
As	0.031-0.094	**0.047** ± 0.018	**-**	0,0000345	0,0003	0,1149
Hg	0.375 - 0.635	**0.416** ± 0.053	**0.500**	0,0003051	0,0004	0,7627
					TOT	**0,9094**
∑6PCBs	0,020-0,069	**0.037** ± 0.021	0.075	**2,7133E-05**		

Table 24. Range, mean concentration with standard deviation, EDIs of PCBs, EDIs and HRIs of trace elements in the flesh of European hake from five areas of the Mediterranean Sea, considering Italian acute data available for adults.

Acute consumption

Pollutant	**Range** (mg/Kg ww)	**Mean ± Std. Dev.** (mg/Kg ww)	**MTL (Max.Tolerable Limit)** (mg/Kg ww)	**EDI** (mg/kg b.w./day)	**Reference oral doses** (mg/kg b.w./day)	**HRI**
Cd	0.001-0.020	**0.006** ± 0.008	**0.050**	0,0000127	0,001	0,0127
Pb	0.109-0.156	**0,131** ± 0,018	**0.300**	0,0002777	0,0035	0,0793
As	0.031-0.094	**0.047** ± 0.018	**-**	0,0000996	0,0003	0,3321
Hg	0.375 - 0.635	**0.416** ± 0.053	**0.500**	0,0008819	0,0004	2,2048
					TOT	**2,6290**
∑6PCBs	0,020-0,069	**0.037** ± 0.021	0.075	0,00007844		

Relatively to PCBs, the results in table 23 showed that the estimated daily intake of PCBs from *M. merluccius* chronic consumption varies between 1,47E-05 e 5,06E-05 mg/kg b.w/day, with an average of 2,71E-05 mg/kg b.w/day in the Mediterranean basin. The six PCBs selected in this study are contaminant indicators, subjected to a maximum tolerable level for their sum in the seafood. According to the regulation on the "Official Journal of the European Union 2011" such maximum tolerable is of 75 ng/g wet weight, indicating that contaminants in the flesh of European hake were below the conventional permissible limit in all samples examined. Such finding is in agreement with the literature, where similar EDI values are reported (Arnich *et al.*, 2009; Cirillo *et al.*, 2009; Perelló *et al.*, 2012; Barone *et al.*, 2014). In addition, studies showing the average estimated levels of adult dietary exposure to indicator PCBs in European countries showed low level of PCBs contaminants. In particular, from Italian diet food it has been estimated an average EDI of 10.9 ng/kg b.w./d and a median of 11.0 for the six congeners (EFSA, 2005; Fattore *et al.*, 2005, 2008), with the fish products that are the main contributors to the total intake of NDL-PCBs for children (38%), women of childbearing age (42%) and adults (47%) (Arnich *et al.*, 2009).

These values appear high, according to the tolerable daily intake proposed by French Food Safety Agency (ANSES/AFFSA) for this group of six congeners, that is 10 ng/Kg/ bw/day (ANSES, 2010). However, within this frame, the EDI reported for European hake in both scenarios seems quite low with respect to the overall values.

In fact, also in the second scenario (Table 24) the EDI of PCBs from *hake* acute consumption varies between 0,000042 and 0,000146 mg/kg b.w/day, with an average of 0,000078 mg/kg b.w/day that it is below the permissible level.

As for other contaminants with adverse effects on human health, FAO, WHO and EU have established the maximum permitted concentrations of heavy metals in foodstuffs (Chary *et al.,* 2008; Xue *et al.,* 2012;). Accordingly, EFSA in the Commission Regulation (EC) set the maximum tolerable limit (MTL) for total mercury, cadmium and lead in fish muscle in 0.50, 0.050 and 0.30 mg/kg wet weight, respectively (Commission Regulation, 2006). On the contrary, there is no a MTL for arsenic in foods, but the "EFSA Panel on Contaminants in the Food Chain" conducted a risk assessment on arsenic in food and recommended reducing dietary exposure to inorganic arsenic (EFSA 2009).

The samples collected from different sampling areas in the Mediterranean basin did not vary widely in their heavy metal contents. Mean concentrations of heavy metals and their EDIs varied between very low (Cd) and low values (Pb, As) while reaching higher values for total Hg. In any case, all the reported EDIs were below the maximum values recommended by the EU (Tables 23, 24).

Total HRIs of the single TE examined in the fish muscle was calculated by summing the individual HRIs of the elements. Total HRI value, showed in table 23, is 0.90, a value lower than 1, indicating that there is no health risk for humans due to the intake of the studied TEs from Mediterranean European hake chronic consumption. Although the total HRI of heavy metals is below 1, the HRI value of total mercury is quite high and has a strong influence on the arithmetical sum. This is in agreement with the data reported by Storelli (2008), founding a higher incidence of Hg, with respect to Cd and Pb, in the European hake from Adriatic Sea.

In the case of the second scenario (Table 24), the situation is fairly complicated since total HRI is greater than one, with a worrying value of total mercury HRI.

However, as evidenced in other studies, the intake of Hg from many Mediterranean species (including European hake) is not cause of concern (Brambilla *et al.*, 2013).

The comparison of the results obtained from different studies is rather complicated because numerous factors and their interaction can influence the tissue contamination levels of seafood (Barone *et al.,* 2014). In addition, there are relevant methodological differences, including the set of contaminants examined, and the comparison of intake estimations in different countries is further complicated by a strong bias in the seafood consumption rate adopted: as an example, in Italy this rate is 18 g/week according to Barone *et al.* (2014) and FAO (2011) but raises to 48 g/day according to Akoto *et al.* (2014). However, there is an overall agreement of the studies in considering the consumption of European hake a safe and healthy practice.

CONCLUSIONS

There is a contrast between the benefits coming from fish food intake, due to its content in omega 3 fatty acids, and the potential risk due to the associated dietary exposure to contaminants. This contrast has fostered a number of studies on both the contents of organic and inorganic contaminants in many fishery products and of the health risk associated with their consumption. Such studies have been carried out all around the word, including the Mediterranean area where fish food represents a relevant part of the diet, generally showing that the intake due to fish food does not constitute a risk, although some caution should be paid to some species and elements (Rodriguez-Hernandez *et al.*, 2016).
The European hake is one of the most consumed species of white fish, sustaining commercially important fisheries throughout the Mediterranean Sea, and has been the focus of many regional studies, suggesting a moderate to negligible contamination by toxic elements and compounds (Belcari *et al.,* 2006; Bocio *et al.,* 2007; Bodiguel *et al.,* 2008; Cresson *et al.,* 2014).
According to our results the concentration of organic and inorganic contaminants in hake flesh is below the recommended international limits, as well as the estimated daily intake for adults and the associated health risk are low despite the non-negligible mercury concentration for chronic Italian hake consumptions.
Our study, although designed to simply provide further data on this widely studied species, suggests that the European hake is a safe fish food among the white fish group, which contributes to the desirable balance between healthy diet and contaminants avoidance. To further monitor the level of hake contamination and to extend the research to other sampling stations would allow to better assess the environmental quality and the risk in the food chain.

