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CHEMICAL TOOLS FOR FOOD QUALITY ASSESSMENT

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Abstract

There is nowadays more awareness on the impact on health of pollutants emitted even during cooking both from commercial as well as from domestic activities. In this study, it has been set up a new system allowing to analyse by solid-phase microextraction and gas chromatography coupled to mass spectrometry (SPME-GC-MS) the volatile organic compounds (VOCs) emitted during cooking. This could be done by aspiring into a polyethylene terephthalate (PET, Nalophan) bag the air over a cooking process. The bag allows to transport the sample to the instrument location and to perform the SPME extraction of the sampled air. The efficiency of different systems to perform the SPME extraction from the air contained in the bag was assessed by using a standard mixture of alkanes in order to obtain a sufficient sensitivity. Then the defined system was used to extract and analyse VOCs in air obtained during frying fries in sunflower oil. Several SPME extraction times (1h, 3h, 5h, 7h and 24h) were evaluated bringing to results that can be useful both with short extraction times and with long extraction times. Then the evaluation of three different filters was performed. Thus, the developed system, combining the use of olfactometric bags and the SPME-GC-MS, is applied for the first time to the study VOCs emitted during cooking and it allows to perform the analysis, even on samples produced in sites far from the instrument location, in an easy way and with instrumentations available in most of the laboratories. The results show a different retention effect for each filter under investigation on the classes of molecules detected. In particular, it has been found that, one of the three examined filters gives better filtering performance than the other two, confirmed also by the statistical analysis.

Chapter 1.

A new system to analyze volatile organic compounds (VOCs) produced during cooking

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1.1 Introduction

1.1.1 Indoor air quality (IAQ)

Indoor air quality (IAQ) is a common term generally used to describe the air quality within a building environment. IAQ theme has become an important current issue for the community due to the increased amount of personal time spent in indoor environment. Nowadays in fact, people spend approximately 90% of their time in an indoor environment such as home, office, school, car, shopping center and public buildings. In a recent study, it was seen that in the USA for example, adults spend around 21 h/day indoors while children spend, on average, 17-19 h/day indoors; consequently, the attention to safety has grown [1]. A large number of studies have shown that the level of pollutants in indoor environment is higher than that in outdoor environment [2]. For many years, the damaging effects of contaminated air on human health have been known. Environmental studies have initially focused on outdoor air and the potential problems of elevated levels of particulate and gas contaminants on health. However, over the last 30 years, there have been significant developments in this field: in fact, the evident impacts of the IAQ on people health makes important to find new approaches in order to reduce adverse health consequences, to improve quality of life and the work environment with consequential human benefits [3]. The main air pollutants contributing to decrease IAQ are carbon dioxide (CO_2), carbon monoxide (CO), formaldehyde (HCOH), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), volatile organic compounds (VOCs) and particulate matter ($PM_{2.5}$ and PM_{10}) [2]-[4]. In particular, $PM_{2.5}$ and PM_{10} (fraction of particulate matter with aerodynamic diameter range smaller than 2.5 μm and 10 μm, respectively) are usually chosen as indicators of air pollution rather than total suspended particulate matter (TSPM), since such particles are small enough to enter the thoracic region [5]. In addition to this, air temperature, velocity and humidity levels are further IAQ factors associated with thermal comfort zone. The accumulation of these contaminants, in combination with other factors, likes low ventilation and moisture in building materials, leads to “sick building syndrome” (SBS), which cause sensory irritation or invoke effects on the central and peripheral systems, and to other reported diseases in affected spaces as respiratory problems (children are particularly affected), immune - allergy to dust mites, skin and mucous membranes diseases (especially to formaldehyde), cardiovascular problems, liver, kidney and gastro-intestinal diseases and cancer (connected to tobacco smoke and radon) [3]. Therefore, many countries’ national organizations and worldwide influential committees, as an example World Health Organization (WHO), have stipulated standard and guideline values that encourage improving IAQ management. In general, IAQ standard guidelines establish values

which are recommended during the development of a facility. An IAQ target value is defined as value that one should seek to achieve by a set date, while an IAQ limit value is a legally binding value in legislation that must not be exceeded to avoid risk of penalty or punishment. Considering these different values' definitions, to understand the application of IAQ guidelines and standard values to minimize health effects and reducing the probability of SBS, has become of considerable importance. International agencies that establish air quality guidelines and standards are listed in Table 1. Some of these are the American Society of Heating, and Refrigerating and Air-Conditioning Engineers (ASHRAE), the National Health and Research Council of Australia (NHMRC), the American Conference of Governmental Industrial Hygienists (ACGIH), Health Canada and the Deutsche Forschungs Gemeinschaft (DFG/MAK) Commission [4].

Country	Organization	Acronym of Organization (Reference)
Australia	The National Health and Medical Research Council https://www.nhmrc.gov.au/	NHMRC (NHMRC, 1996a, 1996b; TEC Green Office, 1997)
Australia	The National Occupational Health and Safety Commission http://det.wa.edu.au/policies/detcms/policy-planning-and-accountability/policies-framework/web-references/national-occupational-health-and-safety-commission-nohsc-procedures.en#main-content	NOHSC (NOHSC, 1995)
Belgium	Air Infiltration and Ventilation Centre http://www.aivc.org/	AIVC (2003)
Canada	Health Canada www.hc-sc.gc.ca	Health Canada (Federal–Provincial Advisory Committee, 1989; Schuh, 2000; NRC, 2005; Health Canada, 2006; Light House Sustainable Building Centre, 2007; Phipps, 2007; Dales et al., 2008; Government of Alberta, 2009; Salthammer et al., 2010)
China	Administration of Quality Supervision, Inspection and Quarantine http://english.aqsiq.gov.cn/ http://english.gov.cn	AQSIQ (AQSIQ, 2002)
China	State Environment Protection Agency http://english.mep.gov.cn/ http://www.sepa.gov.cn/	SEPA (You, 2013)
Hong Kong	Hong Kong Environmental Protection Department http://www.iaq.gov.hk/index_eng.asp	HKEPD (Environmental Policy Working Group, 1999; HKEPD, 1999; Lee et al., 2002; You, 2013)
Hong Kong	Hong Kong Indoor Air Quality Objectives http://allie.dbcls.jp/short/exact/Any/HKIAQO.html	HKIAQO (Lee and Chang, 2000; Li et al., 2001; Lee et al., 2002; HKSAR, 2003)
Hong Kong	Government of the Hong Kong Special Administrative Region http://www.epd.gov.hk/epd/english/top.html	HKSAR (HKSAR, 2003a; Charles et al., 2005; Muhamad–Darus et al., 2011)
Denmark	Danish Society of Indoor Climate http://www.dsic.org/dsic.htm	DSIC (Eweda, 2012)
Europe	European Commission * http://ec.europa.eu/index_en.htm	EC (2013)
Finland	Finnish Society of Indoor Air Quality and Climate http://www.sisailmayhdistys.fi/portal/fisiaq_in_english/	FiSIAQ (Environmental Policy Working Group, 1999; FiSIAQ, 2001; HKSAR, 2003; Air Duct Cleaners, 2013)
Germany	Deutsche Forschungs Gemeinschaft http://www.dfg.de/en/	DFG/MAK (Federal Republic of Germany, 2000; ANSI/ASHRAE, 2004; Charles et al., 2005; Light House Sustainable Building Centre, 2007; Hedrick, 2010 Eweda, 2012)
Japan	Ministry of Health, Labor and Welfare http://www.mhlw.go.jp/english/	MHLW (Charles et al., 2005)
Kuwait	Kuwait Environmental Protection Agency or Kuwait Environment Public Authority, http://epa.org.kw/	Kuwait EPA (Tang and Al–Ajmi, 2006)

Country	Organization	Acronym of Organization (Reference)
Korea	Korea Environmental Industry and Technology Institute http://www.keiti.re.kr/en/index.do	KEITI (Jeong, 2008; Colbeck, 2012)
Malaysia	Department of Occupational Safety and Health http://www.dosh.gov.my/index.php?lang=en	DOSH (DOSH, 2010; Muhamad–Darus et al., 2011)
Singapore	Singapore Indoor Air Quality Guideline http://www.nea.gov.sg	SIAQG (Ministry of Environment, 1995)
Singapore	Institute of Environmental Epidemiology, Ministry of the Environment http://www.zaobao.com.sg/english	Institute of Environmental Epidemiology (Institute of Environmental Epidemiology, 1996; Muhamad–Darus et al., 2011)
UK	Health and Safety Commission http://www.hse.gov.uk/	HSC (HSC, 2002)
US	American Conference of Governmental Industrial Hygienists http://www.acgih.org/	ACGIH (ACGIH, 1995; Environmental Policy Working Group, 1999; Schuh, 2000; ANSI/ASHRAE, 2004; ACGIH, 2005; Charles et al., 2005; Light House Sustainable Building Centre, 2007; Hedrick, 2010; Eweda, 2012; Air Duct Cleaners, 2013; TSI, 2013)
US	American Society of Heating, Refrigerating and Air–Conditioning Engineers, https://www.ashrae.org/	ASHRAE (Environmental Policy Working Group, 1999; Schuh, 2000; ANSI/ASHRAE, 2004; Light House Sustainable Building Centre, 2007; Hedrick, 2010; Sebesta, 2011; Eweda, 2012; Air Duct Cleaners, 2013; TSI, 2013)
US	Illinois Department of Public Health http://www.idph.state.il.us/	IDPH (Sebesta, 2011)
US	Occupational Safety and Health Administration https://www.osha.gov/	OSHA (Schuh, 2000; ANSI/ASHRAE, 2004; Charles et al., 2005; OSHA, 2006; Light House Sustainable Building Centre, 2007; Hedrick, 2010; Sebesta, 2011; Eweda, 2012; Air Duct Cleaners, 2013; TSI, 2013)
US	Office of Environmental Health Hazard Assessment (California EPA) http://oehha.ca.gov/	OEHHA (Schuh, 2000; AIVC, 2003; Salthammer, 2011)
US	Texas Department of Health	TDH (2003)
US	The National Ambient Air Quality Standards http://www.epa.gov/air/criteria.html	NAAQS (U.S. EPA, 1996; ANSI/ASHRAE, 2004; Charles et al., 2005; Light House Sustainable Building Centre, 2007; Hedrick, 2010; Eweda, 2012)
US	The National Institute for Occupational Safety and Health http://www.cdc.gov/niosh/	NIOSH (Environmental Policy Working Group, 1999; Schuh, 2000; Light House Sustainable Building Centre, 2007; NIOSH, 2004; Charles et al., 2005; Hedrick, 2010; Eweda, 2012; Air Duct Cleaners, 2013; TSI, 2013)
US	US Environmental Protection Agency http://www.epa.gov/	U.S. EPA (U.S. EPA, 1996; Environmental Policy Working Group, 1999; Schuh, 2000; ISIAQ–CIB, 2004; Charles et al., 2005; Light House Sustainable Building Centre, 2007; Hedrick, 2010; Berenguer, 2011; Salthammer, 2011; Eweda, 2012; U.S. EPA, 2012; Air Duct Cleaners, 2013; TSI, 2013)
Worldwide	World Health Organization http://www.who.int/en/	WHO (Environmental Policy Working Group, 1999; Schuh, 2000; WHO, 2000; AIVC, 2003; HKSAR, 2003; WHO, 2003; ISIAQ–CIB, 2004; Charles et al., 2005; Light House Sustainable Building Centre, 2007; Bluyssen, 2010; Hedrick, 2010; Berenguer, 2011; Salthammer, 2011; Eweda, 2012; Air Duct Cleaners, 2013; TSI, 2013)

Table 1. International bodies involved in setting air quality guidelines and standards [4]

[a] It consists of 28 member states, including the UK, Italy, Spain, Portugal and Germany as well as other international bodies such as WHO.

1.1.2 Hazardous and odorous pollutant emission during cooking activities

As a consequence of healthcare science progress, human life expectancy has increased gradually over the years; many risks that previously threatened human life, with the advances of civilization and urbanization have been reduced or eliminated. Nevertheless, other kinds of risk are arising in our normal everyday life; in fact not many people are aware of the risks associated with cooking activities. The impact on human health of pollutants emissions from domestic and commercial cooking activity are often overlooked throughout the world; all populations have become exposed to this cooking-related risk, regardless of their race, age, wealth, cultural food preferences, etc. [6]. Humans can be subject to cooking-related risks via various intake routes either directly (overcooked foodstuffs) or indirectly (fumes). The cooking processes like frying, roasting, grilling, steaming, barbecuing, smoking, microwaving, boiling and broiling, contribute to emissions of pollutants, particularly in closed, poorly ventilated areas where air quality is deeply influenced on their presence. Furthermore, considering that the most of fire-based cooking cannot be carried out without fuels, the effect of fuel combustion can add to the risks of cooking activities: cooking fuels are one of the most important causes of indoor air pollutants (IAPs), especially in developing countries [6]. Pollutant emissions from food mainly result from heating and cooking operation through which organic materials in the food are volatilized. The nature and quantities of pollutants emitted from those sources would highly depend on the cooking stuff, cooking styles, and even on cooking fuel [6]-[7]. However, food cooking not only is a known source of IAP, but also of odor emissions. Odor nuisance is generally defined by the four factors: frequency, intensity, duration, and offensiveness. These key properties can be defined briefly as follows: frequency refers to the number of times an odor occurs, intensity refers to the strength of an odor, duration refers to the period of time an odor is encountered, and offensiveness refers to the character or hedonic tone of the odor (pleasant or unpleasant) [7]. Different kinds of pollutants such as reduced sulfur compound (RSC), aldehydes, organic acids, ketones and polycyclic aromatic hydrocarbons (PAHs) are common components found in cooking oil fumes (COFs) [8]. These volatile organic compounds (VOCs) are the principle known IAP. The WHO classifies VOCs as indoor organic pollutants with a boiling point range between 50/100°C and 240/260°C [1]. Many VOCs are known to be toxic and are considered carcinogenic, mutagenic or teratogenic. In fact, as known in the literature [8], PAHs and hydrocarbons (HCs) are carcinogenic for people and may produce respiratory symptoms or local irritation in the airways. Studies regarding the activities of cooking in China and Taiwan have confirmed the development of these diseases due to exposure to the COFs, which has potential adverse effects on human health, because of the presence of compounds such as PAHs,

heterocyclic amines and unsaturated aldehydes [9]-[10]. Umano et al. [11], focused their attention on the acrolein effects. Acrolein is an *α*,*β*-unsaturated aldehyde, which is formed from the dehydration of glycerol when animal and/or vegetable fats are heated to high temperatures, and it is a liver toxic substance and an irritant for the gastric mucosa. In addition to this aspect, other studies, considered the odorant behavior of these molecules. Kabir et al. [7], studied the emissions from the roasted coffee seeds of six sample types, indicating that RSCs like hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), aldehydes (such as acetaldehyde, propionaldehyde, butyraldehyde and isovaleraldehyde), organic acids (like propionic acid, butyric acid, isovaleric acid and valeric acid) and other VOCs such as toluene, styrene, *p*-xylene, methyl ethyl ketone (MEK), methyl isobutyl ketone (MIBK) are the principle odorants. On the contrary Blanda et al. [12], demonstrated that the generation of off-odours and off-flavours in boiled potatoes (POF) are strongly correlated with the presence of 2-pentanal, 2-hexanal, 2-heptanal, 2-pentylfuran and 2-decenal. As regards fishy odor, it is known that 1-penten-3-one, 1-octen-3-one, (*Z*)-4-heptenal, (*E*,*Z*)-2,6-nonadienal, (*E*,*Z*)-2,4-heptadienal and (*E*,*Z*,*Z*)-2,4,7-decatrienal are molecules responsible for the bad odors [13]. Therefore, for all these reasons, the monitoring of these substances both in private environments (as for example in the domestic kitchen environment) and in public environments (such as restaurants, fast-food, etc.), is particularly important in order to safeguard the public and private health.

1.1.3 The principles of air flow, air pressure and air filtration

Comfort air conditioning is described as "*the processes of treating air to simultaneously control its temperature, humidity, cleanliness and distribution to meet the comfort requirements of the occupants of the conditioned space*" [14]. Air conditioning used in other fields than comfort is classified as "industrial air conditioning". Anyway, the four requirements of temperature, humidity, cleanliness and distribution control are applied equally to industrial conditioning. The air we breathe is a mixture of gases, composed of 21% oxygen (O_2), 78% nitrogen (N_2), 1% argon (Ar) and carbon dioxide (CO_2), and traces of other gases (Figure 1).

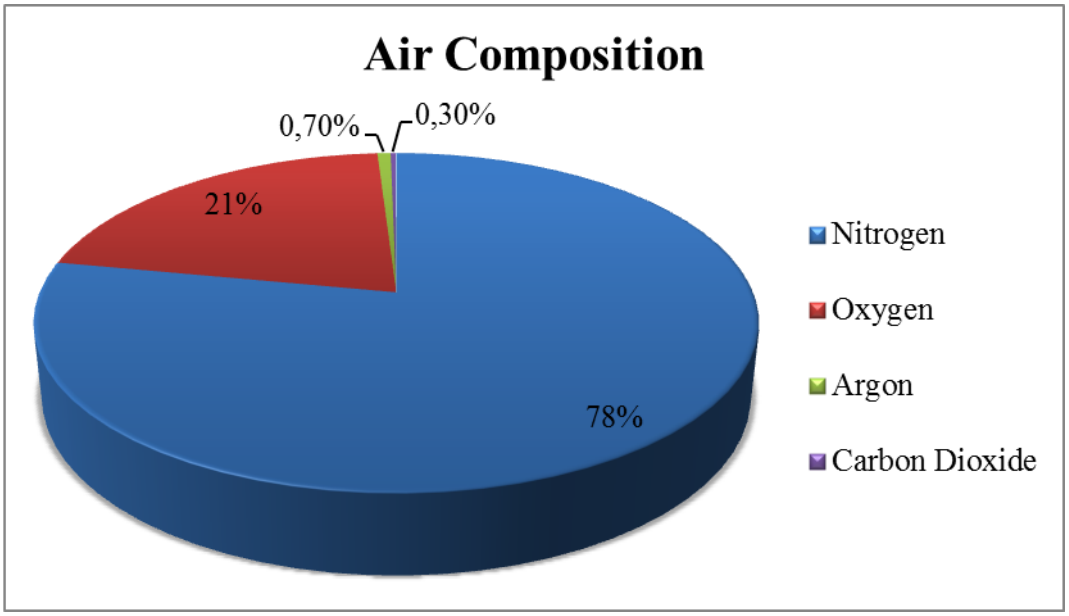


Figure 1. Air composition

Air we breathe also includes VOCs, particulate material and gases generated by nature, by man, industrial processes, building materials and finishes, building contents such as furniture and fixtures, cooking odors, that influence our health or comfort, that damage the spaces we occupy, or that affect the products or components we are manufacturing. Therefore, air filtration becomes particularly important, because it supplies the means to obtain the level of particulate and molecular cleanliness required by any definition of air conditioning. It ranges from the simple task of preventing larger particles from plugging heating/cooling coils, to removing particles which can become a respiratory irritant or hazard, or molecular contaminants and particles as small as 0.1 µm and smaller which could cause a short circuit on a microchip [14]. For this reason, facility managers should always look to air filtration and cleaning as the best way to protect the health and safety of the occupants in a facility by removing contaminants from the

air. The flow of air between two points is due to the occurrence of a pressure difference between the two points. This pressure difference results in a force placed on the air, usually by fan, causing the air to flow from the area of higher pressure to the area of lower pressure. The quantity of air, usually referred to in cubic feet per minute (CFM) is represented by the symbol Q. The speed of flow or velocity of the air, usually referred to in feet per minute (FPM), is represented by the symbol V. The size of the conduit through which the air flows, usually ductwork, is referred to as area expressed in square feet and is represented by the symbol A. The air flow through a conduit, ductwork or a filter is expressed by the formula: $Q = V \cdot A$ [14]. As air travels through a conduit, it creates a pressure called velocity pressure (VP). There is a relationship between velocity of the air and the velocity pressure based upon the density of the air. This relationship is expressed by the formula: $V = 4005 \sqrt{VP}$, where V is the velocity in FPM, 4005 is the standard density of air derived from gravitational acceleration (32.2 ft/sec^2 and air density of 0.075 pounds per cubic foot) and VP is the velocity pressure in inches of water. VP is measured in the direction of flow through a conduit and is always positive. Air confined in a conduit whether in motion or not, creates another type of pressure which exerts itself in all directions at the same time. Sometimes referred to as "bursting pressure", this pressure is called static pressure (SP). SP is independent of the velocity of the air and can either be positive or negative depending on where it is measured in the conduit. This pressure can be measured using a device known as a Pitot tube. The figure below (Figure 2) shows the relationship of VP and SP, that is expressed by the formula: TP = SP + VP, where TP is the total pressure [14].