5.4 CASE STUDY VI

A PERSPECTIVE ON THE POTENTIAL HEALTH RISKS FROM HEAVY METALS CONTAMINATION OF ERBORISTIC PREPARATIONS OF *Malva sylvestris*

A BRIEF INTRODUCTION

Malva sylvestris is a member of family *Malvaceae* and its genus is represented by 40 taxa in all over of the world. It is an annual plant native to Europe, North Africa and South-west Asia, with lobed leaves and purple flowers which bloom in late spring, that grows spontaneously and prefers wetlands, such as meadows, the sides of the ditches and the banks of the rivers (Razavi *et al.*, 2011).
Commonly known as common mallow, *M. sylvestris* is a medicinal herb that has always been used in a traditional way, for its emollient and laxative properties (Barros *et al.,* 2010). Numerous surveys conducted in Europe highlighted the potential of a local resource often overlooked; the extracts of some parts of the plant of *M. sylvestris*, in particular leaves and flowers, have been compared for their nutraceutical potential, for their antioxidant properties and for their chemical composition (Ferreira *et al.*, 2006; Natali and Pollio, 2007; Guarrera *et al.*, 2007; Quave *et al.*, 2008; Leporatti *et al.*, 2009; Neves *et al.*, 2009). The mallow leaves have revealed very strong antioxidant properties, due to the presence of phenols, flavonoids, carotenoids and tocopherols, unsaturated fatty acids (α-linolenic acid) and mineral components.
For this reason mauve has been used for a long time (and still today) for medicinal applications and its roots, shoots, leaves, flowers are applied as infusions, decoctions, liniments, lotions, vapour baths and gargling.
These medicinal applications treat specific disorders of several systems of the human body, such as the digestive, the respiratory, the urinary, the muscular and skeletal systems, as well as skin disorders. Besides the anti-inflammatory properties, some pharmacological and clinical effects are recognized. It also has antioxidant and defense actions, essential for survival, in fact from epidemiological studies its use can protect against cancer and cardiovascular diseases (Mozaffarian, 2005; Geronikaki, 2006; Collins, 2009; Rackova, *et al.*, 2009; Della Greca *et al.*, 2009).
However, in addition to all uses for therapeutic purposes, M. *sylvestris* is also used for food purpose; specifically edible uses are concerned with folk gastronomy and with those uses generally included in “minor nourishment” (Guarrera*,* 2003; Carvalho 2005); while the young leaves are eaten raw in salads, the leaves and sprouts are consumed in soups and as boiled vegetables (Barros *et al.,* 2010).
The literature (Cooper and Johnson, 1984; Rivera and Obón de Castro, 1991) shows that the leaves are perfectly safe and that there are no negative effects for human consumption, although some authors have reported harmful effects due to the growth of mauve in contaminated areas.

The plants are sensitive to the absorption of organic and inorganic molecules, increasingly widespread in the environment due to urban and industrial emissions.
In fact plants represent a sort of vehicle for these substances, playing an important role in the bioaccumulation and biomagnification processes (Shinwari *et al.*, 2009).
The contaminants that can accumulate in the soil are numerous and of different nature; in recent years particular attention has been paid to the following classes of organic and inorganic compounds: heavy metals, residues of plant protection products, polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and mycotoxins. It is necessary to better understand the mechanisms by which contaminants are absorbed and accumulated by the plant species that are used for the production of phytotherapics and food supplements.
In the last years, the contamination of food from metals has become a major problem, due to the rapid technological progress that has led to an increase in natural emissions of some elements such as lead, cadmium and mercury from production processes. The most important heavy metal contaminations that may affect medicinal plants concern the presence beyond the maximum limits of Cadmium (Cd), Mercury (Hg) and Lead (Pb) and Arsenic (As).
In medicinal plants, the accumulation of heavy metals is due to their presence in the areas where they grow, near the roads or in the places of metals extraction and melting. Furthermore, the contamination may also be due to anthropogenic processes involving the application of synthetic fertilizers, organic fertilizers and industrial wastes, or inadequate hygienic transport and storage conditions (Dual *et al.*, 2009).
On the basis of possible environmental and production contaminations that may affect the safety of plant products, many leader companies in the herbal sector have defined self-regulation, in according to the limits imposed by current national and international regulations, in order to offer to consumers the best possible guarantees. In the present study, the content of heavy metals was analysed in various herbal products based on *M. sylvestris*, sampled from various European countries. Data obtained have been processed and compared and, for each country, the Health Risk Index was calculated in order to evaluate the potential health risk from mauve products chronic consumption.

EXPERIMENTAL PART

Sixty samples from different areas of Europe, from France, Germany, Greece, Spain and Italy (Figure 49) were analysed to determine the presence of heavy metals in the herbal preparations of *Malva sylvestris*. The preparations consist mainly of dried plants, in the form of tisanes, either in filter or in bulk, consisting of leaves and flowers or only flowers. Each sample was weighed, mineralized and its content of heavy metals was determined by atomic absorption spectrophotometer (HG-AAS).

Fig. 49 Sampling area

Less than a gram of each sample (0.2 – 0.3 g) was weighed and mineralized through a wet digestion process, in presence of 3ml of HNO_3 and 0.5ml of H_2O_2 for about 7 hours. After mineralization and cooling, the mixture was brought to a final volume of 10 ml with deionized water (Papetti and Rossi, 2009).
The levels of Cd, Pb were analysed using the atomic absorption spectrophotometer AA-600 (PerkinElmer, USA), while Hg and As have been determined through Hydride Generation Atomic Absorption Spectrometry (HGAAS). The TEs (Trace elements) calibration was made using appropriate dilutions of stock solutions of Pb, Cd, As, Hg (1000 ppm) (CPAChem). For the determination of hydrides, As and Hg, the following reagents were used: sodium borohydride ($NaBH_4$) at 98%, purchased from ACROS ORGANICS and hydrochloride acid (HCl) at 37% RPE from CARLO ERBA REAGENTS.
To ensure the reproducibility and the accuracy of the method, the same analyses were conducted on certified control samples (NIST1570a Sigma Aldrich) according to Ababneh (Ababneh, 2017) with a mean recovery of about 96±2%.
Instrument detection limits on the GFAA were 0.2 ppb for arsenic, 0.1 ppb for cadmium, 2.0 ppb for lead.
In the graphite furnace 20µl of sample were introduced with the addition of 5µl of a specific matrix modifier composed of magnesium nitrate ($Mg(NO_3)_2$) and ammonium phosphate (NH_2PO_4) to reduce or eliminate interferences in the vapor phase and stabilize the matrix. Subsequently, the analyte was atomized in according to a specific temperature program. The TE concentrations were expressed in milligrams per kilo of dry weight (mg/kg dw). Analysis of variance (ANOVA) tests were conducted for the concentrations of metals, using as independent variables the metals considered, the sampling areas, and the interactions. According to what resulted as significant, Tukey's HSD post-hoc tests were performed.
Subsequently, the estimated daily intake of each TE depends on both the metal concentration in food and the daily food consumption. In addition, the human body weight can influence the tolerance of contaminants. EDI was calculated as follows:

$$EDI = (F_{IR} \times Cm)/W_{AB}$$

Where F_{IR} is the mauve ingestion rate (g/person/day), which was considered to be 0.77 g/day, that is the average of European available data of the chronic consumption of mauve products such as tea and herbal infusions (Table 25) (Chamannejadian *et al.*, 2013; EFSA, 2015); Cm is the TE concentration in foodstuffs; W_{AB} is the average body weight (bw). The body weight was set to 60 kg in this study (Zazouli *et al.,* 2008).

Table 25. Data of mauve chronic consumption from EFSA database.