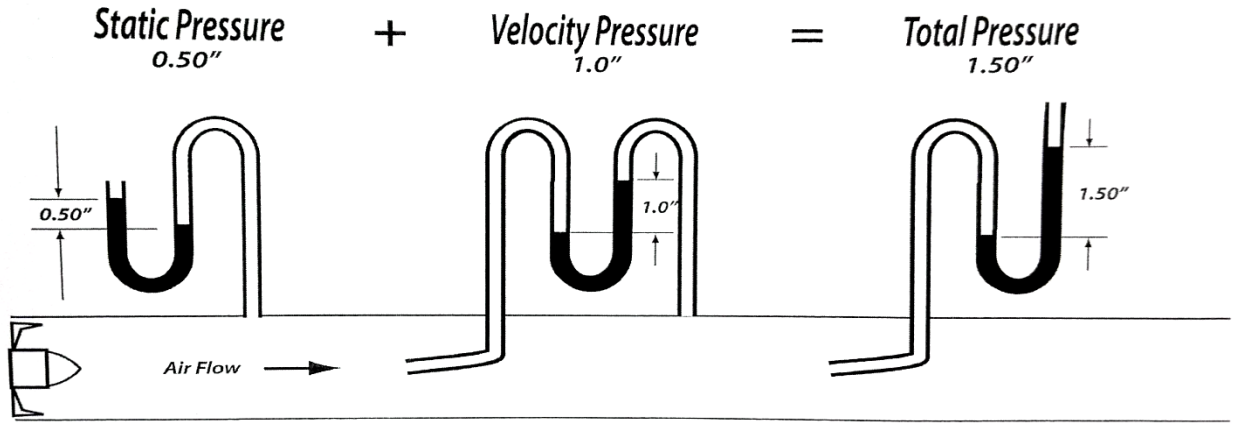


Figure 2.

Air filters are devices that remove aerosols from an air stream as the particulate contaminated air passes through them. There are three different types of air filtration categories: mechanical air filters, filters incorporating electrostatically charged filter media and electronic air cleaners.

1. **Mechanical air filters** capture particulate on the filter media, the material that comprises the filter elements. There are four different processes responsible for the capture of particulates in a mechanical filter:
    - Impingement is the mechanism by which large, high-density particles are captured. When air flows through a filter, it must bend or change direction many times to flow around the filter fibers. However, due to their inertia, larger particles resist to change in direction and attempt to continue on in their original directions. For this reason, they collide with, and adhere to the fibers (Figure 3).

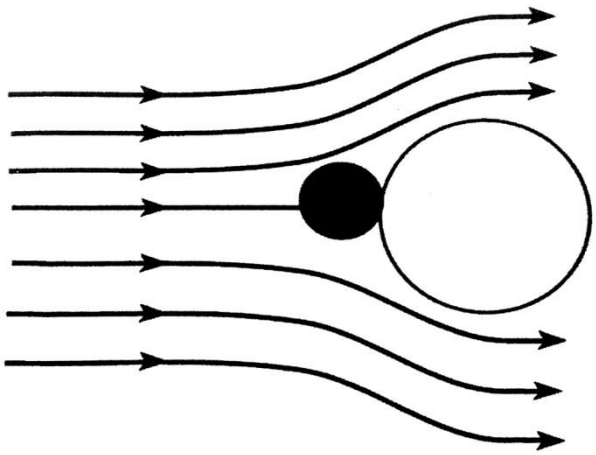


Figure 3. Impingement process

- Interception occurs when a particle follows the air stream, but still comes in contact with the fiber as it passes around it. If the attraction forces between the fiber and the particle are greater than the force of the airflow to dislodge it, the particle will stick to the fiber. The interception process is increased when the sizes of the fiber and the particle are similar (Figure 4).

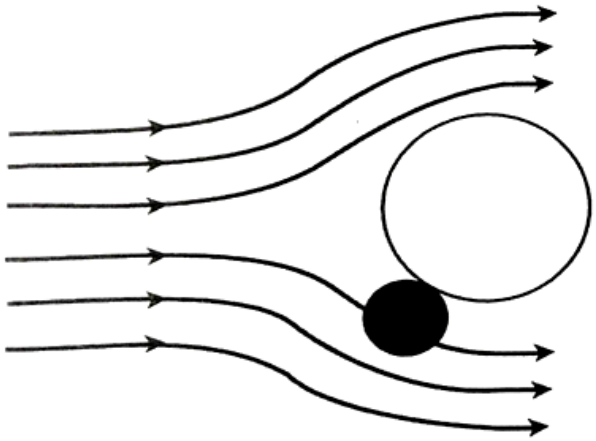


Figure 4. Interception process

- Diffusion is typical for the capture of very small particles at low air velocities. While the contaminated air passes through the filter media, the small particles will take an irregular path described as Brownian Motion. This path increases the probability that particles will come in contact with fibers and will stay attached to them (Figure 5).

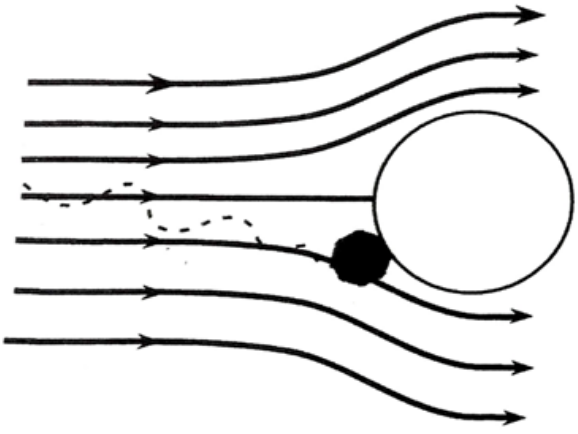


Figure 5. Diffusion effect

- Straining is the phenomenon that happens when the smallest dimension of a particle is greater than the distance between adjoining filter media fibers (Figure 6).

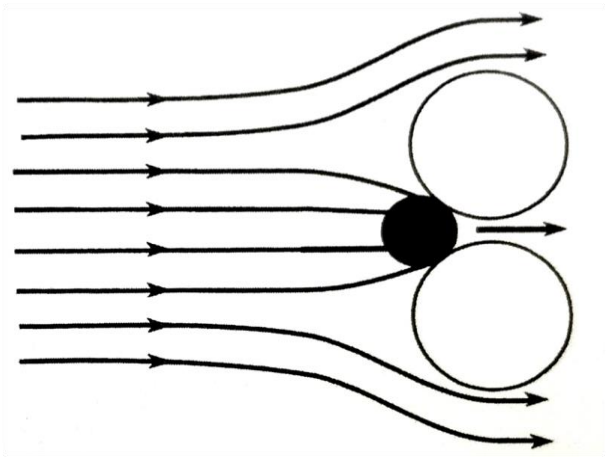


Figure 6. Straining process

2. **Electrostatically charged filter media (passive and active)** were used for several decades. Their advantage is that the charge on the fibers increases initial filtration efficiency without affecting resistance to airflow. In the case of electrostatic attraction active, the synthetic filter fibers can be actively charged during manufacture to be either positively and/or negatively charged. This technology can be classified by the method used to create the electrostatic charge in:
   - Triboelectric charging;
   - Corona charging;
   - Charging by induction.

   In the case of passive electrostatic attraction, the fiber media become electrostatically charged by the flow of air (especially dry air) through it. These filters are described as passive electrostatic filters. Most particles are charged naturally, and are held by strong electrostatic forces to the oppositely charged fiber with which they come in contact (Figure 7). The smaller a particle or a fiber is, the relatively stronger the electrostatic forces will be.

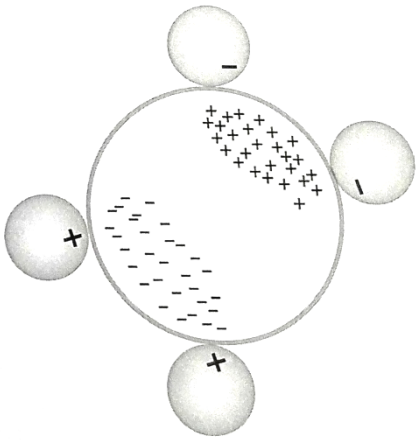


Figure 7. Particle attraction to charged fiber

3. **Electronic air cleaners (two-stage)** are externally powered devices. Air entering a two-stage air cleaner must first pass through a permanent screen or pre-filter, which catches the larger airborne particles. After passing through the pre-filter, the air enters the so called ionizer (ionizing section), or first stage, where the airborne particles receive an intense positive electrical charge. The positively charged airborne particles subsequently enter the collection (collecting sections), or second stage, which consists of a series of collector plates. These collector plates are metal plates or screens alternately charged with positive and negative high voltages. Because the airborne dust and dirt particles received a positive charge when they passed through the first stage of the electronic air cleaner, they are repelled by the positively charged plates in the second stage and propelled against the negatively charged collector plates where they adhere until washed away. The airborne particles are removed from the negative collector plates by periodic vacuuming or washing. Some electronic air cleaners are equipped with washing systems that flush the particles off the plates.

1.1.4 Cooking hoods

As discussed previously, VOCs are among the principle pollutants. The VOC sources can be distinguished in intermittent (Figure 8 and Figure 9) or continuous (Figure 10 and Figure 11) according to the time trends of the emission levels. Cooking food is a typical example of an intermittent and recurrent source, whose emissive profile is shown in Figure 8.

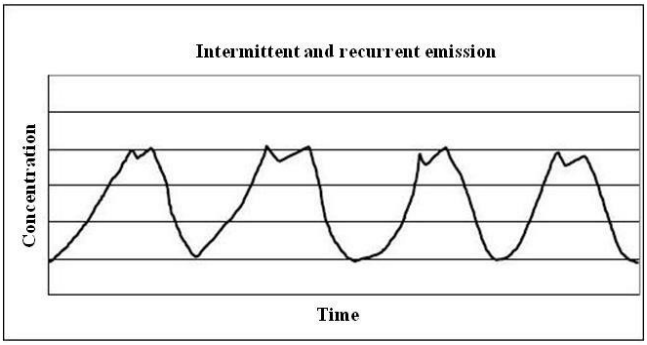


Figure 8. Intermittent and recurrent emission

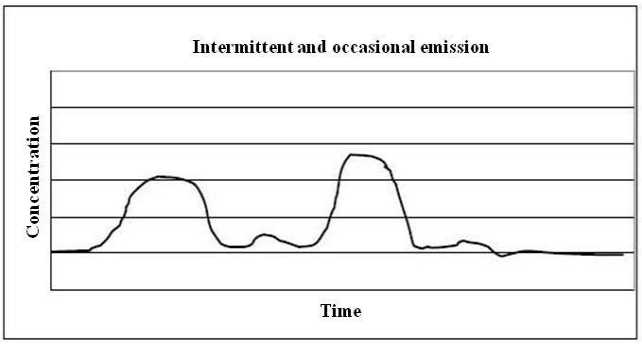


Figure 9. Intermittent and occasional emission

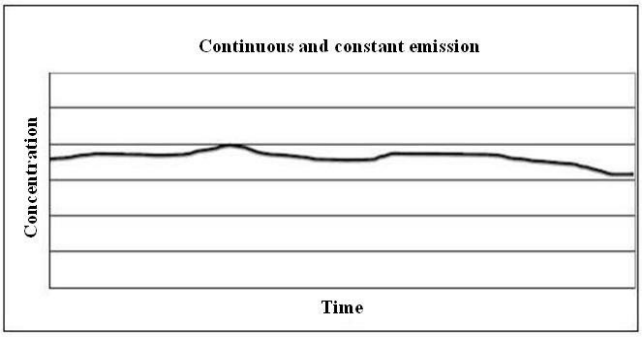


Figure 10. Continuous and constant emission

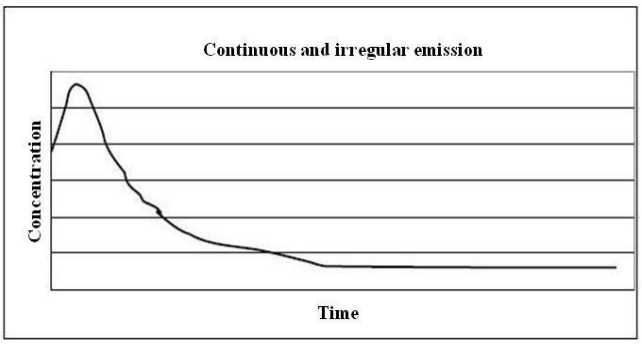


Figure 11. Continuous and irregular emission

Therefore, the cooking hood represents a local indoor air filtration device. The hood is one of the most widespread household appliances, both in Italy and abroad. Its main function is the evacuation of the vapors generated by the domestic activities carried out in the kitchen, or the treatment of the same for the reinsertion of the fluid in the domestic environment. Based on these primary requirements, the product differs in a suction hood and in a filter hood. In both cases, the appliance eliminates fats by mechanical filtration systems. In the **aspiration hood** version, the fluid is directly discharged into the external environment by means of suitable drain pipes that connect the engine to the environment (Figure 12). In the **filtering hood** version (Figure 13), the aspired air after filtration is further treated to eliminate odors with a technology based on activated carbon. The purified air is returned to the kitchen again.

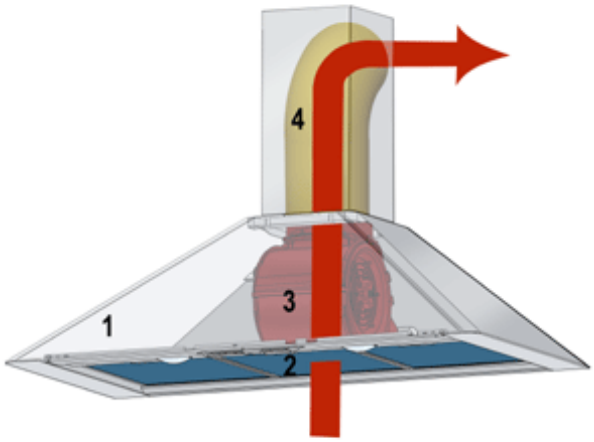


Figure 12. Aspiration hood

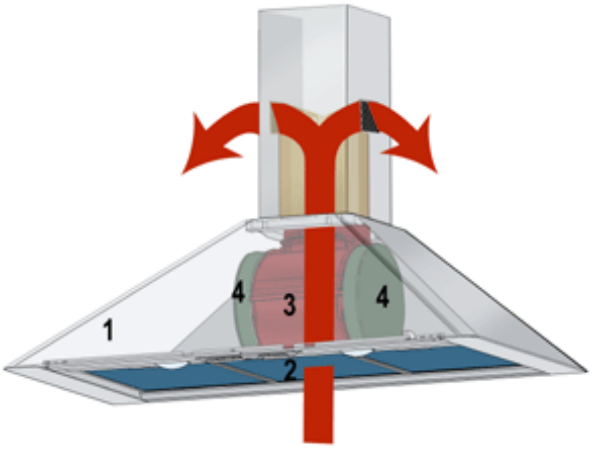


Figure 13. Filtering hood

Thus, the kitchen hood is designed to capture cooking food fumes, consisting of a mixture of vapors, odors and particles of oil and/or fat. To perform this function, the hood is equipped with two different filtering systems:

- **Mechanical filters** to prevent that the particles of oil and/or fat can contaminate the environment as well as damaging the hood itself;
- **Odor filters** that absorb odorous gases in order to avoid high concentrations of unpleasant odors during cooking foods.

1.1.5 Filters

The filtration systems are described by different kind of factors. The Standard International (SI) system of measurements, used in most countries of the world, has established these parameters. In the following table (Table 2), SI units are reported.

MEASUREMENT	SYMBOL	DESCRIPTION
Air volume flow rate [a]	m^3/s	Cubic meters per second
Filter pressure drop	Pa	Pascal
Face area	m^2	Square meters
Filter dimensions	mm	Millimeters
Particle size	µm	Micrometers [b]
Velocity	m/s	Mater/second
Temperature	°C	Degrees Celsius [c]

Table 2. SI units

[a] Air volume flow is also given in liters per second (L/s).

[b] "Micron" is not used in the SI system to give dimensions of very small dust particles. Instead the world "micrometer" is used.

[c] The temperature in Celsius degrees can be obtained by subtracting 273.15 from Kelvin degrees. At 0°C the thermodynamic temperature is 273.15°K.

The most common material used in heating, ventilating and air conditioning (HVAC) system to remove the airborne molecular contaminants (AMC) is activated carbon. A variety of materials such as coal, petroleum coke, wood, and coconut shells are used in its preparation. Each raw material imparts its own special characteristics to the activated carbon which, in turn, may make it the preferred product for a specific application. However, activated charcoal is composed mostly of carbon atoms of vegetable or mineral origin; it is characterized by an extremely high porosity and has a black color; it can be found in powder (PAC) (Figure 14) or in granules (GAC) (Figure 15). Although many different kinds of activated carbon are available, there are a number of different physical properties which can be measured and used for determination of the applicability of various carbons for different uses.

Figure 14. Powder activated charcoal (PAC)

Figure 15. Granules activated charcoal (GAC)

As discussed in the paragraph 1.1.4 the cooking hoods are provided of two different filtering systems: mechanical filter and odors filter. The grease filters of the hoods are generally metallic. The working principle of mechanical air filters is based on the capture of oil and/or fat particles present in the cooking food fumes, as it is possible to see in the figure below (Figure 16).