Countries	Mean (g/day)	SD
Austria	1,47	4,13
Finland	1,40	3,14
Hungary	0,52	1,78
Ireland	0,24	1,34
Netherlands	0,82	3,28
Romania	1,81	3,54
Sweden	0,01	0,24
UK	0,16	0,96
UK	0,32	1,65
Germany	2,23	1,44
Italy	0,09	0,54
Latvia	0,16	0,59
Average	**0,77**	**0,76**

Health risk of consumers due to intake of TE contaminated mauve was assessed by using HRI (Health Risk Index). The HRI was calculated by using the equation:

$$HRI = EDI/R_fD$$

where RfD is the reference oral dose expressed in mg/kg/day and it represents an estimation of the daily exposure of a contaminant to which the human population may be continually exposed over a lifetime without an appreciable risk of harmful effects. The R_fD is 3.0x10-4 for As, 4.0x10-4 for Hg, 1.0x10-3 for Cd and 3.5x10-3 for Pb (Akoto *et al.*, 2014; USEPA, 2009).

The exposure to two or more pollutants may result in additive and/or interactive effects. The total HRI of heavy metals was treated as the arithmetical sum of the individual metal HRI: Total HRI (individual foodstuff) = HRI (toxicant 1) + HRI (toxicant 2) + HRI (toxicant n).

A Total HRI lower than 1 means the exposed population is unlikely to experience obvious adverse effects; whereas a HRI above 1 means that there is a chance of non-carcinogenic effects, with an increasing probability as the value increases (Akoto *et al.*, 2014). HRI was determined for each sampling area.

Results

The study was conducted on 60 samples from different European areas, in particular products based on mallow from different regions of Italy, from Germany, Greece, France, Spain and Mediterranean areas (in this last case the sampling sites are not explicitly specified in the labels of the products), (Table 26).

Table 26. Origin of mauve and number of collected samples

Simpling Sites	Total n. of samples collected N=60
France	11
Germany	8
Greece	9
Italy	15
Spain	9
Mediterranean areas	8

The table 27 shows the data of the average concentrations of As, Cd, Hg and Pb, obtained from 3 replicates performed on each sample, expressed in mg/kg (ppm).

Table 27. Mean concentrations of heavy metals and Standard deviation (mg/kg^{-1})

Sites	Pb	Cd	As	Hg
France	0,096±0,019	0,339±0,083	0,012±0,003	n.d
Germany	0,167±0,044	0,116±0,048	0,068±0,019	n.d
Greece	0,132±0,024	0,290±0,060	0,063±0,019	n.d
Italy	0,136±0,019	0,177±0,029	0,014±0,005	n.d
Mediterranean areas	0,271±0,060	0,311±0,131	0,014±0,011	n.d
Spain	0,116±0,057	0,102±0,036	0,022±0,008	n.d

From the data obtained, the highest concentrations were found for cadmium, particularly in samples from France, Greece and in the Mediterranean areas, where the cadmium concentration varied from 0.303 to 0.513 ppm on a dry weight basis. Overall, among the 60 samples analysed for cadmium, about 27% of the samples contained high levels of cadmium exceeding 0.3 and 0.2 ppm, respectively the permissible limits set by FAO/WHO for medicinal herbs and plants, and the EU Regulation for foodstuffs. Similar results of high levels of cadmium in Egyptian and Iranian medicinal herbs and plants have been reported in earlier studies (Dghaim *et al.*, 2015; Ziarati, 2012).

The level of lead was higher in the samples from the European Mediterranean area and Germany, with a range in between a minimum of 0.10 and 0.347 ppm on a dry weight basis.

The results related to the samples obtained from Mediterranean areas, are in line with the data on the most common medicinal herbs reported in literature, which showed the highest average concentrations of lead and cadmium in samples found in the Egyptian, Iranian and Jordanian regions (Ziarati, 2012; Abou-Arab *et al.* 2000). The FAO/WHO maximum permissible limit of lead in consumed medicinal herbs is 10 mg·kg−1, therefore all the values found were below the allowed limits. According to Dghaim *et al.*, (Dghaim *et al.*, 2015), cadmium is the metal present at the highest concentrations in the traditional herbs, while arsenic and especially mercury are present in lower quantities.

In some cases, the concentration of heavy metals and in particular of cadmium has exceeded the permitted international levels. According to previous studies, the different metal concentrations detected in the products that have been analysed in this work could be attributed to differences in the environmental pollution, the growth stage, the type of soil and the sampling site (Dghaim *et al.* 2015 and Orisakwe *et al.* 2012).

Besides Chizzola *et al.*, (2003), in their research on the monitoring of micronutrient and heavy metals in herbs, spices and medicinal plants from Austria, found that some medicinal herbs have a greater tendency to accumulate cadmium. In fact, higher levels of cadmium were detected in medicinal plants similar to mauve, such as chamomile *(Matricaria chamomilla)*, yarrow *(Achillea millefolium)*, mint *(Mentha spicata)* and sage *(Salvia officinalis)*.

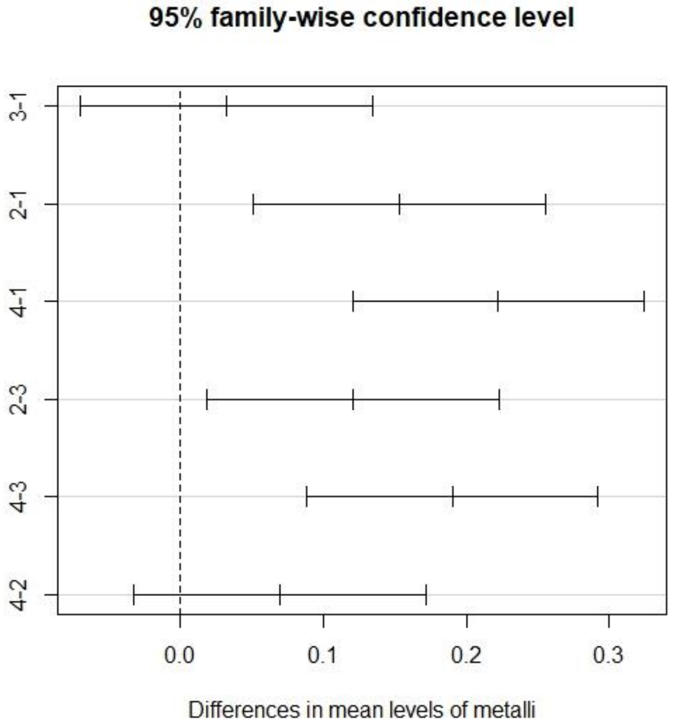
The arsenic contamination levels are less than 0.001 ppm for all samples under examination, in particular higher concentrations were detected in the samples from France, Italy and Greece. Mercury levels in all the samples analysed were below the detection limits.

The results obtained are in line with previous works and reflect the level of soil contamination in the sampling areas, in relation to natural and anthropogenic contributions (Tóth *et al.* 2016). From the literature, the arsenic is more abundant in the substratum of the mountainous areas such as the Alps, the Carpathians, the Massif Central and the Pyrenees, and in the fluvial areas (Pianura Padana), while, high concentrations of cadmium have been found in the Bavarian Alps and in the south-eastern Alps.

High levels of lead can be found in many regions of Europe, mostly the most industrialized ones; such cases are evident in southern Saxony, in the North Rhine in Germany, in Bristol and Manchester in England and in Rome in Italy (Tóth *et al.,* 2016).

On the average metal concentrations obtained, statistical analysis were conducted. According to ANOVA analysis results, related to metals concentrations, only the variable “Metal” was found significant; instead both “Area” variable and the interaction between the two variables, weren’t found significant. Hence, Tukey’s HSD post-hoc test was performed for the variable “Metal” and results were showed in figure 50.

Fig. 50 Tukey's Test Plot (95% family-wise confidence level) – Differences in mean levels of metals. A number is assigned for each metal: Hg (1); Pb (2); As (3); Cd (g).