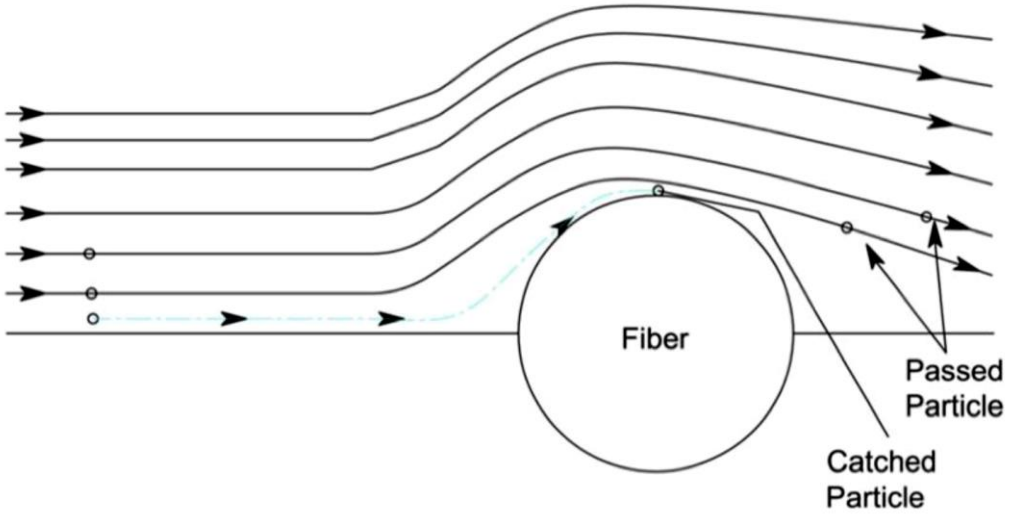


Figure 16. Particle capture with mechanical air filters

This capture involves two different considerations: the first is the probability that the particles collide or are removed from the filter medium; the second is the probability that the particles, once in contact with the filter, continue to adhere to it. The presence of mechanical filters introduces a pressure drop in the hood system, which results in an increase of energy consumption. Therefore, when mechanical air filters are chosen, it is necessary to find the right compromise between the following factors:

- Efficiency: the ability of a filter to remove the highest number of particles from an air stream;
- Pressure drop: the resistance that the filter opposed to the air flow. The higher the pressure drop, the greater the amount of energy required to overcome it;
- Capacity: the air flow rate that a filter can handle at a specific pressure drop.

The odors filter is the second filtration system involved in the cooker hood. The operation of odor filters, used in hoods for the removal of gaseous contaminants, is based on a reversible physical process known as adsorption. Adsorption is the process by which a substance is attracted and held on the surface of another and it is usually described in terms of energy per unit area of a solid. Surface energy is caused by molecules in the surface layer of the solid, which are

subjected to unbalanced external forces. When the surface energy exceeds the kinetic energy of the passing molecule, the molecule is adsorbed by the solid. The adsorption capacity of solids is a function of their total surface area. The solid adsorbents are porous materials (Figure 17); therefore the useful surface extends also inside the solid itself. Activated charcoal is one of the most common adsorbent materials, in which the total surface area can reach up to 1400 m^2/g.

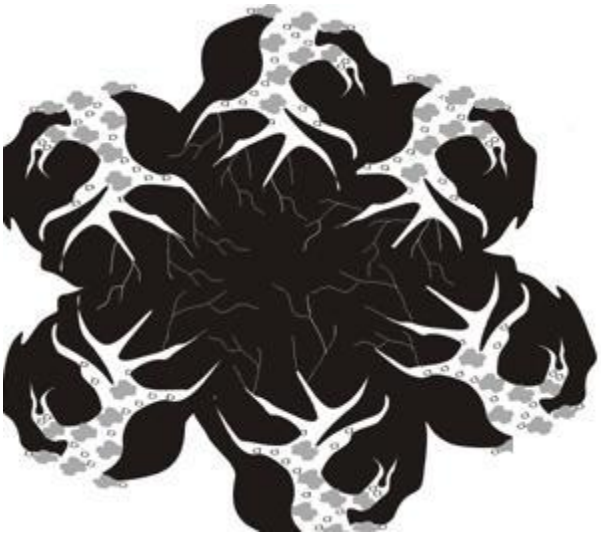


Figure 17. Porous structure of activated charcoal

The efficiency of odors filters is dependent of the following parameters:

- Efficiency of contact: the total contaminant percentage that is in contact with the adsorbent medium;
- Permanence time: the amount of time that the contaminated air takes to pass through the filter, without considering the resistance opposed by the filter medium;
- Adsorption efficiency: the probability that the gas molecules, coming in contact with the adsorbent particle, are retained by it;
- Removal capacity: depending on the total surface area available in the adsorbent medium.

1.1.6 Olfactometric bags

As discussed in the previous sections, VOCs are a large group of carbon-based chemicals that easily evaporate at room temperature, but while some VOCs are odorous, many of them can not be detected by our senses, even if their inhalation may be associated with different health risk levels. Thus, it become very important to find a new analytical method that allows to detect, determine and quantify these molecules, in order to assess and improve the indoor air quality. Various analytical approaches can be used. Solid phase micro-extraction (SPME) has been developed in 1989 by Janusz Pawliszyn, as a rapid, inexpensive and easy alternative to other techniques of extraction-preconcentration, such as liquid-liquid extraction, solid phase extraction, purge and trap technique. The low cost of the device, the possibility of automation and the simplicity of coupling with instrumental methods of analysis, are additional advantages of this sampling strategy. However, the extraction is not quantitative because amount and composition of analytes extracted with this procedure depend on the equilibrium distribution between the fiber coating and the sample. Anyway, the applicability of this technique both for qualitative and quantitative purposes is shown in many literature studies. Estevez et al. [15] for example, have analyzed volatile compounds generated in meat from Iberian and lean pigs, using the SPME technique, combined with gas chromatography and mass spectrometry (GC-MS). Blanda et al. [12] and Sanches-Silva et al. [16] have used this kind of analytical method to analyzed respectively volatile components that generated off-odors and off-flavors in boiled potatoes and during the storage. SPME coupled to GC-MS has been used also to analyze volatile aroma compounds present in freshly cooked turkey breast muscle [17]. Nevertheless, numerous studies related to VOCs determination involve the use of such device in combination with a previous sampling of the air in bags typically used for olfactometric analysis where odors are evaluated both qualitatively and quantitatively using the human nose as the detector [18]. These bags (Figure 18) are made up of different types of polymeric films. In the European Committee of Normalization (CEN) standard EN 13725 (EN 13725, 2003) three bag materials are referred to as qualified for olfactometric sampling bags: tetrafluoroethylene-hexafluoropropylene copolymer (FEP), polyvinylfluoride (PVF, Tedlar) and polyethyleneterephalate (PET, Nalophan). Polymer bags are popular and commonly accepted sampling containers in different kind of analysis due to their moderate price, inertness, relatively good durability and reusability.

Figure 18. Olfactometric bag

In the literature, different kind of studies, regarding the use and the application of olfactometric bags, are reported. As an example, SPME combined to sampling in olfactometric bags, and subsequent GC-MS analysis has been applied to analyze fragrances from live plants [19]. In order to evaluate gas purification installations at landfills and sewage treatment plants, the correct quantification of siloxane levels in biogas is essential [20]. Also in this case, olfactometric bags are by far the most widely used biogas sampling method in Germany and have recently been included in the Association of German Engineers (VDI)-guidelines for the measurement of landfill gas. The combination of sampling olfactometric bags and GC-MS analysis was evaluated to quantify the emission concentrations of aromatic VOCs and carbonyl compounds during the combustion of commonly used barbecue charcoal [21] or else to analyze particulate matter and trace metals emitted through charcoal combustion in cooking activities [22]. This sampling system has been also used in the analysis of VOC emissions from historic plastics and rubbers [23] by placing the objects in olfactometric bags, extracting and analyzing them using SPME-GC-MS. VOCs, and in particular volatile sulfur compounds (VSCs), are often the source of malodorous fumes at waste dumping sites and at biogas-production and sewage treatment plants [24]. These compounds are toxic and may cause health problems, even when present at low concentrations. In order to analyse sulfur compounds in air, also in this case, olfactometric bags and SPME coupled to GC-MS have been used. Furthermore olfactometric bags have been applied for sampling volatile sulphur compounds, relevant to breath analysis [25], to be subsequently extracted and analysed by SPME-GC-MS. E. M. Gaspar et al. [26], demonstrated the presence in expired breath of potential biomarkers for cancer detection,

especially lung cancer, which is a major cause of death among adults and its incidence is increasingly global. The breath from healthy volunteers, smokers and non-smokers, and lung cancer patients without treatment and under radio and/or chemotherapy, was collected using olfactometric bags and then analyzed by SPME followed by GC-MS. The use of breath analysis for monitoring human physiology and exposure to toxic substances or environmental pollutants has been recently reviewed [27]. The SPME-GC-MS combined to sampling in olfactometric bags has been exploited also for the analysis of acetone in breath as a diagnostic tool for diabetes [28] or the determination of isoprene in expired breath as a marker of body cholesterol synthesis [29].

1.1.7 Aim of the work

This research project rises from a collaboration between University of Camerino and Elica company of Fabriano (AN, Italy); the final purpose of this collaboration is to improve or design new aspiration filters for cooker hoods. Cooking processes contribute to emissions of pollutants, in particular in closed areas and poorly ventilated, where air quality is influenced on their presence. These substances, in particular VOCs, present in the environment, are accumulated in the human body and can cause important diseases to humans, as well as generate bad smells in environments such as kitchen. To address this problem and the discomfort due to bad cooking odors, generally aspiration system or filtering system are used, accompanied, when possible, by the prolonged opening of the windows, in order to favor the escape of odors and/or pollutants. This is the central point of this research project, aimed at the planning and production of a new and innovative filtering system that allows to remove selectively or mainly substances having bad odors and/or being toxic. In order to achieve the objective of research work, it is necessary to conduct a qualitative and quantitative study of the main VOCs found in the samples of air emitted during cooking. This is the first step and the principal point of this thesis work, connected to a detailed study of which substances are retained by the aspiration filters of the kitchen hoods, and the study of the air composition before and after the passage from the filter. SPME-GC-MS can be applied for the determination of these substances, if this system is combined with a previous air sampling in olfactometric bags. The bag allows to transport the sample to the instrument location and to perform the SPME extraction of the sampled air. However, despite several applications have been developed in different fields (see the paragraph 1.1.6), this kind of sampling system combined to SPME-GC-MS, has never been exploited to study the emissions of VOCs formed during cooking. Thus, given the importance of monitoring VOCs emissions during cooking activities, the objective of the present study was to assess the applicability of this system in such application. In order to do this, the efficiency of different systems to perform the SPME extraction from the air contained in the bag was assessed by using a standard mixture of alkanes from *n*-C5 to *n*-C18 in order to obtain a sufficient sensitivity. Then, the defined system was subsequently used to extract and analyse VOCs in air obtained from deep-fat frying. The evaluation of different SPME extraction times was performed in order to choose the most appropriate timing for our purpose. Then the evaluation of the effect of several types of filters (three different odors filters) on the composition of VOCs produces during frying of potatoes, determining their composition before passing on the hood aspiration filter and after passing on the hood aspiration filter, was performed. Quantitative (relative) and qualitative composition were evaluated for each examined filter. Especially, as regards

quantitative analysis, the absolute areas obtained before passing filter and the absolute areas obtained after passing filter were compared for each detected analyte; additionally, percentage of dejection, for the analyte passing through the filter, was calculated. However from a qualitative point of view, the percentage areas of each single category of molecules before and after passing through the filter were calculated and compared.

1.2 Materials and Methods

1.2.1 Reagents and standards

A standard mixture solution of alkanes (from *n*-C5 to *n*-C18) was purchased from Agilent Technologies (Milan, Italy).

1.2.2 Samples and sample preparation

The SPME fiber assembly was purchased from Supelco (Bellefonte, PA, USA) and had a 50/30 µm thickness divinyl-benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) coating with 2 cm length stationary phase. The experiments to evaluate the best SPME extraction conditions were performed by inserting a mixture of alkanes into a 8L, olfactometric PET bag (LOD s.r.l., Udine, Italy) and performing the SPME extraction in two different ways: in the first case the cap of the bag's side tube (both cap and tube are made up of PET) was substituted with a pirceable septum cap in order to insert the fiber needle and to expose the fiber inside the tube; in the second case the SPME fiber assembly was connected to a modified holder equipped with a holed steel fiber cap which can be used to protect the fiber coating during the extraction from a possible contact with the bag walls (see paragraph 1.3.1). This holder, as well as the protecting fiber cap, are made up of steel and were realized in our institution for this kind of applications. The comparison of the efficiencies of the different procedures was performed by inserting 1 µl of the solution of pure alkanes (from *n*-C5 to *n*-C18) inside the bag. Subsequently, a further modification for the extraction phase was made: that is, a vial with a pierceable septum was cut and replaced directly to the side tube of the bag. This change allowed to avoid a large loss of volatile substances following the opening of the bag, and at the same time, allowed the entire exposure of the SPME fiber to the VOCs, while remaining protected inside the bag. Once the best SPME extraction conditions were identified (SPME inserted inside the cut vial), air over a deep fat fries frying was sampled connecting the bag side tube to the hood. First of all, five different bags were transferred to the laboratory where the evaluation of different extraction times was performed. Then, the fiber holder with the fiber exposed inside the holed cap was inserted and samples were analyzed at 1h, 3h, 5h, 7h and at a longer fiber exposure time of 24 hours. Then, the bag was opened, the holed protecting cap quickly removed from the holder and the fiber exposed inside the GC injector for the analysis of extracted VOCs. Once the best SPME extraction time was identified (24 hours), air before passing on the hood aspiration filter and after passing on the hood aspiration filter, was sampled and analyzed. Three different types of

filters were tested. For each type of sample (air before filter and air after filter, for each filter) six replicates were analyzed. Moreover, blank analyses were carried out. Olfactometric bags, filled up with nitrogen, were analyzed at room temperature, exposing the fiber for 1h, 3h, 5h, 7h and 24 hours, to monitor the possible release of volatile substances. Anyway, due to the high sensitivity of the SPME fiber a thermal cleaning before its use is necessary to avoid the presence of interferences. Therefore, before the samples analysis, the fiber was inserted into the hot injector of the GC-MS at 260 °C for 15 minutes after which cleaning run is performed. From the chromatogram obtained, it is possible to observe the condition of the fiber. In this way we can evaluate if optimal conditions are reached to proceed with the samples analysis.

1.2.3 Hood aspiration filters

Three different types of filters were tested:

1. **Activated charcoal AC 90** (Figure 19) filter is an activated charcoal produced by extruding. It is of mineral origin and physically activated.

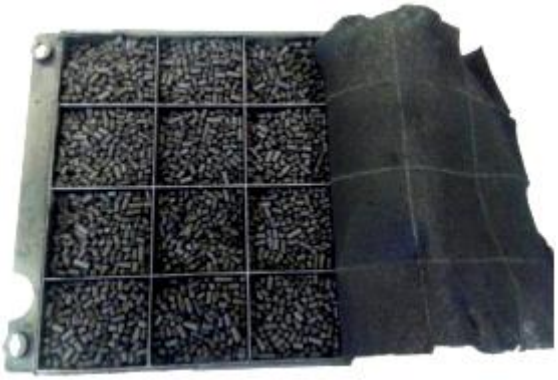


Figure 19. Activated charcoal AC 90

2. **Washable filter 1100-6 1400-11** (Figure 20) is an open cell polyurethane foam, coated with granular activated charcoal and inserted in black polyamide sock (Nylon).

Figure 20. Washable filter 1100-6 1400-11

3. **Helsa-Sorbexx-CS** (Figure 21) filter is a composite of activated charcoal reinforced by ceramic.

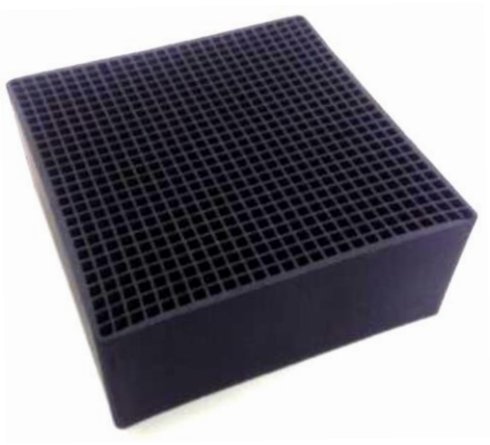


Figure 21. Helsa-Sorbexx-CS

1.2.4 Headspace-solid phase microextraction and gas chromatography-mass spectrometry analysis (SPME-GC-MS)

Volatile compounds were analysed by GC-MS using a 6890N Network GC System coupled to a 5973 Network Mass Selective Detector both from Agilent Technologies. For the initial attempt, the chromatographic column was a 5% phenylmethylsiloxane coated capillary column (30 m x 0.25 mm x 0.25 µm film thickness, HP-5MS, Agilent Technologies). The initial carrier gas (helium) flow rate was 1.2 ml min^{-1}. Injector temperature was 260 °C, splitless time was 4 min. Oven temperature was held at 35 °C for 5 min, then raised to 300 °C at 15 °C min^{-1} and held for 3 min, for a total run time of 25.67 min. Mass analysis was performed in scan mode in the range of 29-400 Da. Transfer line was maintained at 300 °C, ion source at 230 °C and quadrupole at 150 °C. Subsequently, a capillary column coated with polyethylene glycol (60 m x 0.25 mm x 0.25 µm film thickness, DB-WAX, Agilent Technologies) was used. The initial carrier gas (helium) flow rate was 1.2 ml min^{-1}. Injector temperature was 260 °C, splitless time was 4 min. Oven temperature was held at 35 °C for 4 min, then raised to 120 °C at 2.50 °C min^{-1} and then went up to 250 °C at 15 °C min^{-1} and held for 3,33 min, for a total run time of 50.00 min. Mass analysis was performed in scan mode in the range of 29-400 Da. Transfer line was maintained at 260 °C, ion source at 230 °C and quadrupole at 150 °C. Thermal desorption of volatiles compounds was carried out by exposing the SPME fiber in the injector for 10 min. Straight chain alkanes from *n*-C5 to C18 were used to calculate retention indices. Thus, the volatile compounds detected in the air sample, were identified by comparison of their retention indices and their

mass spectra with those of standard solution of alkanes, with reference spectra from the US National Institute of Standards and Technology (NIST) and with retention indices from literature (NIST, 2008). A blank test was performed before each analysis to prevent the release of undesirable compounds.

1.2.5 Statistical analysis of data

Significant differences among the different model systems were determined for each compound by one-way ANOVA statistical analysis. Differences with $P < 0.05$ were considered significant. Data elaboration was carried out using PAST software package [30].

1.3 Results and Discussion

1.3.1 Evaluation of different SPME extraction methods

The aim of the work was to set up a new system to determine the composition of the main volatile organic compounds (VOCs) which are formed during cooking. The study was specifically aimed at evaluating a suitable method allowing to carry out the GC analysis of VOCs in air samples produced during cooking even far from the analytical instrument location and to achieve a good sensitivity and reproducibility. Thus, it was decided to perform the air sampling in olfactometric bags which can be transported to the laboratory where to perform the extraction by SPME and the subsequent gas chromatographic analysis. In general, these bags can be filled with the air sample under investigation by means of a side tube which can be capped after the sampling. In the present study, the cap of the side tube has been initially substituted with a pierceable septum cap in order to allow the insertion of the SPME needle (Figure 22a). However it was found that the SPME extraction carried out by exposure in the side tube did not give satisfactory results in terms of sensitivity. In fact, air sampled over a hob during fries frying, analysed by SPME-GC-MS inserting the SPME needle in the side tube, did not allow to detect any substance, even extending extraction time to several hours. The problem can be attributed to the poor circulation of the VOCs inside the side tube (having relatively small internal diameter). Thus, it has been decided to the expose the fiber directly inside the bag (Figure 22b).

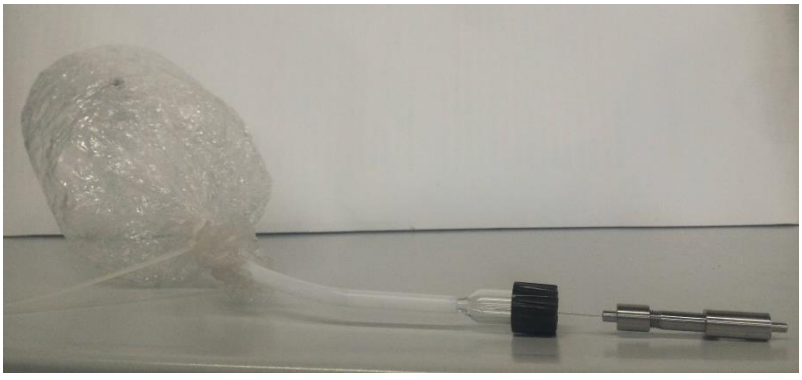


Figure 22a. System used to perform extraction with SPME fiber exposed in the side tube of the bag

Figure 22b. System used to perform extraction with SPME fiber exposed inside the bag

To do this, it has been used a modified SPME holder, realized in our institution, having a holed steel cap (Figure 23) protecting the fiber during the exposure and allowing to place the holder inside different types of container avoiding the risk of damaging the fiber by a possible contact with the container wall.

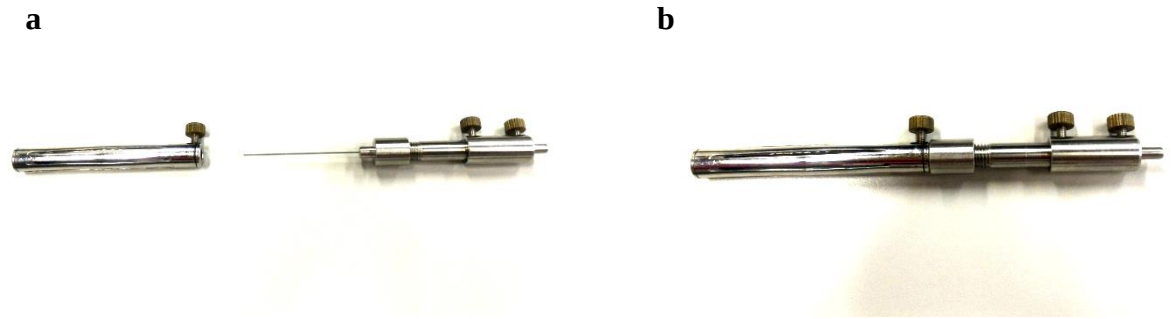


Figure 23. Protecting holed cap and SPME holder disassembled (**a**) and assembled (**b**)

Thus, by using this type of modified SPME holder assembly, the efficiency of the extraction performed from the side tube was compared with that from the extraction carried out inside the bag. A mixture of *n*-alkanes (from *n*-C5 to *n*-C18) was used to assess the different extraction efficiency. In Figure 24a and Figure 24b the chromatograms obtained from the two different extraction methods are reported. The experiments were conducted at a conditioning and extraction temperature of 20°C. The extraction carried out with the SPME inside the bag affords a significantly higher extent of extraction for most of the analytes (Figure 24a, Figure 24b and Figure 25). The differences in the extent of extraction carried out with the fiber exposed inside the bag or inside the side tube are particularly high for the heavier alkanes; from octane upward the differences are always statistically significant ($P < 0.05$).

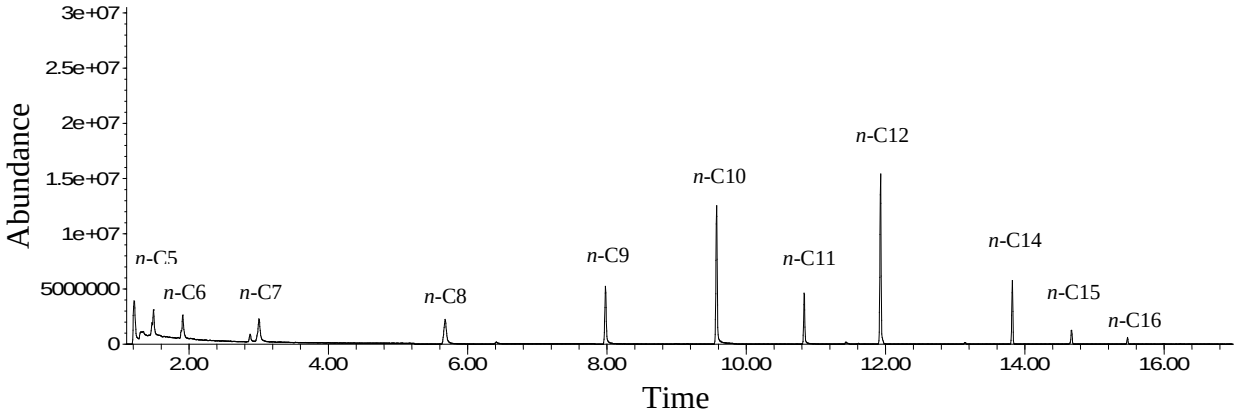


Figure 24a. Chromatograms obtained by extracting a mixture of linear alkanes (*n*-C5-*n*-C18) with SPME fiber exposed in the bag side tube

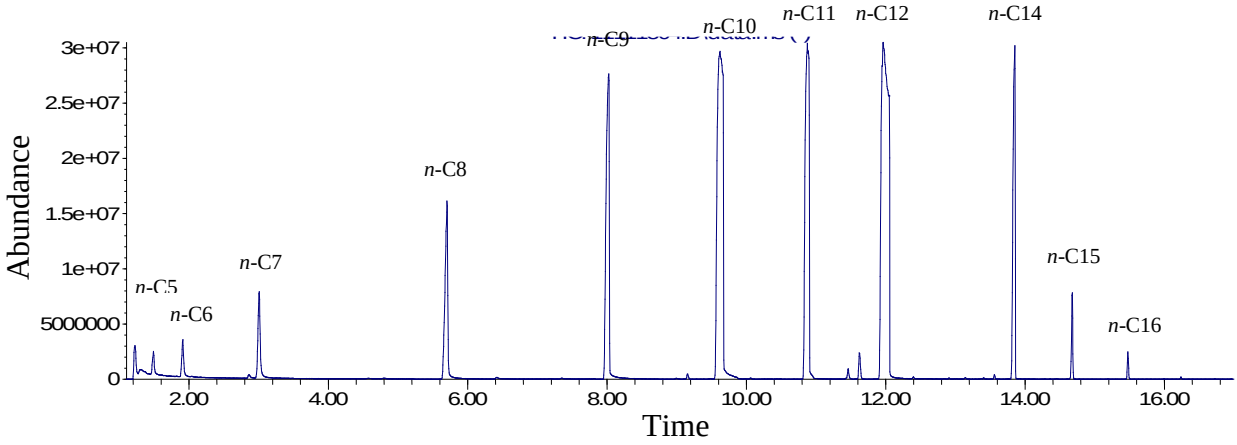


Figure 24b. Chromatograms obtained by extracting a mixture of linear alkanes (*n*-C5-*n*-C18) with SPME fiber exposed directly inside the bag

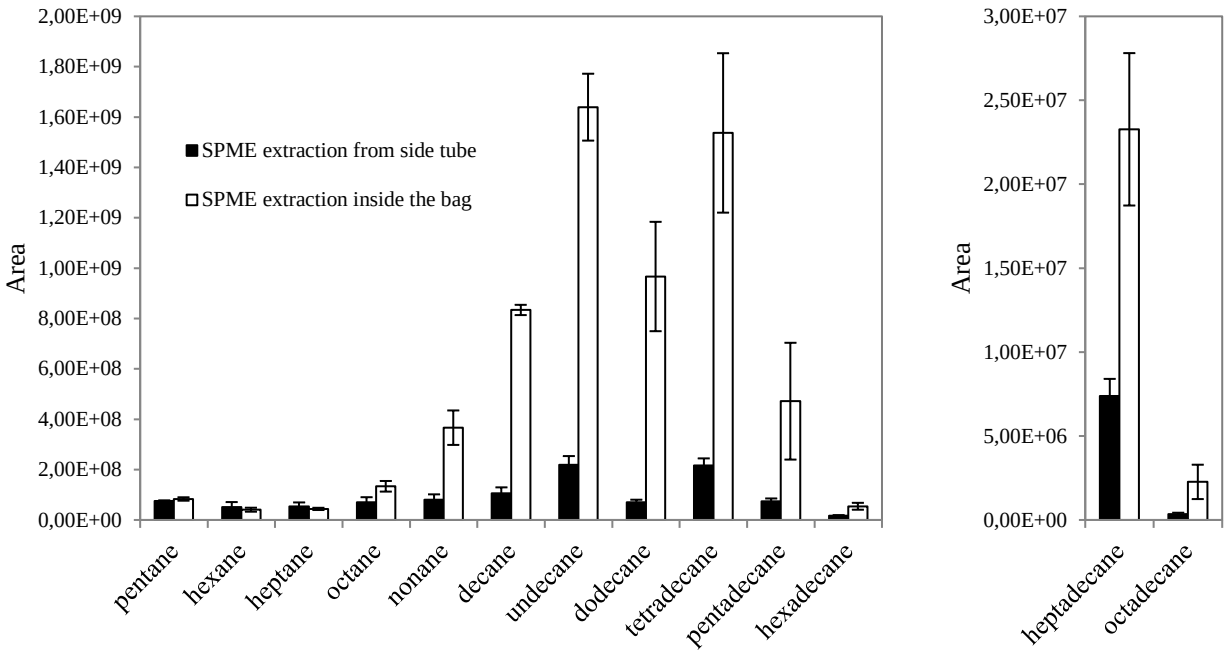


Figure 25. Comparison of the average peak areas (± standard deviation) of alkanes extracted with SPME fiber exposed in the bag side tube or directly inside the bag. Significant differences ($P < 0.05$) are indicated by different letters.

After demonstrating the necessity of performing the extraction exposing the fiber inside the bag, the analysis was performed on real samples of air produced frying potatoes in sunflower oil. However, to avoid possible loss of sample, the new system of extraction (Figure 26) has been applied (as discussed in the paragraph 1.2.2).

Figure 26. New system of extraction: SPME fiber inserted inside the cut vial, through a pierceable septum

1.3.2 Blank samples analysis

Blank analysis was performed in order to detect and extract possible volatile compounds released by the bag wall. This kind of analysis was performed in the same way as the sample: at room temperature and with a fiber exposure time of 1h, 3h, 5h, 7h and 24 hours. Thus, first of all the olfactometric bag was filled up with nitrogen, then the SPME extraction phase was carried out. The analysis was repeated several times to evaluate the system reproducibility. From the overlapping of the chromatograms obtained by carrying out the extraction at 1h, 3h, 5h, 7h and 24 hours (Figure 27a), it is possible to see that the SPME extraction time increase causes an increase in the quantity of substances detected released by the bag.

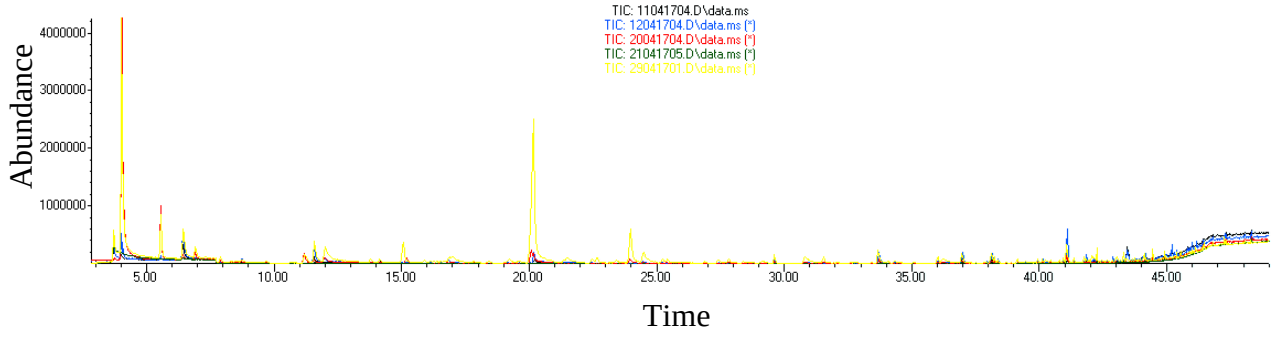


Figure 27a. Overlaid blank chromatograms at 1h (in black), 3h (in blue), 5h (in red), 7h (in green) and 24 hours (in yellow)

Furthermore, from the overlapping of the chromatograms, obtained from analyzes carried out at the same SPME extraction time (Figure 27b), it is noted that there is a high reproducibility. The three replicates, carried out under the same conditions (fiber exposure time of 7 hours), give chromatograms with the same profile and with signals roughly of the same intensity.

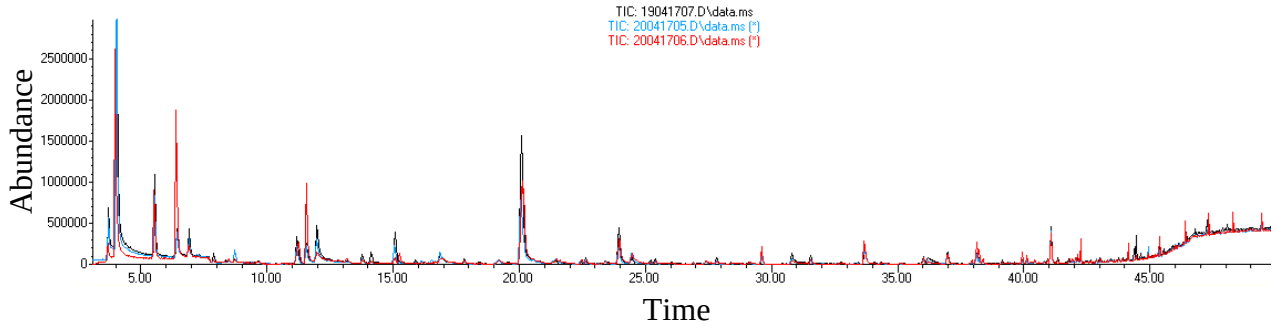


Figure 27b. Overlaid chromatograms of three blank replicates after 7 hours of SPME extraction

The chromatograms obtained from the blank analyses demonstrated that the substances detected are negligible if compared to the sample analyses (Figure 27c). The detected compounds are siloxanes derivatives, aldehydes, esters (like decanal and decanoic acid 2-ethylhexylester) and volatile compounds with low molecular weight (like ethyl acetate and hexane), that could derive also from an environmental contamination of the laboratory.

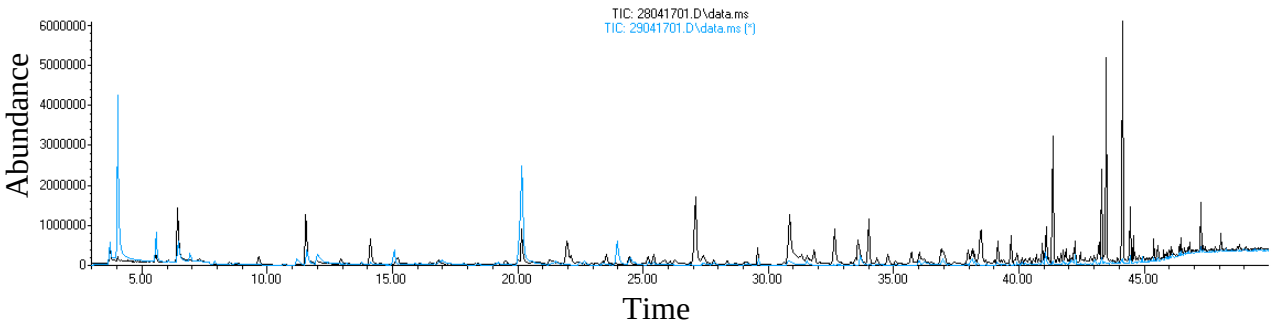


Figure 27c. Overlaid sample chromatogram (in black) with blank chromatogram (in blue) both at 24 hours of SPME extraction time

1.3.3 Evaluation of different SPME extraction times

Once decided the most appropriate SPME extraction method, the evaluation of different SPME extraction times was performed. In order to do this, air sampled from fried potatoes in different olfactometric bags, was analyzed. The air sample was analyzed at 1h, 3h, 5h, 7h and at more prolonged SPME exposure time of 24h. In the figures below examples of obtained chromatograms are reported. In particular, the Figure 28a shows the chromatogram obtained from the analysis of air collected while frying fries using an SPME extraction time of 1h; in the Figure 28b the chromatogram obtained with an SPME extraction time of 24 hours is reported. In both cases, carrying out the extraction with SPME fiber exposed inside the bag.

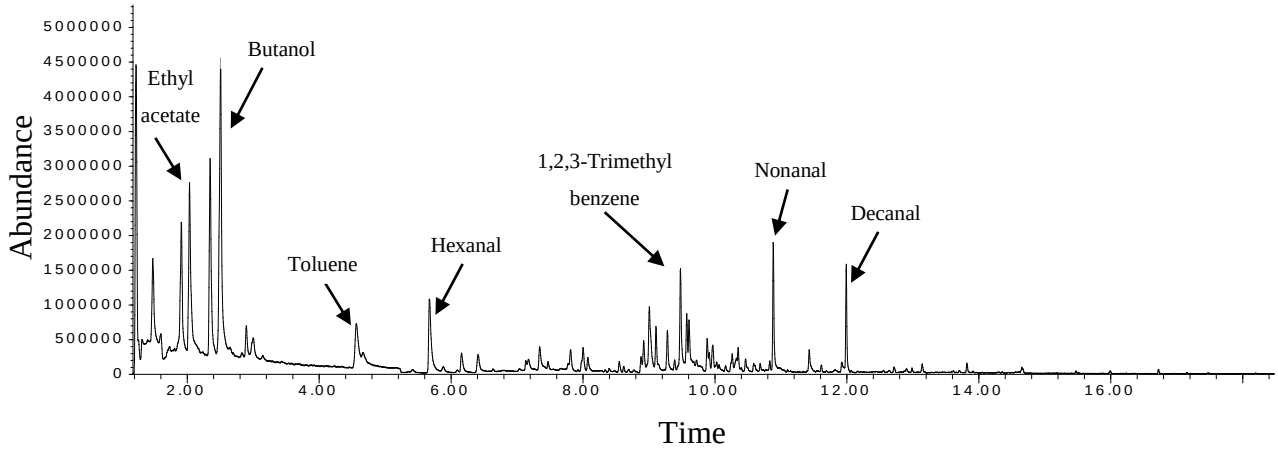


Figure 28a. Chromatogram obtained from the analysis of air collected while frying fries with an SPME extraction time of 1h, carrying out the extraction with SPME fiber exposed inside the bag

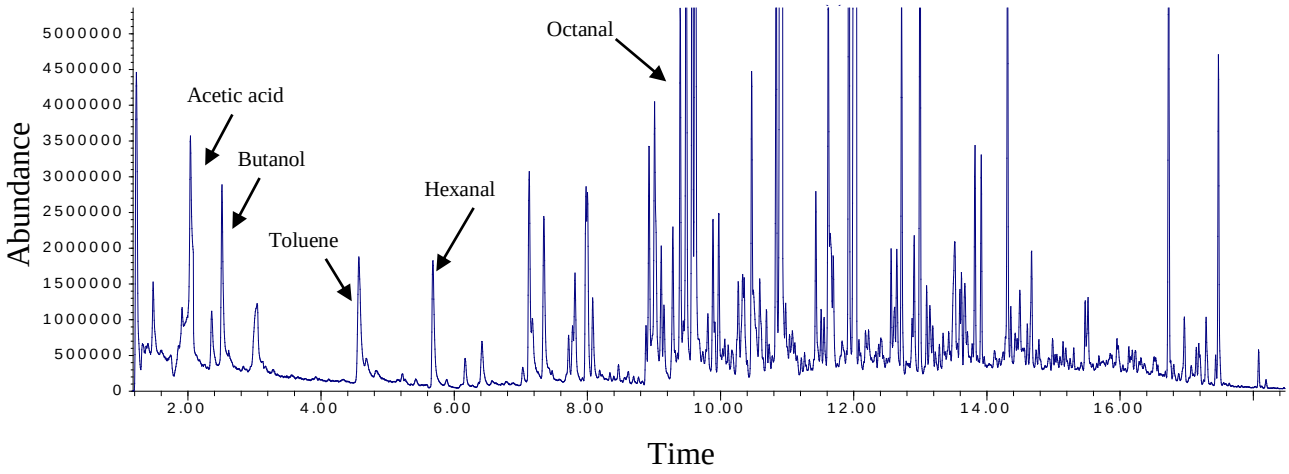


Figure 28b. Chromatogram obtained from the analysis of air collected while frying fries with an SPME extraction time of 24h, carrying out the extraction with SPME fiber exposed inside the bag