As showed in figure 50, only two pairs did not show any significant difference: 3-1 (As-Hg), 4-2 (Cd-Pb), for which the corresponding 95% family-wise confidence intervals include zero. Regarding the risks for human health, the EDI (Estimated daily intakes) and the HRI (Health risk Index) values of trace elements in mauve products, considering data of chronic consumption for adults, were calculated and summarized in Table 28.

Table 28. Mean concentration, EDIs and HRIs of trace elements in mauve products, from different European areas, considering data of chronic consumption for adults.

	Pollutant	Mean (mg/kg^{-1})	EDI (mg/kg b.w./day)	Reference Oral doses (mg/kg b.w./day)	HRI
ITA	Cd	0,176	2,25867E-06	0,001	0,0023
	Pb	0,136	1,74533E-06	0,0035	0,0005
	As	0,014	1,79667E-07	0,0003	0,0006
	Hg	-	-	0,0004	0,0000
					0,0034
SPA	Cd	0,102	1,31E-06	0,001	1,31E-03
	Pb	0,115	1,48E-06	0,0035	4,22E-04
	As	0,022	2,82E-07	0,0003	9,41E-04
	Hg	-	-	0,0004	0,00E+0
					2,67E-03
GER	Cd	0,116	1,49E-06	0,001	1,49E-03
	Pb	0,167	2,14E-06	0,0035	6,12E-04
	As	0,068	8,73E-07	0,0003	2,91E-03
	Hg	-	-	0,0004	0,00E+0
					5,01E-03
FRA	Cd	0,339	4,3505E-06	0,001	0,0044
	Pb	0,096	0,000001232	0,0035	0,0004
	As	0,012	0,000000154	0,0003	0,0005
	Hg	-	-	0,0004	0,0000
					0,0052
GRE	Cd	0,29	3,72167E-06	0,001	0,0037
	Pb	0,132	0,000001694	0,0035	0,0005
	As	0,063	8,085E-07	0,0003	0,0027
	Hg	-	-	0,0004	0,0000
					0,0069
EU Med	Cd	0,311	3,99117E-06	0,001	0,0040
	Pb	0,271	3,47783E-06	0,0035	0,0010
	As	0,014	1,79667E-07	0,0003	0,0006
	Hg	-	-	0,0004	0,0000
					0,0056

All the data obtained were compared with the limits established by European and International Regulations (Reg.EU 4020/2011 and FAO/WHO) and compared with those proposed by *ASSOERBE*, a national association representing Italian companies in the field of medicinal and aromatic plants, spices, plant extracts, essential oils and their derivatives (*www.assoerbe.eu*). The health risk due to the contamination of metals depends on the daily average dietary intake, and is not negligible.

From a study conducted in 2008 by Shen and Chen on the risk assessment of heavy metals in tea infusion, the HI values were very low and were within the limits of safety. However, to date there is still no systematic way to compare the health risk of heavy metals in tea infusions with tea leaves or flowers (Li *et al.*, 2015); many authors reported that EDI values of tea infusions decreased with the increase of the infusion times (Karimi *et al.*, 2008, Li *et al.*, 2015; Martin and Griswold, 2009). Experimental results of tea infusions show that about 40-52.8% of the total Cd has been released by some varieties of black tea (Shen and Chen, 2008), and the transfer ratio (release rate) of Cr (VI) in the green tea leaves by infusion was reported to 37.8% from Li *et al.* (2013).The percentage of Pb and Hg, which were released form black tea to infusions, were 2,6% and 70%, respectively (Karimi *et al.,* 2008).

The tea derived from the leaves of Camellia sinensis is one of the most popular beverages in the world; according to a study of Shekoohiyan *et al.* about 18-20 billion cups of tea are consumed every day in the world. Therefore, the risk associated with the assimilation of heavy metals through the consumption of tea could be quite high. The health aspects related to tea consumption are very important and consumers should be very confident about the absence of any pollutants, especially for imported products. Differences in metal concentrations may depend on conservation methods, processing of leaves and concentrations of metals in soil. For safety, it is recommended to prepare tea with water free of heavy metals and water with a low content of fluorine and preferably contained in the bottle (Shekoohiyan *et al.,* 2012).

Finally, the sum of HRI values determined for each metal are less than 1 for all sampling area, indicating that the consumption of the studied *M. sylvestris* samples do not constitute a health risk for tea consumers.

Conclusion

The purpose of this work was to determine the levels of heavy metal contamination in *Malva sylvestris* products, sampled from different European countries, in order to compare them and evaluate the potential health risk from mallow chronic consumption in adults. The study was carried out on tisanes and mauve based preparations, widely used on the market, and the levels of heavy metals detected were compared with the European and International limits and the guidelines proposed by *ASSOERBE*.

The arsenic and mercury levels are below the current limits provided by European Commission and FAO in all samples, despite the not negligible concentrations of cadmium and lead especially in samples from South Mediterranean areas.

Finally to evaluate the potential risk for human health in the case of mallow products chronic consumption the “Estimated Daily Intake” (EDI) and the “Health Risk Index” (HRI) were calculated. Total Health Risk Index values resulted lower than 1, indicating that there were no risks to human health for each sampling area. It is certainly important to carry out studies and chemical analyses aimed at ensuring the quality of commercialized herb products, especially if they are used in tea infusion, for their aromatic and medicinal properties. Particular attention must be paid to good practices, from collection to consumption, in particular for imported products. Further studies are necessary for determining the presence of toxic metals, chemicals and other dangerous pollutants for assessing the cumulative long-term risk to tea consumer health.

5.5 CASE STUDY VII

A PERSPECTIVE ON THE POTENTIAL HEALTH RISK OF ARSENIC VIA DIETARY INTAKE OF RADISH AND LETTUCE FROM LATIUM.

A BRIEF INTRODUCTION

In some area of Lazio region, most arsenic in groundwater and soils is present naturally. Although arsenic was once used as a pesticide, the predominant source in natural waters is sediments from volcanic rock. Over long periods of time, these arsenic bearing sediments can add arsenic to groundwater. Under the right subsurface chemical conditions, concentrations of arsenic may be very high and substantially exceed the drinking water standard of 0.010 ppm. The presence of naturally occurring elevated arsenic concentrations in the environment is particularly difficult to manage.

Indiscriminate application of inorganic arsenical pesticides, desiccants and wood preservatives led to pollution of many agricultural soils and to reduction of their productivity (Carbonell-Barrachina *et al.*, 2009; Fitzmaurice *et al.*, 2009). Since the 1960s, the use of As-based insecticides/pesticides in Europe has been sensibly reduced, but 4.6 million m3 of As-containing wood preservative are still annually produced (Zevenhoven *et al.*, 2007). Elevated geogenic As concentrations in groundwater have been reported in geothermal areas in Argentina, Chile, France, Greece, Italy, Japan, Mexico, New Zealand, and the USA (Yoshizuka *et al.*, 2010).Water is the next important input to fertilizer for crop production. If water is polluted, it may be dangerous for plants, animals as well as for human being. If arsenic contaminated water is used for irrigation, it may create hazard both in soil environment and in crop quality. Twenty percent loss of crop (cereal) production due to high concentration (20 ppm) of arsenic in plant body was reported by Farid *et al.* 2003. Like other heavy metals, arsenic is toxic to plant and its discharge into the environment must be carefully controlled and minimized. Mainly, radishes, in a sandy soil, either did not grow or showed 90 percent to 96 percent reduction in growth at a soil concentration of 500 ppm. At concentrations of 100 ppm in loamy sand, silty clay and clay soils, radishes showed 67 percent, 17 percent and 7 percent reductions in growth, respectively. Therefore, it is of utmost importance to determine the arsenic content in vegetables.

Very limited work has been done on the effects of using arsenic contaminated water on crop production and it's carried over effect on food chain. With this view in mind, this study was undertaken to find out the level of arsenic transmission from irrigation water to vegetable food.

EXPERIMENTAL PART

Field experiments were conducted on vegetables as lettuce and radish in plots using arsenic contaminated irrigation water. Plastic pots (2.5 L) with 1.5 kg of soil were used with four replications in each treatment. The soil was fertilized according to the calculation by following the Fertilizer Recommendation Guide and was saturated with 500 mL of water. On a per pot basis, 3 lettuce plants were sown at 0.3 cm, 6 radish seeds were sown at 1.3 cm.
Polythene was used in between two plots to protect horizontal movement of arsenic from arsenic contaminated irrigated plot to arsenic free irrigated plot.
Five arsenic treatments 0.0, 0.01, 0.1, 0.5 and 1.0 mg/L As containing irrigation water were used in this experiment. Sodium arsenate dibasic heptahydrate (Na_2HAsO_4 $7H_2O$) Sigma-Aldrich ≥98.0% was used for arsenic source. The pots were arranged, and the position was changed every day in a completely randomized way so that the plants got equal sun light. During the whole growth period, all visible symptoms were observed and recorded. Only the edible part of the lettuce plant was harvested 60 days after sowing the seeds. The stems of the lettuce plants were cut at 1.0 cm above the soil. While radishes were take up 30 days after sowing the seeds. Vegetables were packed with labeled polythene/brown paper. These labeled packed vegetables were immediately sent to LAMeT laboratory and divided into different pieces with knife. Precautions were taken so that arsenic could not transfer from one sample to another through knife. Labeled and chopped plant parts were kept in the sun for one or two days for removing moisture. The harvested plant part was washed with deionized distilled water. The collected plant samples were air dried subsequently oven dried at 55°C- 60°C ± 5°C to attain constant weight.
A total of 20 vegetables (10 plants of lettuce and 10 plants of radish) were analysed from three three areas of the province of Viterbo (Table 29). All samples were put in polythene zip-bags and transported to the laboratory on the day of sampling. Each vegetable sample was carefully washed with distilled water and the edible parts were cut into small pieces and then oven dried at 55°C- 60°C ± 5°C to attain constant weight.
The dried plant samples were grinded by a electrical grinder. After grinding one sample, the grinder was cleaned so that arsenic could not transfer from one sample to another. The dry weights of plant samples were measured and recorded.

Fig. 51 Microwave digester CEM, MARS6

A microwave digester (CEM, MARS6, fig.51) was used to digest all food samples. Ground food sample (approximately 0.5 g of each) was weighed directly into a Teflon vessel, then adding 5mL concentrated HNO_3 (from Fischer Chemicals). Samples were allowed to predigest by standing open for 30 min before sealing vessels. Each vessel was sealed, placed into the rotor. The microwave conditions were: microwave power 1020–1800 W, ramp time: 20–25 min, hold time: 15min at210°C.

After cooling for 30 min, the vessels were opened carefully. Each digestion solution was transferred to a 50-mLplastic centrifuge tube and diluted to 10 mL using milli-Q water. The samples were analyzed on the same day of preparation-otherwise stored in fridge at 4°C.
At the end of mineralization, deionized water was added to the samples to achieve a final volume of 10 mL
The digested plants were analyzed for As using the Atomic Absorption Spectrophotometer AA-600 PerkinElmer; reagent blanks and internal standards (from Kanto Chemical Co. Inc., Tokyo, Japan) were used to ensure the accuracy and precision of the analyses, according to the previously published protocols (Smith *et al*l 1997). Standard reference materials (SRMs), trace elements in natural water (SRM 1640) and Trace Elements in Spinach Leaves SRM (1570a) from the National Institute of Standards and Technology(NIST) were used to verify the results for As. All samples were collected and analyzed in triplicate, and the average results were used to represent the data.
Contents of the elements are shown as mg kg-1. Accumulation of As in the edible part of the plant is shown as mg kg^{-1}s.s.
The estimated daily intake of each TE depends on both the metal concentration in food and the daily food consumption. In addition, the human body weight can influence the tolerance of contaminants. EDI was calculated as follows:

$$EDI = (EF \times ED \times FIR \times Cf \times Cm)/(WAB \times TA)$$

Where EF is the exposure frequency (365 days/ year); ED is the exposure duration, equivalent to average lifetime (64 years); FIR is the lettuce/radish ingestion rate (g/person/day), which was considered to be 27.25 g/day and 16.92 g/day in the case of a chronic consumption of lettuce and radish, respectively (Ali and Hau, 2001; Akoto *et al.*, 2014; EFSA, 2015); Cf is the conversion factor for wet weight (ww) to dry weight (dw); Cm is the TE concentration in foodstuffs (mg/kg dw); TA is the average exposure time for non-carcinogens (equal to EF × ED); WAB is the average body weight (bw): according to a recent survey of Codacons (Coordinamento delle associazioni per la difesa dell’ambiente e dei diritti degli utenti e dei consumatori) the average weight of an Italian adult is 74.1 kg, so the authors decided to approximate this value to 75 kg (https://codacons.it/crisi-codacons-italiani-mangiano-meno-cala-peso-medio/).
Health risk of consumers due to intake of TE contaminated fish was assessed by using HRI (Health Risk Index). A HRI lower than 1 means the exposed population is unlikely to experience obvious adverse effects; whereas a HRI above 1 means that there is a chance of non-carcinogenic effects, with an increasing probability as the value increases (Akoto *et al.*, 2014). The HRI was calculated by using the equation:

$$HRI = EDI/RfD$$

where RfD is the reference oral dose expressed in mg/kg/day and it represents an estimation of the daily exposure of a contaminant to which the human population may be continually exposed over a lifetime without an appreciable risk of harmful effects. The RfD is $3.0x10^{-4}$ for As (Akoto *et al.*, 2014; USEPA, 2009).

In the first part of the study authors have investigated if there were differences in the arsenic accumulation capacities between leaf and root vegetables and tried to identify and estimate the correlation between the content of arsenic in irrigation water and its accumulation in vegetables The graphical presentation of relative arsenic content of different vegetable grown in the greenhouses of CREA, irrigated with contaminated water, is shown in Figure 52.

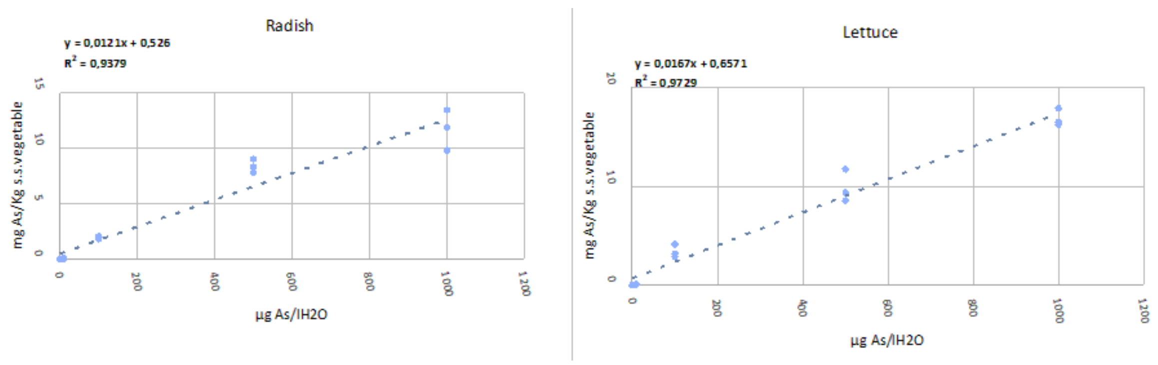


Fig. 52 Arsenic content in vegetables (radish and lettuce) irrigated with water contamined by Sodium arsenate dibasic heptahydrate