Most of the compounds detected could be clearly identified by interpretation of their electron ionization mass spectra and by comparison of their mass spectra with those reported in the NIST library and also by means of their linear retention indices (Table 3). As could be expected, due to the typical autoxidation process of unsaturated fatty acids [31], the main components detected were aldehydes, in particular hexanal, heptanal, octanal, nonanal, decanal and undecanal, typically associated with negative sensorial attributes. Beyond producing off flavors (such as fatty, fishy), these aldehydes are toxic and can be responsible for the development of diseases and pathologies for the human organism [32], thus their monitoring in the kitchen environment and in places often frequented, is especially important to safeguard private and public health. Another category of analytes detected is that of aromatic hydrocarbons. Some of them, such as styrene and *p*-xylene, are known to be carcinogenic and it is known that they can be found in cooking oil fumes [33] due to an incomplete combustion or pyrolysis of organic substances. Other compounds belonging to the same class, as toluene and propyl benzene [34], have been also detected in the sample. The abundance of each VOC after the cooking process depends on its initial amount in the original oil, on its formation rate throughout the heating time, on the rate at which it escapes into the atmosphere during heating and on the rate of its possible degradation to yield other compounds. The initial VOCs abundances are, in general, small in relation to those generated by the cooking process. Frying temperature and time, frying oil type, antioxidants, and the type of fryer affect the hydrolysis, oxidation, and polymerization of the oil during frying. These are the chemical reactions of frying oil that influence the presence and the abundance of VOCs in the oils [32]-[34]. The flavor of oil formed during deep-fat frying is described as fruity, grassy, buttery, burnt, nutty, and fishy. The oxidation of linolenic acid during deep-fat frying increases fishy odor and decreases fruity and nutty odor. Sensory quality generally decreases with the number of fryings. The optimal concentration of oxygen produces the typical desirable fried flavor. Low amounts of oxygen produce poor and weak flavor, and high levels of oxygen produce off-flavor. Fried flavor compounds in fried foods are mainly VOCs produced from linoleic acid and they are dienal, alkenals, lactones, hydrocarbons and various cyclic compounds. From the literature [32]-[35]-[36], compounds such as acetaldehyde, pentanal, hexanal, 2-heptenal, 2-octenal, 2-nonenal, 2,4-decadienal, pentane and 1-pentanol are emitted after degradation of linoleic acid (C18:2, *n*-6). In this way, for instance, as reported from Katragadda et al. [35], the emission rates of 2-heptenal and 2-hexanone should be higher in those oils having higher content of linoleic acid: safflower > canola > extra virgin olive > coconut (Figure 29).

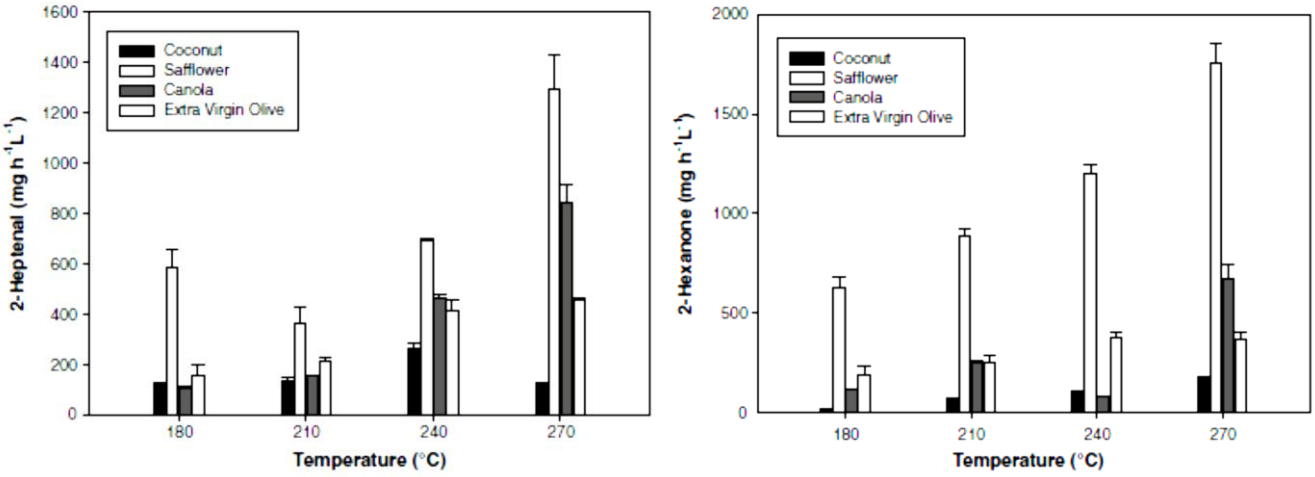


Figure 29. Emission rates of 2-heptenal and 2-hexanone (mg h^{-1} l_{oil}^{-1}) being released from coconut, safflower, canola and extra virgin olive oils heated at four different temperature (180° - 270°C) [35]

Likewise, the abundances of (*E*)-2-butenal and (*E*)-2-pentenal are very closely related to the percentage of linolenic acid (C18:3, *n*-3) in the total fatty acid composition of the original oils; while the abundance of (*E*)-2-propenal and (*E*)-2-hexenal depends on the percentage of linolenic and linoleic groups. Different studies [32]-[35]-[37], have shown that compounds like heptane, heptanol, octane, octanal, nonanal and decanal derive from oleic acid (C18:1, *n*-9), and more precisely from the homolytic fission of the R-O bond of this fatty acid. Moreover, also 2-nonenal, 2-decenal, 1-undecene and 2-undecenal are closely related to the percentage of oleic acid in the total fatty acid composition in the original oils. For instance, it has been seen [35], that the emission rates of 2-decenal and nonanal should be higher in those oils having higher contents of oleic acid: extra virgin olive ≥ canola > safflower ≥ coconut (Figure 30).

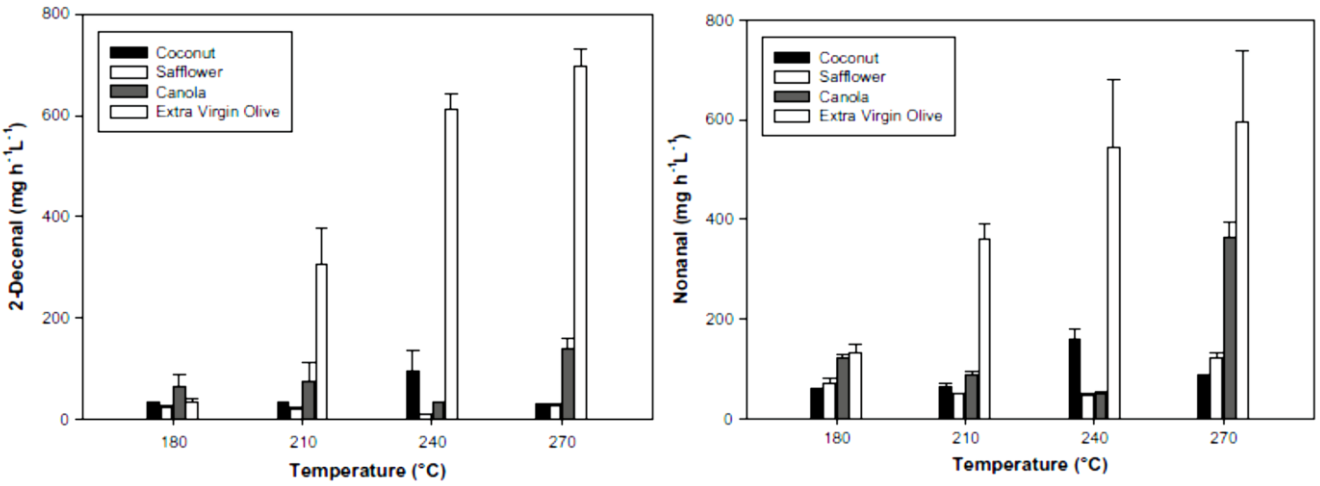


Figure 30. Emission rates of 2-decenal and nonanal (mg h^{-1} l_{oil}^{-1}) being released from coconut, safflower, canola and extra virgin olive oils heated at four different temperature (180° - 270°C) [37]

The analysis was performed also by prolonging to 24 hours the extraction time in order to evaluate if it is possible to detect a higher number of analytes and/or to increase the overall sensitivity of the method. In Figure 28a, Figure 28b and Table 3, it is shown that actually the extent of extraction increases significantly for many compounds.

Peak No.	RT (min)[a]	Compounds detected[b]		LRI (exptl)[c]	LRI (lit)[d]
		SPME extraction time			
		1h	24h		
1	1.913	Hexane	Hexane	600	600
2	2.038	-	Acetic acid	611	608
3	2.036	Ethyl acetate	Ethyl acetate	612	608
4	2.358	2-Methyl-1,3-dioxolane	2-Methyl-1,3-dioxolane	641	-
5	2.511	Butanol	Butanol	655	646
6	4.569	Toluene	Toluene	758	760
7	5.679	Hexanal	Hexanal	799	801
8	6.164	Butyl acetate	Butyl acetate	820	815
9	7.030	-	2-Hexenal	857	854
10	7.173	Ethylbenzene	Ethylbenzene	863	859
11	7.347	-	*o*-Xylene	871	885
12	7.341	1,3-Dimethyl benzene	-	873	869
13	7.471	1-Methoxy-2-propyl benzene	-	878	-
14	7.719	-	Acetyl cyclohexene	887	-
15	7.777	Styrene	Styrene	889	895
16	7.814	*p*-Xylene	*p*-Xylene	891	876
17	8.000	Heptanal	Heptanal	899	902
18	8.081	2-Butoxy ethanol	2-Butoxy ethanol	906	-
19	8.553	α-Pinene	-	936	938
20	8.881	Propyl benzene	Propyl benzene	954	950
21	8.925	(*Z*)-2-Heptenal	(*Z*)-2-Heptenal	956	964
22	9.010	1-Ethyl-2-methyl benzene	Ethyl-2-methyl benzene	962	985
23	9.150	-	2-Pentyl nonylester	970	-

24	9.283	-	Ethyl-4-methyl benzene	979	968
25	9.394	-	6-Methyl-5-hepten-2-one	986	987
26	9.388	2-Methyl-5-hepten-2-one	-	988	-
27	9.485	1,2,3-Trimethyl benzene	1,2,3-Trimethyl benzene	991	1018
28	9.624	Octanal	Octanal	1000	1002
29	9.810	-	2-Ethyl butanal	1015	-
30	9.886	1,2,4-Trimethyl benzene	1,2,4-Trymethyl benzene	1021	-
31	9.915	*p*-Cymene	*p*-Cymene	1023	1027
32	9.972	D-Limonene	D-Limonene	1028	1032
33	10.066	Indane	-	1039	1029
34	10.266	Methyl-3-propyl benzene	1-Methyl-3-propyl benzene	1051	1058
35	10.354	-	*o*-Cymene	1058	1050
36	10.467	-	(*E*)-2-hepten-1-ol	1067	-
37	10.588	-	4-Ethyl-1,2-dimethyl benzene	1077	1091
38	10.689	-	1-Ethyl-2,4-dimethyl benzene	1085	1083
39	10.729	-	(*Z*)-Linalool oxide	1088	1088
40	10.921	Nonanal	Nonanal	1104	1108
41	11.510	-	(*E*)-2-Nonenal	1158	1158
42	11.693	-	(*Z*)-9-Methyl-3-undecene	1175	-
43	12.032	Decanal	Decanal	1207	1207
44	12.561	-	(*E*)-2-Decenal	1263	1263
45	12.615	-	Nonanoic acid	1269	1274
46	12.647	-	(*E*)-2-Undecenol	1280	-
47	12.719	-	(*Z*)-9,10-Epoxyoctadecanol	1297	-
48	12.877	-	2,4-Dodecadienal	1297	-
49	12.995	Undecanal	Undecanal	1308	1308
50	13.096	-	(*E*,*E*)-2,4-Decadienal	1320	1319
51	13.518	-	2-Undecenal	1365	-
52	14.309	-	(*Z*)-Geranyl acetone	1456	1455
53	14.358	-	2,6-Dimethyl heptadecane	1462	-
54	14.495	-	2,6-Di-tert-butyl-*p*-benzoquinone	1478	1472
55	14.608	-	α-Isomethyl ionone	1491	1485
56	14.739	-	(*Z*)-8-Hexadecene	1507	-
57	14.986	-	Lilial	1538	1532
58	15.305	-	4,5-Dimethyl-2-pentadecyl-1,3-dioxane	1577	-
59	16.966	-	2-Methylpentyl benzoate	1800	-

Table 3. Volatile compounds detected by HS-SPME-GC-MS, their experimental linear retention indices on a 5% phenyl polydimethylsiloxane column and values reported in literature on 5% phenyl polydimethylsiloxane columns

[a] RT: retention time

[b] Compounds reported are those which had peak area values higher than 500.000

[c] Experimental linear retention index

[d] Linear retention indices reported in literature (NIST, 2008)

Thus, the extraction time can be modulated depending on the specific analytes under investigation. In the specific case under investigation an extraction time of 1 hour (as of 3h, 5h and 7h) is sufficient to detect the most abundant compounds and the most volatile compounds, while an extraction time of 24 hours allows to quantitate and to detect a much higher number of analytes. It is also noteworthy that the shortest extraction time (1h) allows a higher sensitivity for

some of the lightest compounds detected (hexane, 2-methyl-1,3-dioxolane, butanol) and this finding can be explained by the displacement effect occurring when limited adsorption sites are available in the fiber coating, and the analytes with higher affinity displace analytes with lower affinity for these sites, with the establishment of a dynamic equilibrium [38]. Anyway in the present study, where olfactometric bags are used, this behavior could be due also to a preferential diffusion mechanism of the more volatile substances through the bag wall during time [39]. The analyses of blanks, carried out by filling the bags with nitrogen, demonstrated the absence of release and interference of the bag materials. Therefore, considering the results obtained, it was decided to continue the analysis by exposing the SPME fiber inside the bag for a time of 24 hours. In more detail, for each kind of filter, six replicates of air before passing the hood aspiration filter and after passing on the hood aspiration filter were sampled and analyzed.

1.3.4 Qualitative composition

Once all samples were analyzed with the SPME technique coupled to GC-MS instrument, the identification of the different peaks, present in the resulting chromatograms, was performed. The first step was the identification of peaks in the chromatogram relative to alkanes. The identification of compounds present in the analyzed samples was the next work phase. The recognition, of most of the compounds present in the examined samples, was possible by comparing the data obtained from the NIST library and retention indexes. The Kovats retention index (I or RI) indicates where an analyte elutes with respect to straight chain alkanes. For an *n*-alkane, a retention index equal to 100 times the number of carbon atoms is assigned. These values are identical by definition in any chromatographic system. The following expression was applied to determine the value of the retention indexes of the analytes: $RI_X = 100 \cdot Z + 100 \cdot (T_X - T_Z)/(T_{Z+1} - T_Z)$, where Z is the number of carbons of the *n*-alkane having a retention time T_Z, T_X is the retention time of the analyte of interest, T_Z is the retention time of the *n*-alkane which elutes before of the analyte and T_{Z+1} is the retention time of the *n*-alkane which elutes immediately after the analyte. Table 4 shows all the analytes present in the samples. Furthermore, for this study, the analytes detected were subdivided into aldehydes, aromatic compounds, ketones, alcohols, esters, aliphatic hydrocarbons, organic acids and other compounds; then qualitative compositions were evaluated for each examined filter. The percentage areas of each single category of molecules before and after passing through the filter were calculated and compared (as will be seen in the next paragraphs).

Peak No.	RT (min)[a]	Compounds detected[b] SPME extraction time: 24h	LRI (exptl)[c]	LRI (lit)[d]
1	3,847	Formic acid, ethenyl ester	-	-
2	**4,018**	**Hexane (C6)**	**600**	**600**
3	4,080	Ethyl ether	616	616
4	4,089	1,2-diethoxy-ethane	618	-
5	**4,413**	**Heptane (C7)**	**700**	**700**
6	**5,309**	**Octane (C8)**	**800**	**800**
7	5,526	Acetone	811	814
8	5,967	6-methyl-1-heptene	834	-
9	6,027	Cyclohexane	838	732
10	6,333	2-octene	854	858
11	6,857	Ethyl Acetate	881	884
12	7,181	2-butanone	898	900
13	**7,222**	**Nonane (C9)**	**900**	**900**
14	7,234	(*E*,*E*)-6,10-dimethyl-5,9-dodecadien-2-one	900	-
15	7,832	Methylene chloride	918	914
16	7,885	5-methyl-2-phenyl-1H-indole	920	-
17	8,196	Benzene	929	924
18	8,452	Ethanol	936	933
19	8,671	2-methyl-1,3-dioxolane	943	953
20	9,239	2,2,4,6,6-pentamethyl-heptane	960	957
21	9,386	2,6-dimethyl-octane	964	-
22	9,606	Pentanal	970	974
23	**10,609**	**Decane (C10)**	**1000**	**1000**
24	11,168	alpha-pinene	1013	1013
25	11,252	alpha-pinene	1014	1013
26	11,901	Toluene	1029	1033
27	12,035	2-butenal	1032	1038
28	12,876	(*E*)-1,3-nonadiene	1051	1046
29	13,691	Butyl ester acetic acid	1069	1072
30	14,055	Hexanal	1077	1079
31	14,911	beta-pinene	1097	1097
32	**15,067**	**Undecane (C11)**	**1100**	**1100**
33	16,019	Ethylbenzene	1118	1118
34	16,435	*p* -xylene	1125	1125
35	17,202	1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene	1139	1223
36	17,770	1-butanol	1150	1150
37	18,313	1-methylcyclopentyl ester acetic acid	1160	-
38	18,816	1-ethoxy-2-propanol	1169	-
39	19,202	*o* -xylene	1176	1177
40	19,436	Heptanal	1181	1183
41	19,943	D-limonene	1190	1192
42	**20,480**	**Dodecane (C12)**	**1200**	**1200**
43	20,751	Eucalyptol	1205	1209
44	21,202	(*E*)-2-hexenal	1213	1212
45	21,346	1-ethyl-2-methyl-benzene	1216	1222
46	21,705	2-(2-ethoxyethoxy)-ethanol acetate	1223	-
47	21,870	2-pentyl-furan	1226	1231
48	22,049	(*Z*)-3-dodecene	1229	1254
49	22,126	(*E*)-6-dodecene	1231	-
50	22,361	1,2,3-trimethyl-benzene	1235	1327
51	23,050	Styrene	1248	1247
52	23,473	1-pentanol	1256	1256
53	23,851	1-methyl-2-(1-methylethyl)-benzene	1263	1268
54	24,378	1,2,4-trimethyl-benzene	1273	1275
55	24,857	3-hydroxy-2-butanone	1282	1286
56	25,000	2-octanone	1284	1283
57	25,129	Octanal	1287	1287
58	25,323	2,2,4,4,6,8,8-heptamethyl-nonane	1290	-
59	25,810	5-methyl-1-heptene	1299	-
60	**25,844**	**Tridecane (C13)**	**1300**	**1300**
61	26,871	1-ethyl-3,5-dimethyl-benzene	1320	1321
62	26,978	(*Z*)-2-heptenal	1322	1320
63	27,287	1-ethyl-4-methyl-benzene	1327	1261
64	27,413	1,3,5-trimethyl-benzene	1330	1283
65	27,766	6-methyl-5-hepten-2-one	1337	1339
66	28,450	1-butoxy-2-propanol	1350	-
67	28,602	1-methyl-3-(1-methylethyl)-benzene	1353	1250
68	29,019	1-ethyl-2,3-dimethyl-benzene	1360	1362
69	29,062	4-ethyl-1,2-dimethyl-benzene	1361	1364
70	29,973	2-ethyl-1,4-dimethyl-benzene	1379	1364
71	30,739	Nonanal	1393	1396
72	**31,094**	**Tetradecane (C14)**	**1400**	**1400**
73	31,277	4,4-dimethyl-2-pentanol	1404	-
74	31,484	2-butoxy-ethanol	1408	1411
75	31,738	3-ethyl-2-methyl-1,3-hexadiene	1413	-
76	32,003	1,2,4,5-tetramethyl-benzene	1418	1438
77	32,272	N,N-dimethyl-acetamide	1423	1414
78	32,546	(*E*)-2-octenal	1429	1425
79	33,228	Heptyl ester-2-ethylbutyric acid	1442	-
80	33,492	2,6,10,14-tetramethyl-heptadecane	1448	-
81	33,582	Acetic acid	1450	1447
82	33,923	1-octen-3-ol	1456	1456
83	34,269	1-heptanol	1463	1461
84	34,342	Cycloheptane	1465	-
85	34,687	4-ethylcyclohexanol	1472	-
86	35,639	N,6-dimethyl-5-hepten-2-amine	1490	-
87	35,714	3,3-dimethyl-hexanal	1492	-
88	35,974	2-ethyl-1-hexanol	1497	1492
89	**36,119**	**Pentadecane (C15)**	**1500**	**1500**
90	36,159	Decanal	1501	1502
91	36,253	Phytol	1503	2606
92	36,838	3-nonen-2-one	1517	1518
93	36,924	Benzaldehyde	1519	1515
94	37,975	(*E*)-2-nonenal	1544	1542
95	38,093	2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl-1H-trindene	1547	-
96	38,398	2,7-octanedione	1554	-
97	38,621	1,1-dimethylpropyl ester pentanoic acid	1560	-
98	38,947	(1-methylethylidene)-cyclohexane	1568	-
99	39,116	1-octanol	1572	1566
100	39,338	9-octadecyne	1577	-
101	39,905	4-ethyl-cyclohexene	1590	-
102	40,249	2-hydroxy-butyl ester propanoic acid	1599	-
103	**40,308**	**Hexadecane (C16)**	**1600**	**1600**
104	40,399	Methyl ester-1-cyclopentene-1-carboxylic acid	1605	-
105	40,662	*trans* -2-undecen-1-ol	1618	-
106	40,775	1,5-pentanediol	1623	-
107	40,790	D-allose	1624	-
108	40,911	Butanoic acid	1630	1624
109	40,933	2-(2-ethoxyethoxy)-ethanol	1631	1622
110	41,176	1,2-ethanediol	1643	1635
111	41,304	2-cyclohexen-1-ol	1650	-
112	41,549	Pentyl-cyclohexane	1662	-
113	41,660	2-methyl-pentanoic acid anhydride	1667	-
114	41,749	1-decene	1672	-
115	41,757	1-nonanol	1672	1671
116	41,870	1-nonene	1678	-
117	41,878	Heptyl hexyl ether	1678	-
118	**42,319**	**Heptadecane (C17)**	**1700**	**1700**
119	42,435	(*E*,*E*)-2,4-nonadienal	1708	1706
120	43,001	Pentanoic acid	1745	1743
121	43,184	2,3-dihydro-2-methyl-1H-inden-1-ol	1757	-
122	43,282	2-dodecenal	1763	-
123	43,366	2,6-dimethyl-benzenamine	1769	-
124	43,460	2,4-dodecadienal	1775	-
125	**43,836**	**Octadecane (C18)**	**1800**	**1800**
126	43,950	2-[2-(2-butoxyethoxy)ethoxy]-ethanol	1810	-
127	44,107	(*E*,*E*)-2,4-decadienal	1824	1821
128	44,390	4-oxononanal	1848	-
129	44,442	Hexanoic acid	1853	1860
130	44,569	4-(1-methylpropyl)-phenol	1864	-
131	44,634	(*E*)-6,10-dimethyl-5,9-undecadien-2-one	1869	1865
132	44,812	Hexagol	1885	-
133	44,852	Benzyl alcohol	1888	1885
134	44,918	2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester propanoic acid	1894	-
135	44,985	2,5,8,11,14-pentaoxahexadecan-16-ol	1900	-
136	**44,987**	**Nonadecane (C19)**	**1900**	**1900**
137	45,544	Heptanoic acid	1960	1967
138	45,555	2-ethyl-hexanoic acid	1961	1969
139	**45,918**	**Eicosane (C20)**	**2000**	**2000**
140	46,405	Octaethylene glycol	2061	-
141	46,472	Octanoic acid	2070	2070
142	46,605	2-heptenoic acid	2087	-
143	**46,712**	**Heneicosane (C21)**	**2100**	**2100**
144	47,285	Nonanoic acid	2177	2171
145	**48,191**	**Tricosane (C23)**	**2300**	**2300**

Table 4. Volatile compounds detected by HS-SPME-GC-MS, their experimental linear retention indices on a DB-WAX column and values reported in literature on DB-WAX columns

[a] RT: retention time

[b] Compounds reported are those which had peak area values higher than 500.000

[c] Experimental linear retention index

[d] Linear retention indices reported in literature (NIST, 2008)

1.3.5 Quantitative (relative) composition

Completed the process of the peaks recognition present in the chromatograms of the samples, it was possible to perform the relative quantification. During this phase, first of all, it was necessary to integrate manually, in each chromatogram, the peaks previously identified in order to obtain the corresponding area. The chromatograms showed peaks both well defined, and partially overlapping and contiguous. To try to ensure greater precision, in the latter case the integration was tried to follow the progress of the base line; dividing as much as possible the two peaks in the same manner in each chromatogram. The areas obtained by the integration of each chromatogram, finally, were transferred to the *Excel* program where they were subsequently processed. In particular, as regards quantitative analysis, for this kind of research work, the absolute areas obtained before passing filter and the absolute areas obtained after passing filter were compared for each detected analyte. Moreover, the percentage of dejection, for the analyte passing through the filter, was calculated.

1.3.6 Activated charcoal AC 90 filter

In this section the results obtained from activated charcoal AC 90 filter, are reported. In the figures below, an example of chromatogram obtained for air before filter (Figure 31) and air after filter (Figure 32), are shown.

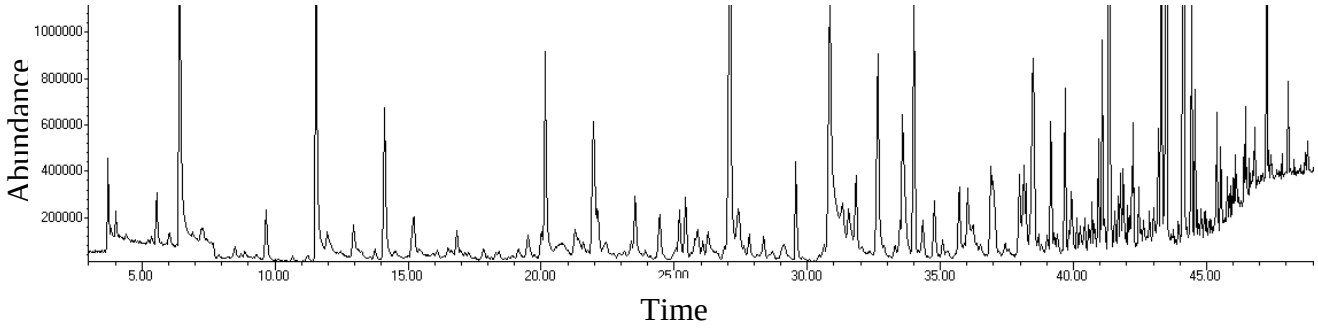


Figure 31. Chromatogram obtained for air before filter

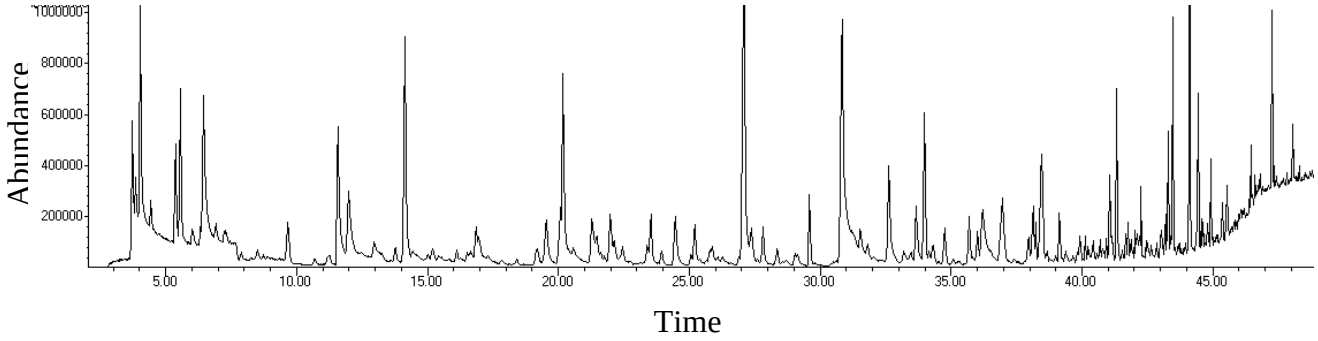
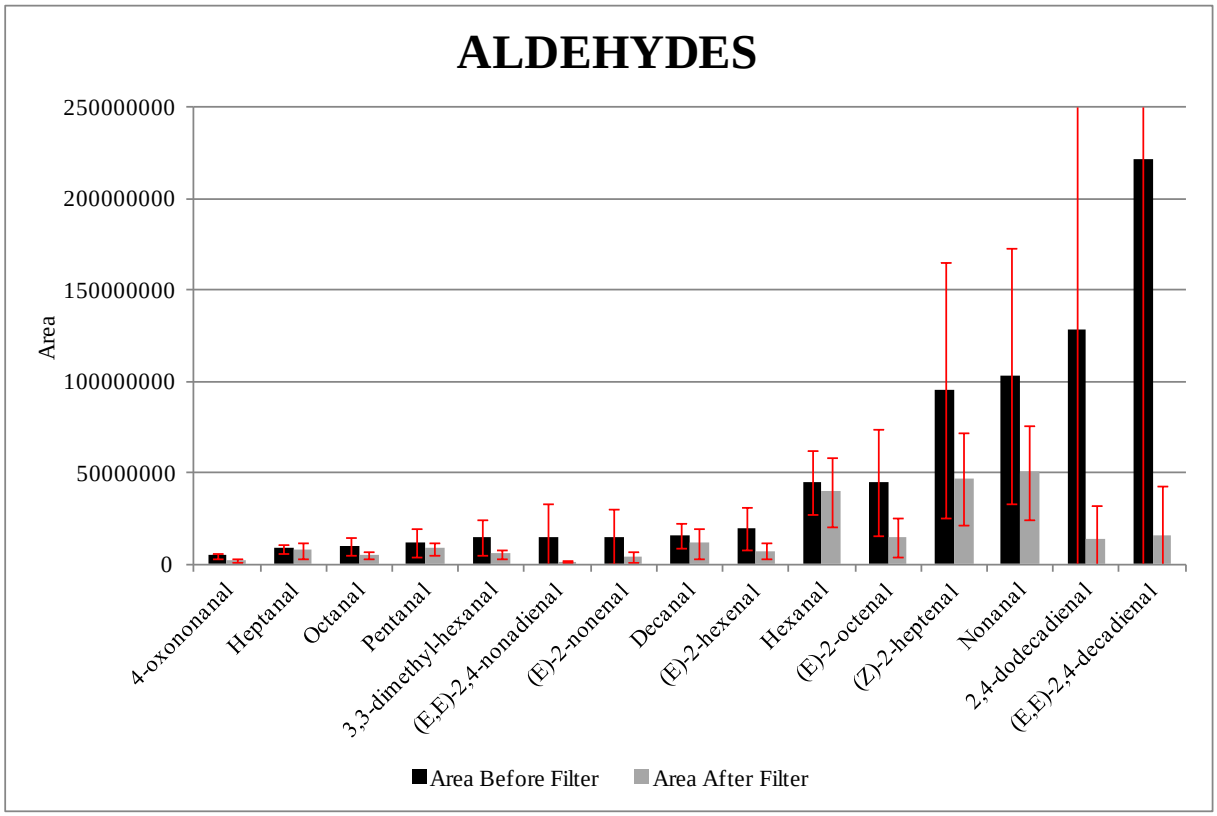


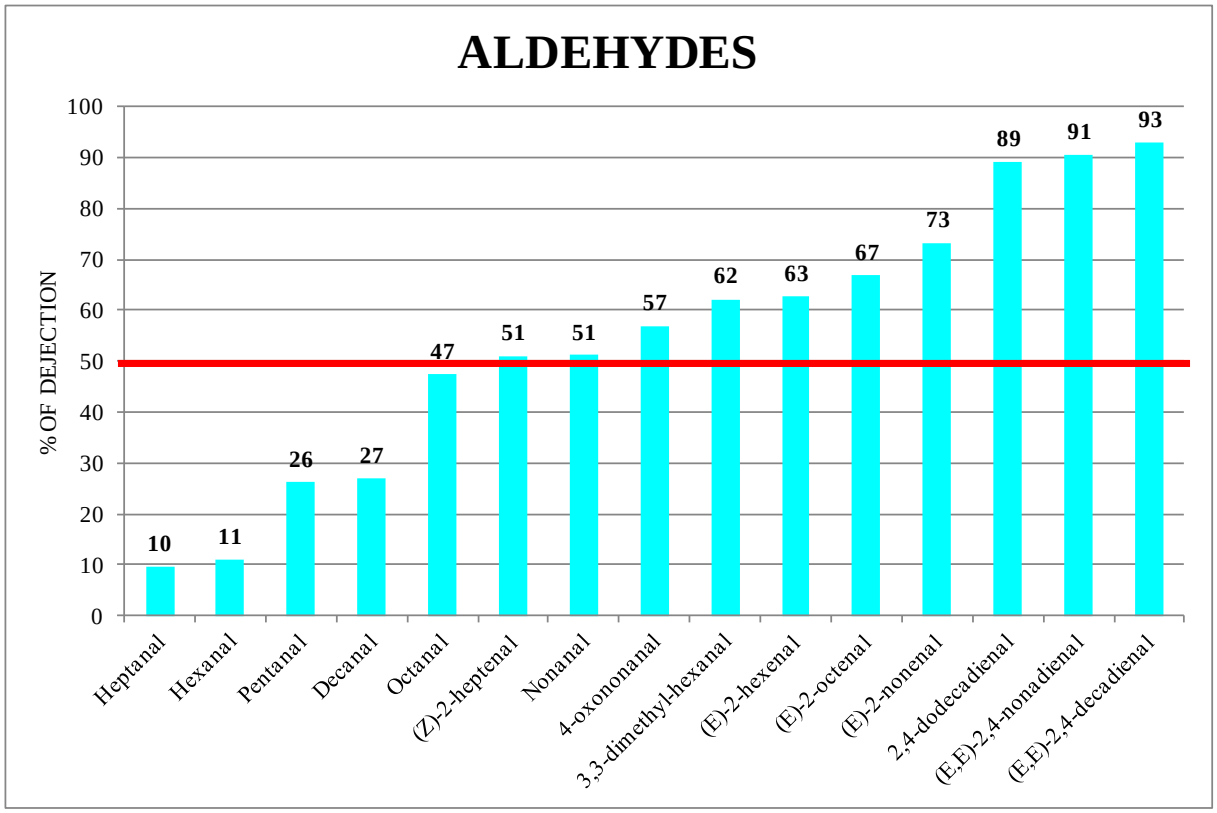
Figure 32. Chromatogram obtained for air after filter

The detected compounds were divided into different classes and for each substance a comparison between the absolute area of the peak obtained analyzing the air before and after passing through the filter, and the dejection percentage, calculated by dividing the difference between the average peak area from the air before the filter and the air after the filter, for the average area from the air before the filter, were reported.

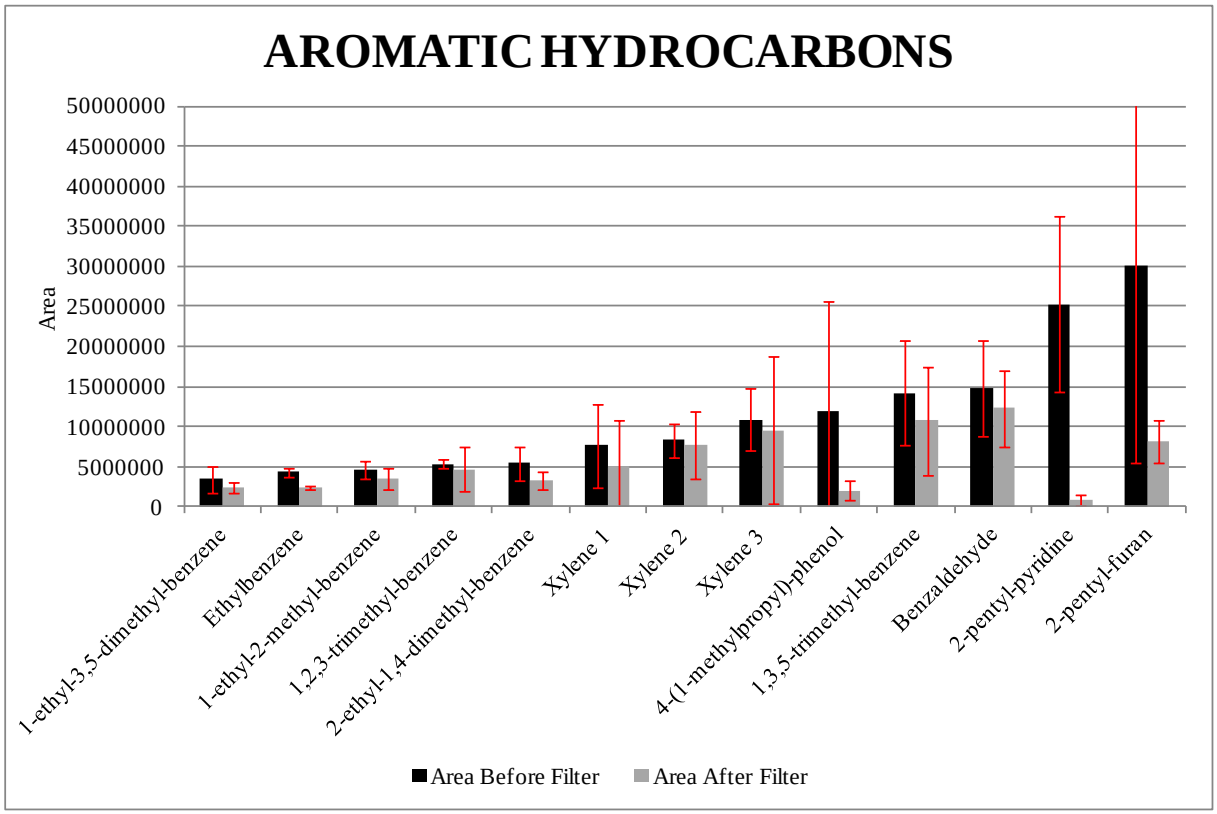
However, before discussing the results, it is important to underline that this type of filter, among the three examined, is the most economical and non-regenerable; therefore it is possible to expect lower performances (in terms of filtering) than the other two filters.