Arsenic content in vegetables, irrigated with contaminated water varied widely. Leafy vegetables accumulate more arsenic than other vegetable. On the other hand, significant differences in metal concentrations among the different vegetables implied that different vegetable species had different abilities and capacities to take up and accumulate different metals. The As concentration (mg kg^{-1} dw) in the edible part of the plants increased significantly with increasing As concentrations in irrigation water. A significant ($p \leq 0.01$) increasing trend of arsenic accumulation in plants was found with increase of arsenic in irrigation water. The highest level of arsenic in fact was observed in the treatment of 1.0 mg/l As containing irrigation water and lowest level in control treatment. Arsenic in irrigation water showed a strong positive correlations with arsenic accumulation into plants, and the trend of accumulation was found as lettuce > radish. Experimental data were evaluated by employing linear-regression model of arsenic contaminated water against arsenic accumulation by edible parts of a few vegetable crops under study. The values of R2 derived from these models give clear indication of strong positive relationship between arsenic availability in irrigation water and arsenic accumulation of vegetable crops.

Considering the growth of lettuce plants (i.e., dry matter production of roots and leaves) was severely impaired by the irrigation with the As-contaminated water. The phytotoxic effect of Arsenic determined a strong inhibition of root growth and, to a lesser extent, leaf development. For instance, lettuce plants irrigated with water containing 10 mg L^{-1} of As produced less than a third of the root biomass of the control. Cozzolino *et al.*, 2010 and Gusman *et al.*, 2013 also reported the sensitivity of this leaf vegetable to As, which resulted in a stunted growth in contaminated soil or nutrient solution. In the conditions used in our study, plants showed symptoms of As toxicity but none of them died from As intoxication. The vegetable samples grown in the greenhouses of the CREA showed different abilities and capacities to take up and accumulate the arsenic. In the second part of this study, analysis were conducted also on field-grown vegetables from different areas of Latium in order to confirm this result, considering that arsenic content in open field-grown vegetables would have been presumably higher than the concentration of those planted in a greenhouse. Specifically, in the second part, the study investigated concentrations of As, in commonly consumed horticultural analysed from three three areas of the province of Viterbo to assess food quality and make recommendations based on an explicit understanding of human health risks. In this area (central Italy) high arsenic concentrations have been detected in soil and phytoavailable geogenic arsenic enters the food chain. As a result, local population may be exposed to inorganic arsenic through consumption of food and the Arsenic contamination of vegetables poses a potential health risk to both humans and animals.

The heavy metal concentrations (mg/kg dw) in the edible part of vegetables are listed in Table 29. The concentration of metals varied greatly among species and sample locations.

Table 29. Concentration of arsenic (mg/Kg dw) in the edible part of vegetables.

		Sito		
Tipologia ortaggi	ID Campione	Viterbo	Tarquinia	Tuscania
Radish 	*Sample n.1*	n.D	0.308 ±0.068	0.399± 0.105
	Sample n.2	0.091±0.01	0.295 ±0.014	0.412±0.211
	Sample n.3	n.D	0.189±0.011	0.301±0.005
	Sample n.4	0.02±0.00	0.300±0.207	0.441±0.002
	Sample n.5	n.D	0.301±0.157	0.453 ±0.005
	Sample n.6	n.D	0.285 ±0.02	0.397±0.011
	Sample n.7	0.075±0.03	0.274±0.111	0.412±0.201
	Sample n.8	n.D	0.291±0.001	0.417±0.032
	Sample n.9	n.D	0.299±0.027	0.396±0.007
	Sample n.10	n.D	0.271±0.004	0.448±0.001
Lettuce 	*Sample n.1*	0.02±0.001	0.478±0.051	0.841±0.421
	Sample n.2	0.01±0.005	0.554 ± 0.42	0.789±0.257
	Sample n.3	n.D	0.511±0.381	0.912±0.003
	Sample n.4	n.D	0.691±0.004	0.689±0.273
	Sample n.5	0.002 ±0.00	0.738±0.260	0.735±0.005
	Sample n.6	n.D	0.862±0.231	1.102±0.008
	Sample n.7	n.D	0.777±0.274	1.005±0.490
	Sample n.8	0.015±0.001	0.745±0.317	0.986±0.224
	Sample n.9	n.D	0.608±0.004	1.271±0.279
	Sample n.10	0.021±0.003	0.804±0.027	0.998±0.007
Data are expressed as mean values ± SD (n = 4)				

Arsenic concentrations in the edible plant portions ranged from less than the analytical detection limit (0.1 µg kg^{-1} dw) to 1.271 mg kg^{-1} dw, with a mean of 0.805 mg kg^{-1} dw for lettuce and 0.344 mg kg^{-1} dw for radish. All samples of radishes, and lettuces grown in the area of Viterbo showed low (or no detectable) arsenic values. The highest levels of arsenic have been detected either for the lettuce and the radish in samples from Tuscania and they were 1.271 mg kg^{-1} and 0.453 mg kg^{-1}, respectively. The As concentration in the plant grown in different levels of As contaminated soil exceeded the maximum limit for vegetables of 0.5 $mgkg^{-1}$ (Alam *et al*, 2003) when the soil As concentration was higher than 20 mg kg^{-1}. A study of roots, stems, leaves and fruits in soils with varying arsenic levels found that the amount of arsenic in root crops, such as potatoes and onions, corresponded with the amount of arsenic in the soils in which they were grown (Dahal, *et al*l. 2008). This is similar to results reported by Huang, *et al* (2006), who found that radishes and onions accumulated arsenic when grown in soils with approximately 1 to 25 ppm arsenic, though not in concentrations of concern. The As concentration in the edible part was two and six times higher than the maximum limit for vegetables of 0.5 $mgkg^{-1}$ when grown in 30 and 50 $mgkg^{-1}$ soil, respectively (Joardar *et al.*, 2014). Another studies by Gaw *et al.* 2008 and Ramirez-Andreotta *et al.* 2014 found that radishes and lettuce grown in soils that had formerly been treated with arsenical pesticides also accumulated arsenic, though not in concentrations that exceeded the FDA standard. Finally, in the study by Baroni *et al.* 2004 the As contents of plants were low, especially in crops and in the most common wild species and roots usually showed the highest content followed by leaves and shoots. Arsenic levels in soils and plants were positively correlated, while the ability of the plants to accumulate the element (expressed by their Biological Accumulation Coefficients and Concentration Factors) was independent of the soil As content. (Baroni *et al.* 2004). Definitely, also in this case (like in the first part of this work) higher arsenic concentrations have been detected in the samples of lettuce than in those of radish.

The exposure pathway of humans to organic and inorganic contaminants through ingestion of contaminated food has been studied by many researchers (Chary *et al.*, 2008; Copat *et al.*, 2012; Xue *et al.*, 2012; Akoto *et al.*, 2014). In this study, the health risk of consumers due to the intake of contaminated vegetable was assessed using the estimated dietary intake (EDI) and HRI index. The data obtained are shown in Fig. 53.