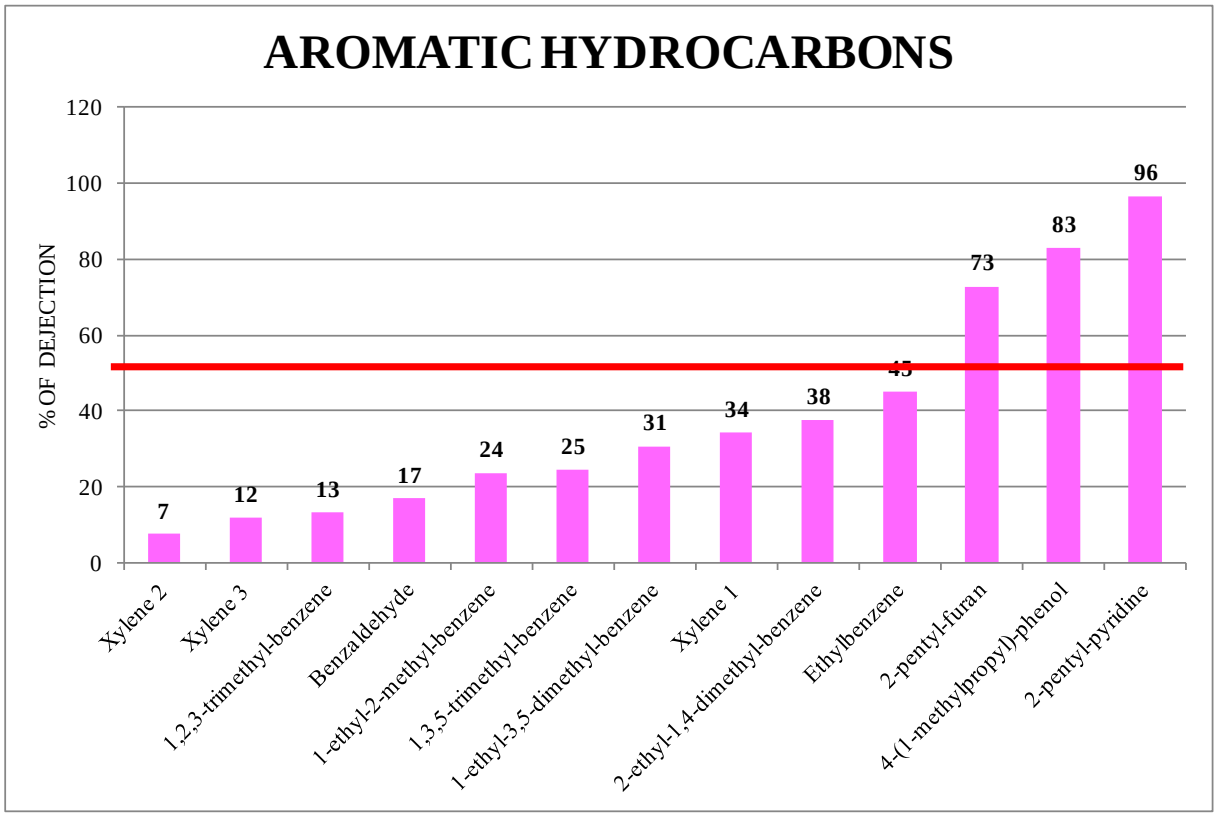
Graph 1. Aldehydes absolute areas before and after passing filter



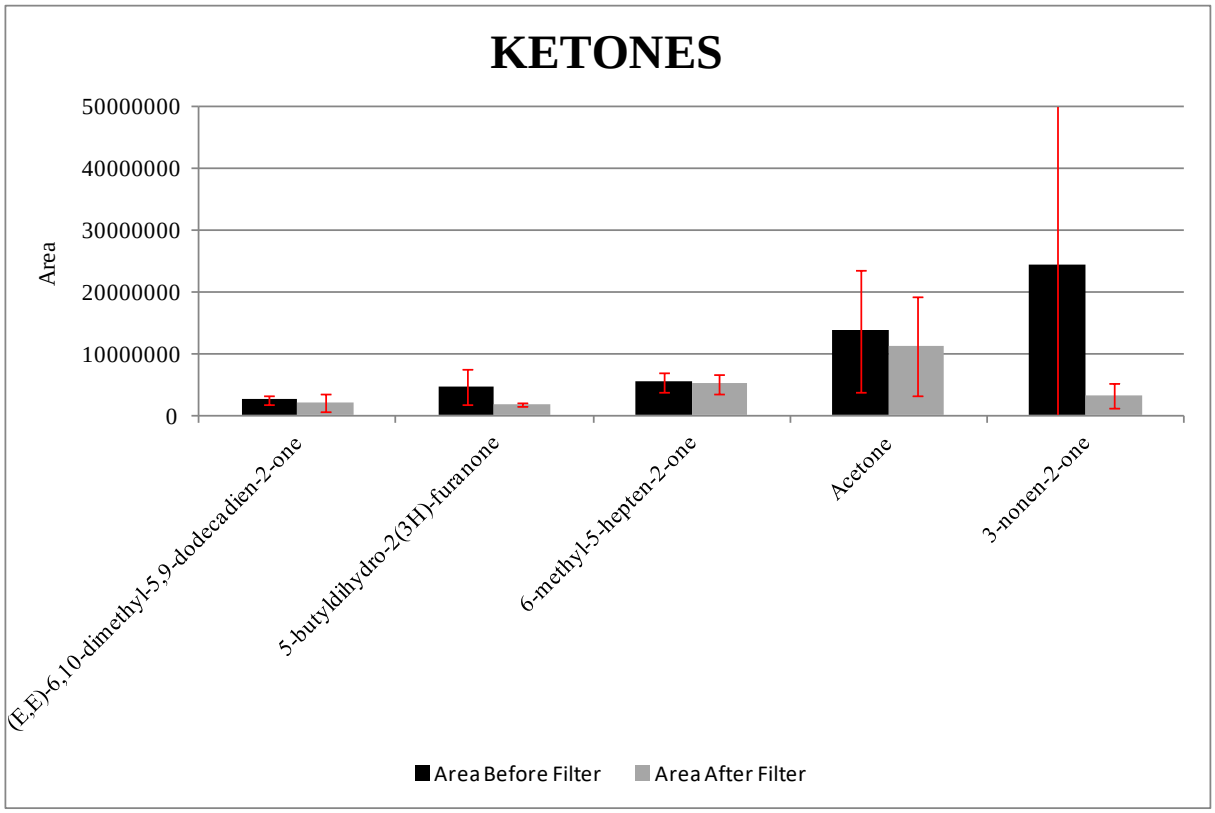
Graph 2. Dejection percentage after filter passage for aldehydes compounds



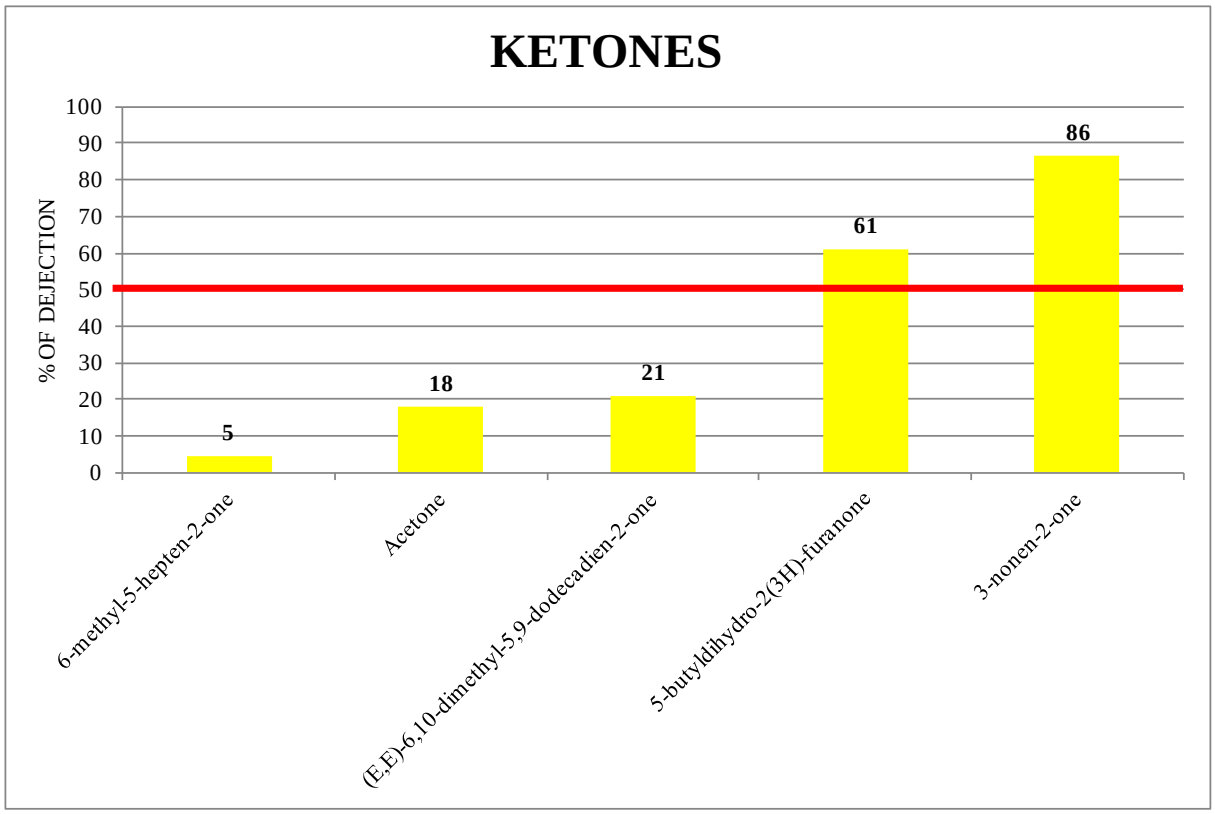
Graph 3. Aromatic hydrocarbons absolute areas before and after passing filter



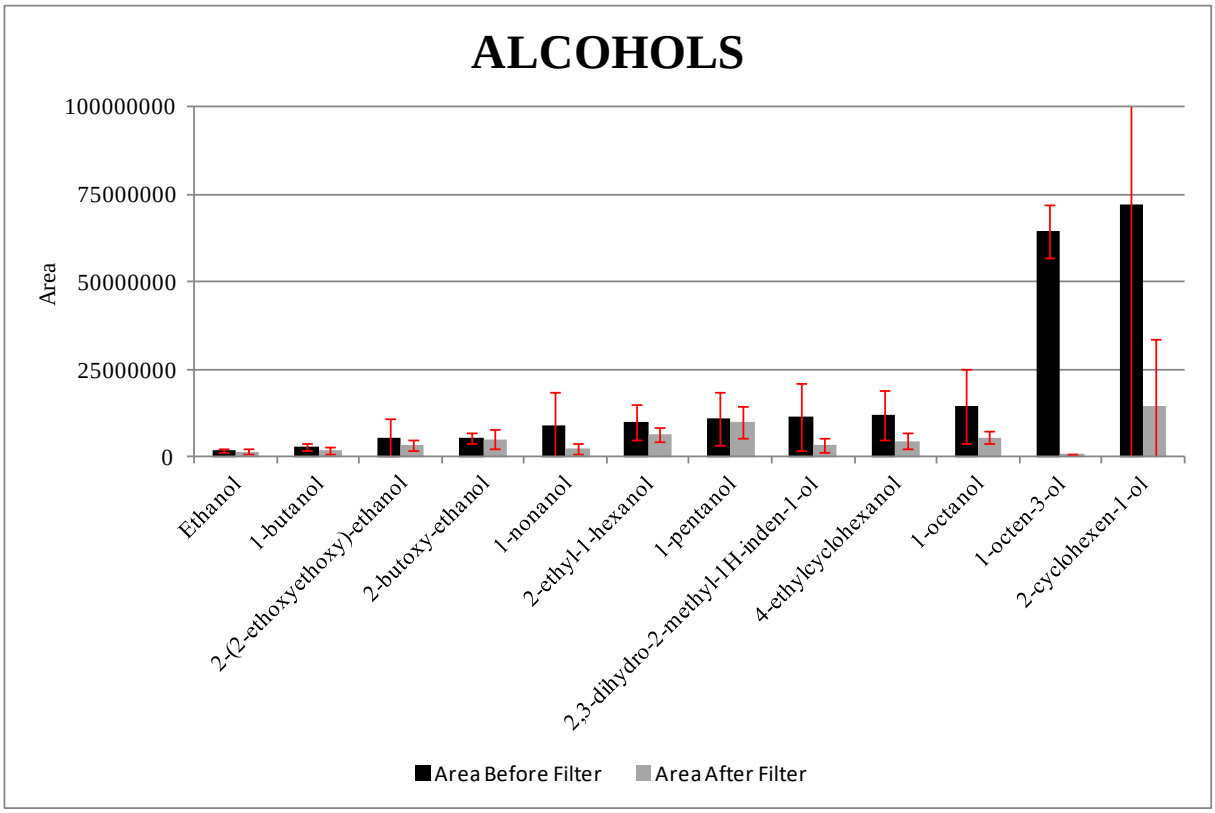
Graph 4. Dejection percentage after filter passage for aromatic hydrocarbons compounds



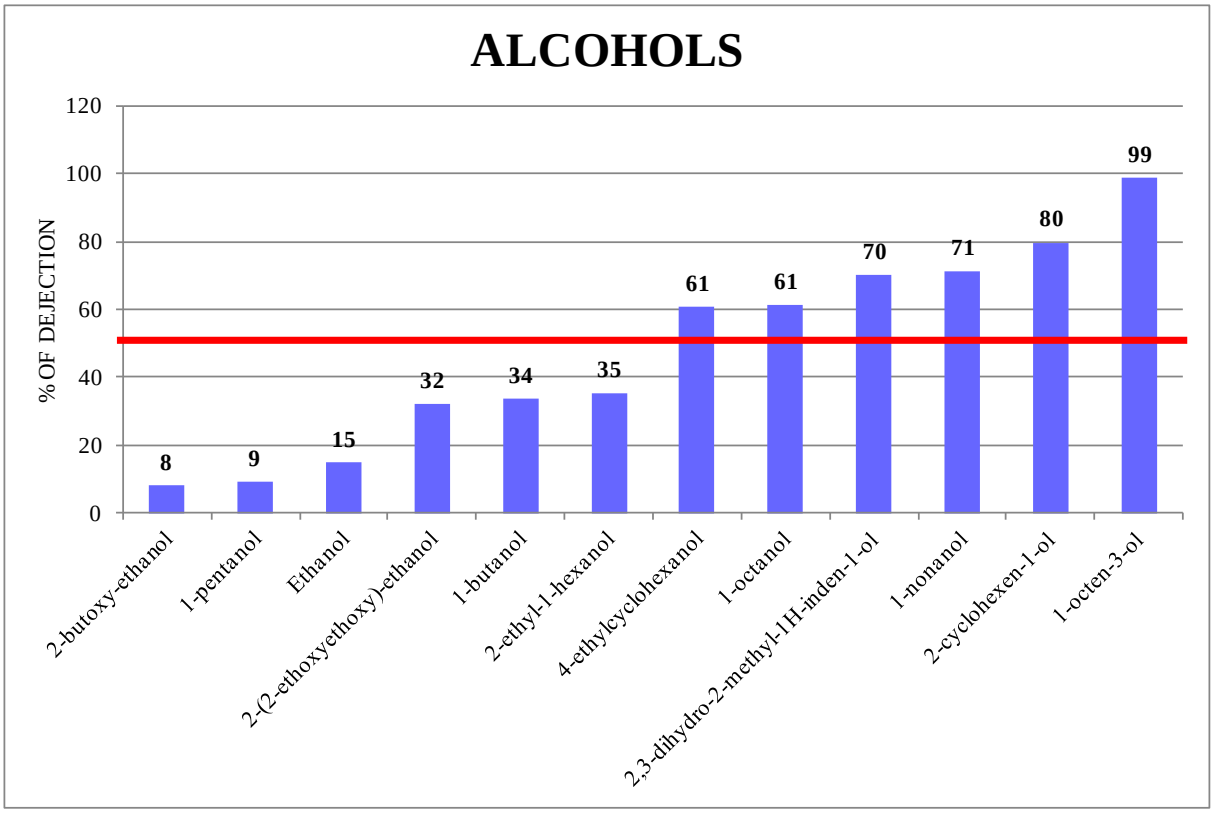
Graph 5. Ketones absolute areas before and after passing filter



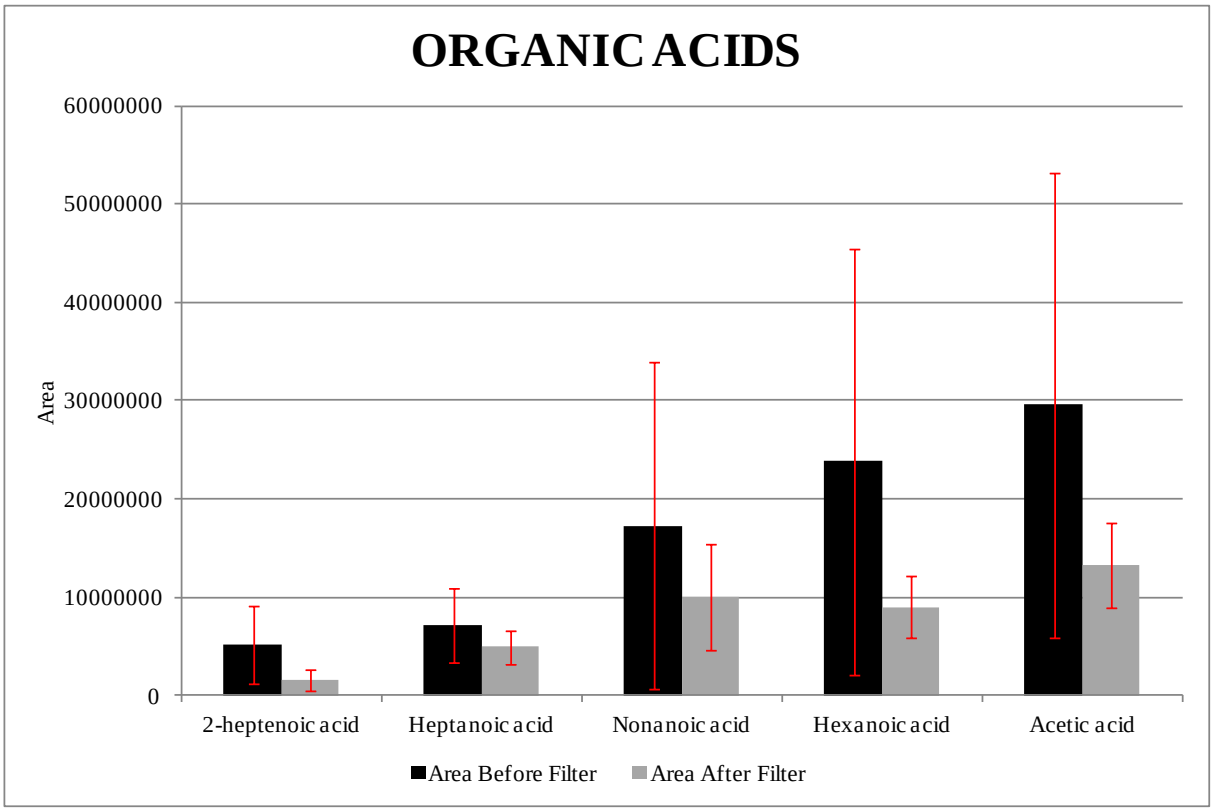
Graph 6. Dejection percentage after filter passage for ketones compounds