Fig. 53 Determination of EDI and HRI values to evaluate the potential health risk from consumption of *Lactuca s.* and *Raphanus s.*

Chronic Consumption - *Lactuca s.*

	Area	Mean ± Std. Dev. (mg/Kg)	EDI (mg/kg b.w./day)	Reference oral doses (mg/kg b.w./day)	HRI
As	Tuscania	0,933 ± 0,177	0,00033899	0,0003	**1,13**
	Tarquinia	0,677 ± 0,132	0,000245977	0,0003	**0,82**
	Viterbo	0,007 ± 0,009	2,54333E-06	0,0003	**0,01**

Chronic Consumption - *Raphanus s.*

	Area	Mean ± Std. Dev. (mg/Kg)	EDI (mg/kg b.w./day)	Reference oral doses (mg/kg b.w./day)	HRI
As	Tuscania	0,408 ± 0,043	9,20448E-05	0,0003	**0,31**
	Tarquinia	0,281 ± 0,035	6,339360E-05	0,0003	**0,21**
	Viterbo	0,021 ± 0,036	4,737600E-06	0,0003	**0,02**

To evaluate the potential health risk from consumption of *Lactuca s.* and *Raphanus s.*, we calculated the estimated daily intake (EDI) for adults, finding that HRI (Health Risk Index) index value for arsenic was low (<1) in the case of chronic consumptions for all samples of radishes, and for the lettuces grown in the area of Viterbo. On the contrary, the lettuces obtained from Tuscania and Tarquinia presented very high concentrations of arsenic and a worrying value of the HRI value (Fig. 53).

CONCLUSIONS

The amounts of the As found in samples of radish (*Raphanus sativus L.*) and lettuce (*Lactuca sativa L.*) were generally higher than those reported for the vegetables and cereals consumed in other parts of the world, exceeding the national standards and international recommendations. An urgent and systematic study of the arsenic in the vegetables traded in Lazio, and specifically in the area of Viterbo is recommended since their consumption could contribute negatively to increase the intake of the arsenic, a toxic element potentially dangerous for the human health. The dietary intake of that metal is not only determined by its level in the food, but also by the amount the people consume and the quantity and quality of the water they drink.
Perhaps different remediation measures should be taken promptly to remove exciding metal contamination in these study areas.

6. CONCLUSIONS

In this study we analysed a series of instruments aimed at enhancing and protecting food products made in Italy, and not only. In order to valorise a product we must first recognize its value, that is the result of intrinsic characteristics and its ability to satisfy consumer demands.

The product quality corresponds to its ability to meet explicit and implicit needs of consumers.

In the past, the quality of the food product simply had to meet compliance requirements; today to be defined as a quality product, it must satisfy sanitary, organoleptic, nutritional, commodity, technological, origin, environmental and ethical requirements.

Quality requirements in the food sector fall into the category of primary needs and are therefore protected, in the first instance, by Italian and international legislation. These regulatory requirements fall in the mandatory scope and their aim is to guarantee safety from the point of view of hygiene and health and in nutritional terms (hygiene package, Regulation (EU) No. 1169/2011).

In the regulated field, reference must be made to all Community regulations aimed at protecting the production of specific geographical areas and the production obtained with traditional techniques and/or using traditional raw materials (Council Regulation (EEC) No. 2081/92, 2082 / 02 of 14 July 1992, Council Regulation (EC) No. 510/2006, Regulation of the European Parliament (EU) No 1151/2012). Finally, there are voluntary legal references drawn up by bodies or Organizations that, with the consent of all the interested parties, define the quality standards of the food product (ISO 9000, 14000, 22000).

Voluntary approaches to quality (regarding "system", "product" and "process") are complementary and synergistic; they are based on conscious and demanding choices of the operators involved, and represent instruments, that are not only preparatory to the respect of the laws, but of improvement.

Unfortunately, these methods are not always sufficient enough for the enhancement and protection of products: there are gaps in regulations, monitoring and sanctions are not always effective and reassuring, and the certifications are sometimes not cofirmed by the choices of Italian consumers.

The present research fits into this scenario, and the first aspect that has been considered is linked to the protection of PDO (protected designation of origin) and PGI (protected geographical indication) Italian products.

The quality, connected to its origin, responds to a series of specifications and is promoted across quality brands on the product. The Italian PDO and PGI products are subject to a series of laws laid down by the regulation 1151/2012 of the European Union, which should protect them from counterfeiting, a debatable form of food fraud, that is punishable by law.

Unfortunately, the same does not happen for an increasingly widespread phenomenon abroad: *the Italian sounding*. This expression refers to the imitation of Italian products or logos, with illicit references to the alleged *"Italianness"* of the product. Despite the efforts made to contain this phenomenon, Italy continues to record significant losses in turnover.
Therefore, in addition to certifications of origin and controls, it is necessary introduce other methods that allow the identification of typical and unambiguous markers of quality of the product, in order to defend it on the market.
In the first case study, we tested analytical and chemometric tools to discriminate PDO buffalo mozzarella from Campania (Italia), obtained with traditional and industrial processing methods.
According to our results, the conjunction of FT-IR spectroscopy with chemometric analysis can provide an exceptional tool to confirm cheese quality, classify products according to their manufacturing process, monitor complex biochemical changes and contrast the counterfeiting.
However, the instrumental analysis are not enough to define in a univocal and objective way the sensorial profile of a food product, but it is necessary to conduct descriptive and discriminatory tests, in which a panel of experts evaluates the intensity of specific attributes related to the various aspects of the perceivable quality of the food examined.
Descriptive tests are often accompanied by hedonistic tests, aimed at assessing sensory acceptability on consumers' behalf. On the other hand, the consumers, often victims of informative asymmetry, are not always able to distinguish similar products, or products obtained by different production processes, or do not have the necessary knowledge to identify positive attributes and defects of the food they consume every day.
In Italy, many people do not know the differences between the different brands of origin, others do not know what the biological production consists of. It is therefore necessary to conduct information campaigns aimed at educating and sensitizing the consumers. In this sense, valorising a product also means making consumers aware of its intrinsic quality.
In the second case study, we investigated the perception of young Italian consumers concerning three different types of olive oil: extra virgin olive oil, PDO and organic extra virgin olive oils, taking into account that olive oil is a traditional product of our peninsula (Italy is in fact the second largest producer and importer of oil in the world). We have conducted consumer science studies to see if Italian consumers can be influenced by the brand of origin or the biological label, so we conducted an information campaign to raise their awareness.
Through the administration of questionnaires we found a significant difference between normal and semi-expert consumers in the recognition of the positive attributes of extra virgin olive oil. In the panel tests, normal consumers associated the term "DOP" with "better quality", but also with "expensive"; on the contrary semi-experts and experts associated this term with words as "certification" and "safety". With reference to organic products, all the panelists used adjectives as "untreated" and "healthy". A few assessors were reluctant to use organic products, defining them as fraud.

If there is still confusion in the field of production specifications and of organic production, on the contrary, more and more consumers are attentive to the nutritional label. The nutrition label contains information that helps consumers understand how different foods can contribute to a healthy and balanced diet. Today, consumers prefer healthy and fat-free products, and it is very important to take into account their demands, by experimenting with innovative products that are able to embrace the new food trends (e.g. the affirmation of vegan products and functional foods).

The rheological, analytical and consumer science tools allow us to understand if the new products have adequate chemical-physical characteristics and embrace the expectations and needs of consumers, in comparison with other products currently on the market. In this sense, valorisation is synonymous with innovation, starting from the expressed needs of the consumers.

In the third case study, we experimented with a new vegan mayonnaise, egg- and soybean-free, combining different ingredients including linseed proteins and beta glucan (functional ingredients). We obtained a good emulsion, with rheological and sensory properties quite similar to those of conventional mayonnaises. In addition to obtaining a good response among the panelists, our mayo showed a competitive nutritional profile while containing fewer ingredients than the others samples and only one allergen (mustard).

Although this emulsion should be improved especially from the point of view of homogenization and sterilization, we decided to test it in making a bakery product (case study IV), and to compare it with cakes made using other types of mayonnaises (the same samples used as controls in the first study).

The characteristics of the texture (firmness and springiness), and those related to the color did not show differences compared to the other samples. On the contrary, in sensory evaluations consumers declared the chocolate cake made by using our mayo the best one for its cocoa aroma, cocoa flavor, as well as for the softness and chewiness. In the future we can think of using mayonnaise for the production of gluten-free desserts and egg-free products.

Protecting consumers also means ensuring that there are no allergens in foodstuff, and/or that there are no illicit nutritional/health claims that can mislead them.

However, at the base of all the characteristics of a food product, there is an essential factor: food safety.

Consumers always take this aspect for granted. In addition to the stringent limits imposed by the Italian food safety authority, several factors as globalization, urbanization, lifestyle changes, industrialization, intensification of agricultural and livestock production continue to increase the responsibility of producers and operators in ensuring food safety.

Food safety is threatened by various kinds of risks: there are some environmental contaminants that are biomagnified along the food chain and could represent a serious danger to human health.

It is important to control food along the supply chain, and to continuously conduct environmental monitoring especially to verify the safety of those products that are marketed

without processing the raw material and/or sold wholesale or in local markets. For this reason, we decided to conduct studies on samples of hakes, mallow herbal preparations and vegetables (such as lettuce and radish) in order to determine the concentration of some environmental contaminants in these products and, thus, evaluate the potential health risks from their consumption.
As for European hake, which is one of the most consumed species of white fish in Italy, the concentration of organic and inorganic contaminants was below international limits, as well as the Estimate Daily Intake for adults and the associated Health Risk were low (despite the non-negligible mercury concentration) for chronic Italian hake consumption.
Low values were also found in relation to the concentrations of some heavy metals in herbal preparations based on *Malva Sylvestris*. In this case, the total "Health Risk Index" value resulted much lower than 1, without risks to human health.
In the last case study, alarming data were recorded only with reference to the consumption of lettuce contaminated by arsenic in the Tuscania area (Lazio, Italy).
It should be borne in mind, however, that most arsenic in groundwater and soils is naturally present because of the volcanic origin of the territory.
The need for in-depth and continuous monitoring to ascertain the quality of the products become increasingly apparent. Particular attention must be paid to good practices, from harvest to consumption, especially for products imported from countries where laws regarding food safety are less stringent than in Italy.
In conclusion, it is necessary to support the certifications, the product specifications
and the efforts made by national and international authorities, with the identification of "marker of quality" able to describe univocally and objectively a food product through instrumental and chemometric analyses.
Moreover, it is absolutely necessary to carry out information campaigns to educate the consumers, so that they can make more informed choices and not be victims of fraud. Another important aspect is to take into account the needs of consumers (also with reference to new food trends), innovating but, at the same time, ensuring the only essential feature of food, that is, food safety.

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9. Appendices

Appendix 1 - QUESTIONNAIRE

I PART – DEMOGRAPHIC SECTION

1.	**Gender**	❑ M	❑ F			
2.	**Educational Level**	❑ Middle School	❑ High School	❑ Degree		
3.	**Age**	❑ 18-29	❑ 30-39	❑ 40-49	❑ 0-59	❑ +60
4.	**Family income(€)**	❑ < 25.000	❑ < 50.000	❑ < 75.000	❑ +75.000	

II PART – OLIVE OIL CONSUMPTION

5.	**Consumption Frequency**	❑ Once a year	❑ 3 or 4 times a month	❑ 2 or 3 times a week	❑ Daily
6.	**Purchase place**	❑ Supermarket	❑ Specialised Store	❑ Local Producer	❑ Own Production
7.	**Reason to buy**	❑ Healthy	❑ Tasty	❑ Good Seasoning	❑ Naturalness
8.	**Most Important Factor**	❑ Packaging ❑ Origin	❑ Experience ❑ DOP/EU organic certification	❑ Label	❑ Price
9.	**Less Important Factor**	❑ Packaging ❑ Origin	❑ Experience ❑ DOP/EU organic certification	❑ Label	❑ Price
10.	**Is the origin of the olives an important factor when buying olive oil?**			❑ Yes	❑ No
11.	**You usually buy olive oil, whose origin is…**	❑ Regional	❑ National	❑ European	❑ Extra-EU
12.	**Generally, are you interested in PDO and / or Organic products?**			❑ Yes	❑ No

13. Would you recognize the Organic production logo of the European Union among them?

❑ ❑ ❑ ❑ ❑ ❑

14. Would you recognize the PDO European logo?

❑ ❑ ❑ ❑ ❑

15. Have you ever bought Olive oil with EU Organic certification?		❑ Yes	❑ No
16. If so, how often?	❑ Once/twice a year	❑ Once/Twice a month	❑ Weekly
17. Have you ever bought Olive oil with PDO European		❑ Yes	❑ No
18. If so, how often?	❑ Once/twice a year	❑ Once/Twice a month	❑ Weekly

19. Which attributes come to mind when you think of Organic food products? Please, provide three answers.

1. **2.** **3.**

20. Which attributes come to mind when you think of PDO food products? Please, provide three answers.

1. **2.** **3.**

21. How much are you willing to pay more for Organic olive oil?	❑ Up to 10%	❑ Up to 20%	❑ Up to 50%	❑ More than 50%

22. Do you consider positive or negative the following attributes of olive oil?	***Fruity***	❑ Positive	❑ Negative
	Bitter	❑ Positive	❑ Negative
	Pungent	❑ Positive	❑ Negative

23. If you had to choose between two olive oils: one characterized by pronounced hints of fruity, bitter and spicy (A) and one in which such attributes are barely perceptible(B), which would you buy?	❑ A	❑ B

24. ***Finally, which one of the following olive oils would you buy?***

Sample	*Price* (€)	*EU Organic Certification*	*PDO Certification*	*Origin*
1.	12,00	✓	✗	Italian
2.	12,00	✗	✓	Local
3.	9,50	✗	✓	Italian
4.	9,50	✓	✗	Local
5.	8,00	✗	✗	Local
6.	8,00	✗	✗	Italian

III PART – *a) Panel Test (Blind Test)*

Parameters	*Sample A*	*Sample B*	*Sample C*
How **fruit** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
How **bitter** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
How **pungent** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
Would you buy it? (Yes/No)	❑ **Yes** ❑ **No**	❑ **Yes** ❑ **No**	❑ **Yes** ❑ **No**
Willingness to pay (WTP) for this sample Select one of the following options:	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€
Which sample did you prefer? Order numerically the samples according to your preferences (**1**,**2**,**3**: "1" the most appreciated, "3" the less appreciated).	❑	❑	❑

III PART – b) Panel Test (Normal test)

Parameters	***EVOO***	***PDO***	***EU Organic***
How **fruit** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
How **bitter** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
How **pungent** is this olive oil sample? Express your opinion, indicating a number between "1" and "5". **1-Very low 2-Low 3-Medium 4-High 5-Very High**	❑	❑	❑
Would you buy it? (Yes/No)	❑ **Yes** ❑ **No**	❑ **Yes** ❑ **No**	❑ **Yes** ❑ **No**
Willingness to pay (WTP) for this sample Select one of the following options:	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€	❑ <3 € ❑ 3 - 5 € ❑ 6 - 8 € ❑ 9 - 11€ ❑ >12€
Which sample did you prefer? Order numerically the samples according to your preferences (**1**,**2**,**3**: "1" the most appreciated, "3" the less appreciated).	❑	❑	❑