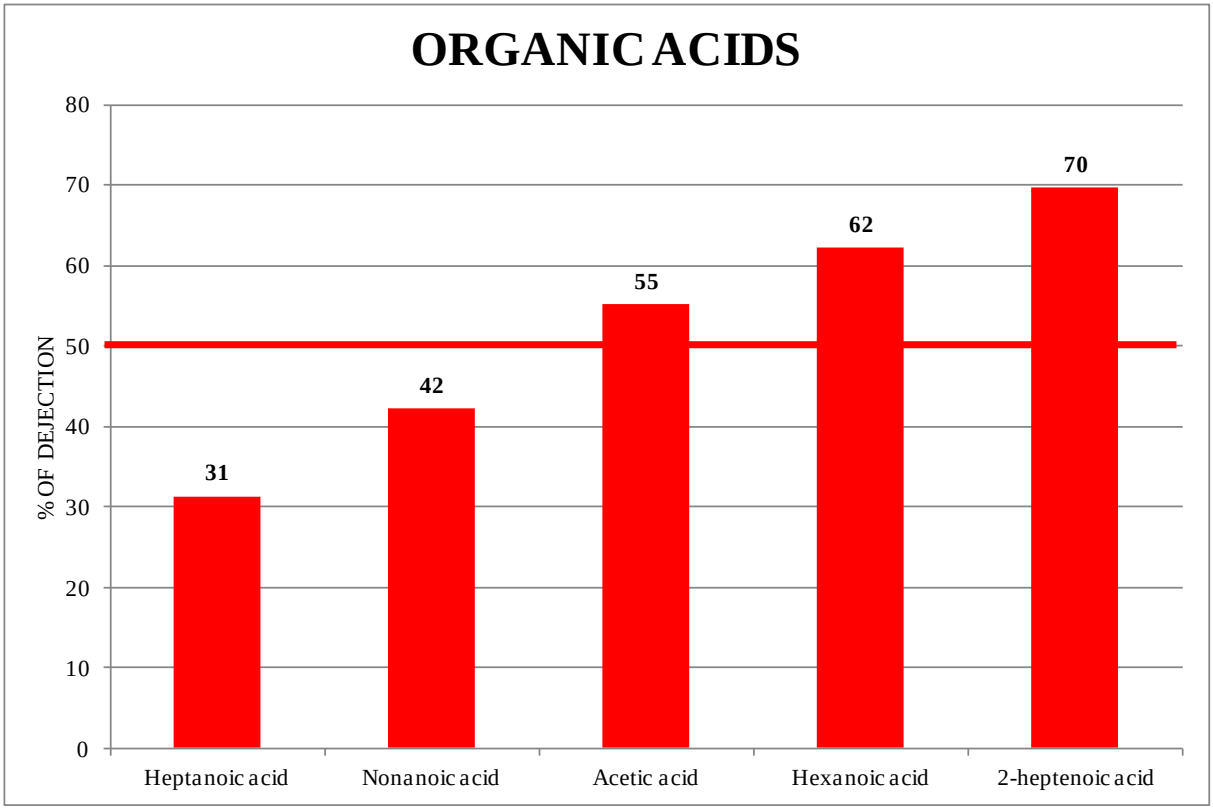
Graph 7. Alcohols absolute areas before and after passing filter



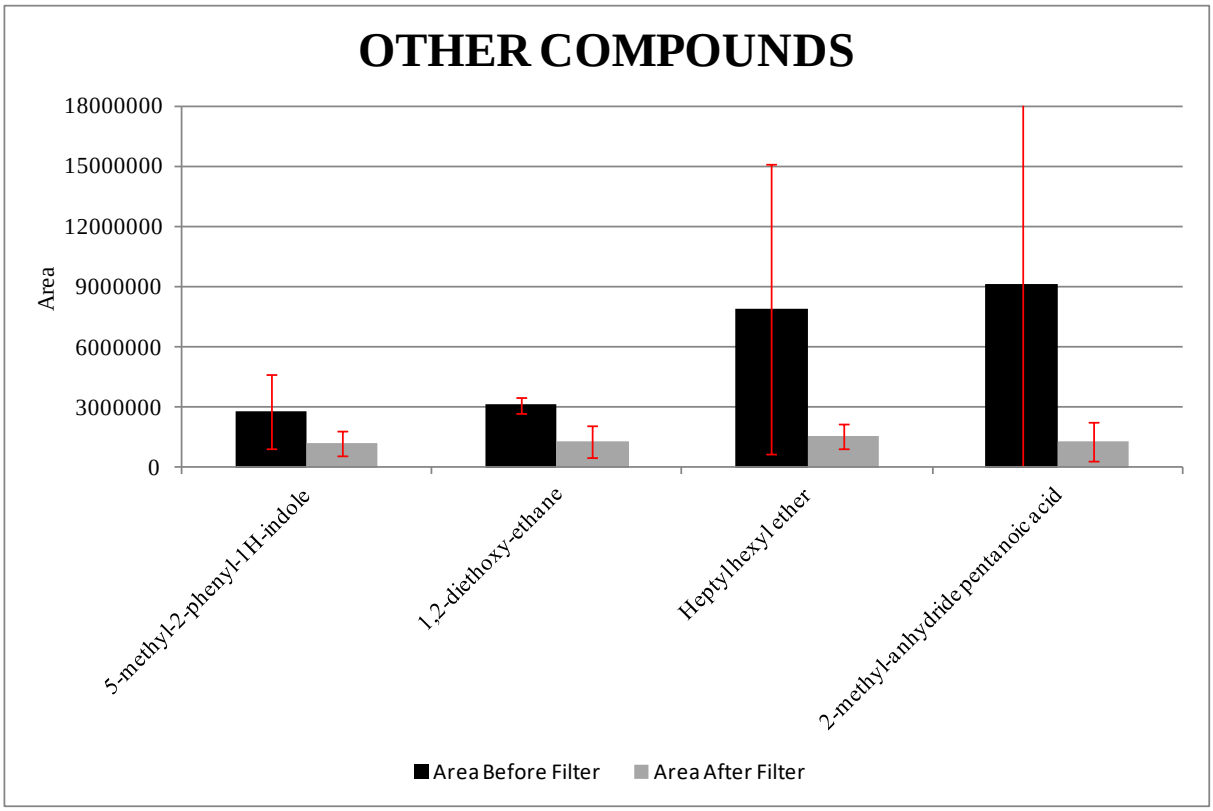
Graph 8. Dejection percentage after filter passage for alcohols compounds



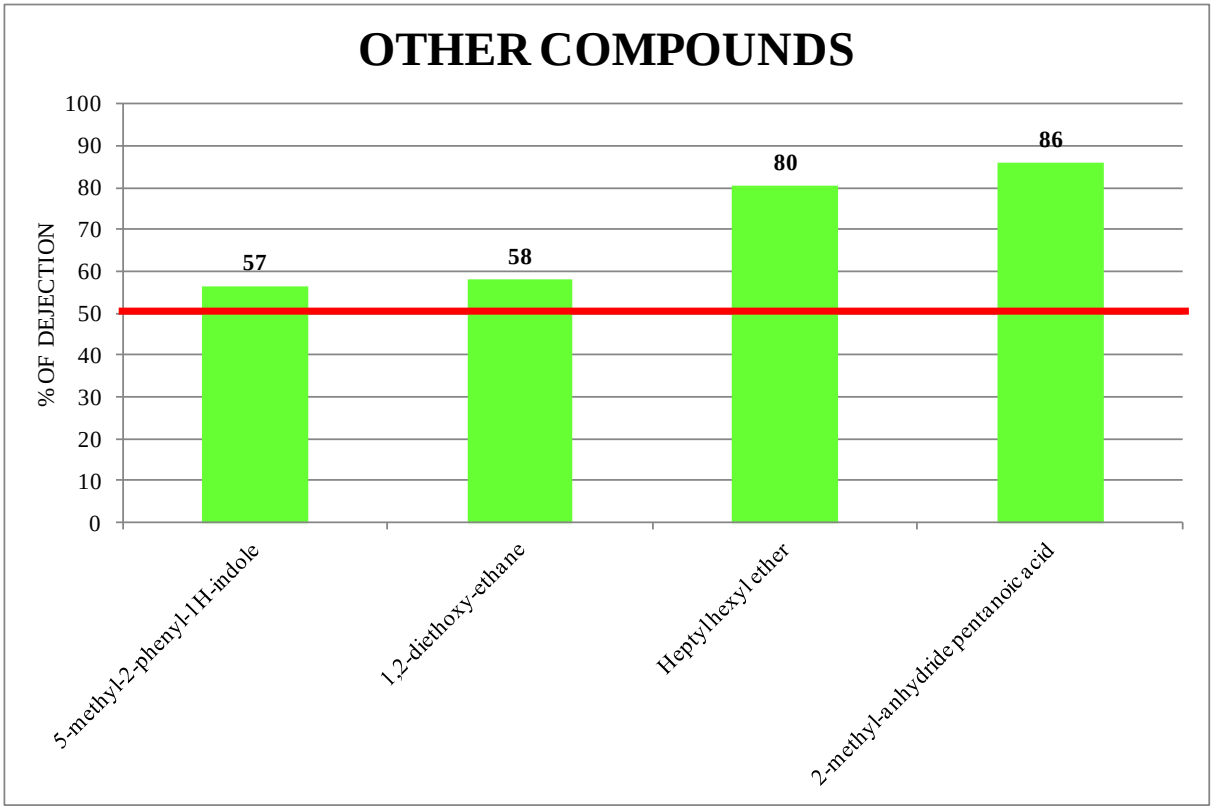
Graph 9. Organic acids absolute areas before and after passing filter



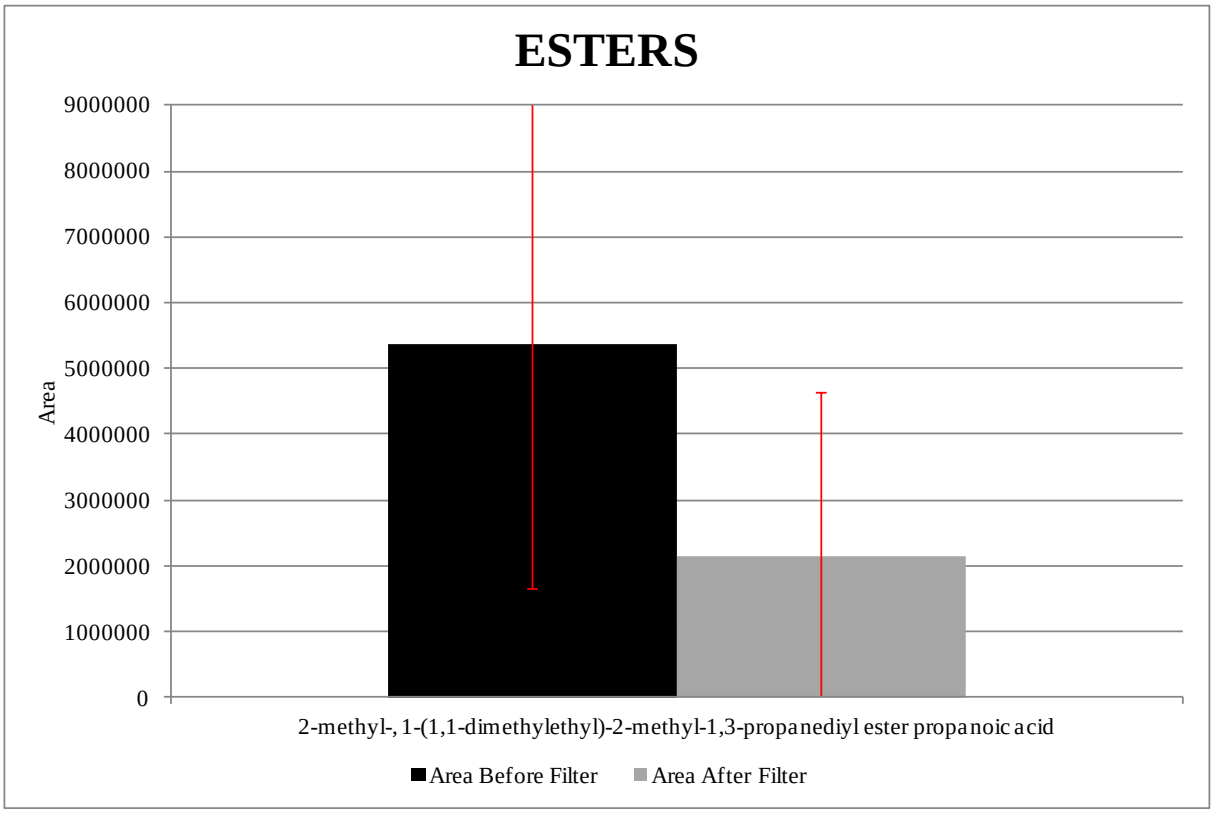
Graph 10. Dejection percentage after filter passage for organic acids compounds



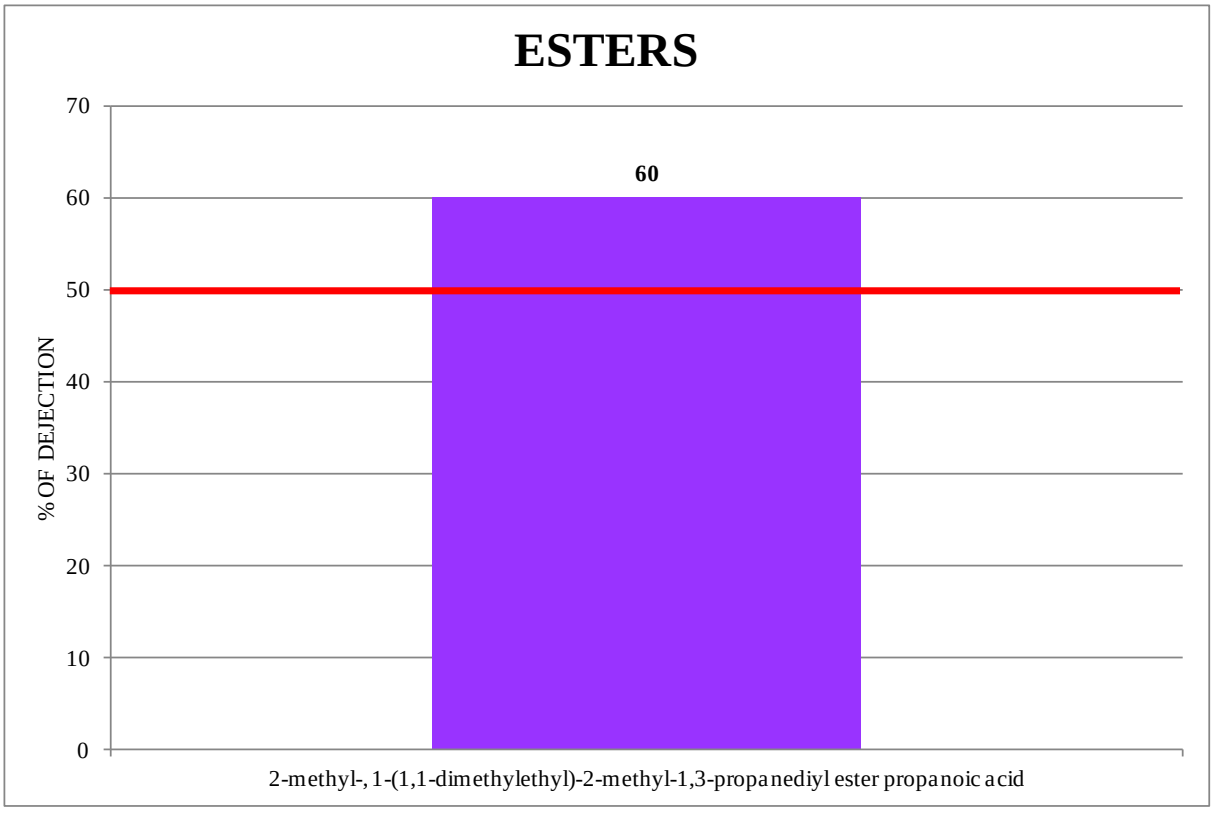
Graph 11. Other compounds absolute areas before and after passing filter



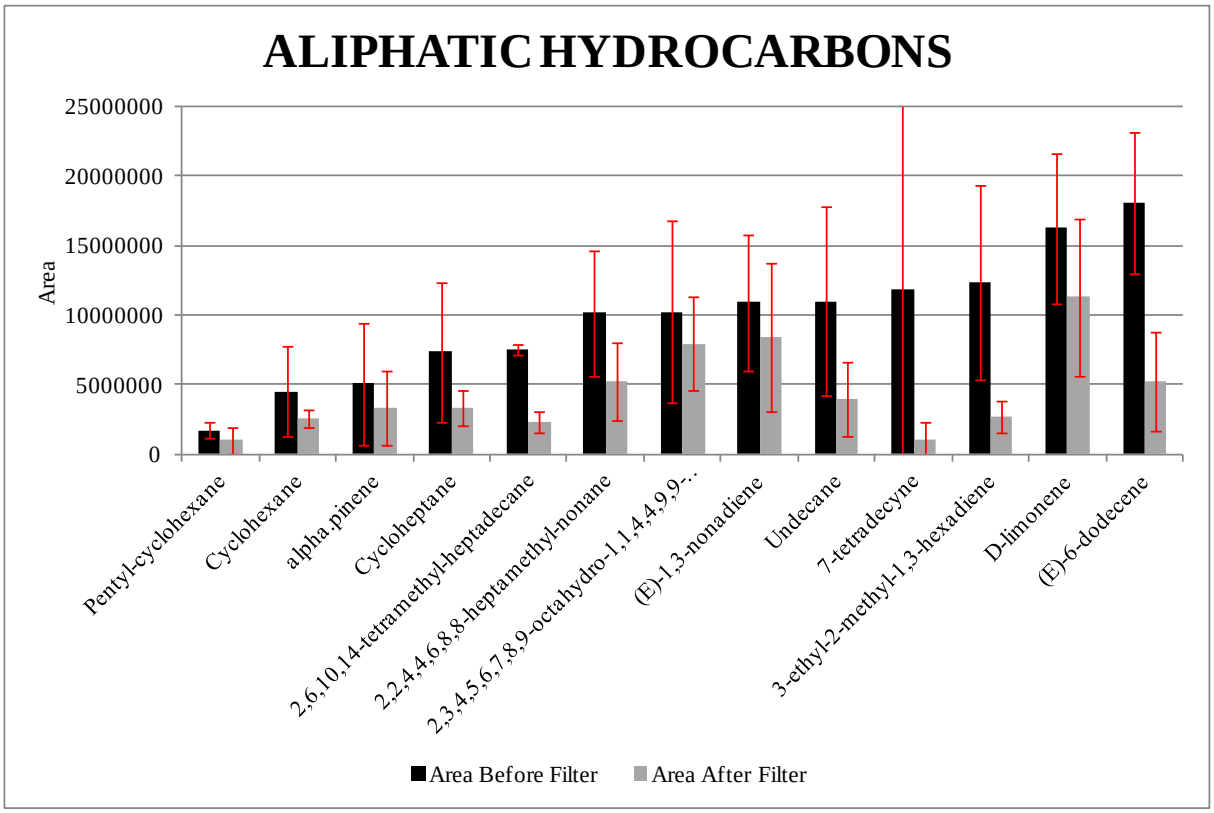
Graph 12. Dejection percentage after filter passage for other compounds



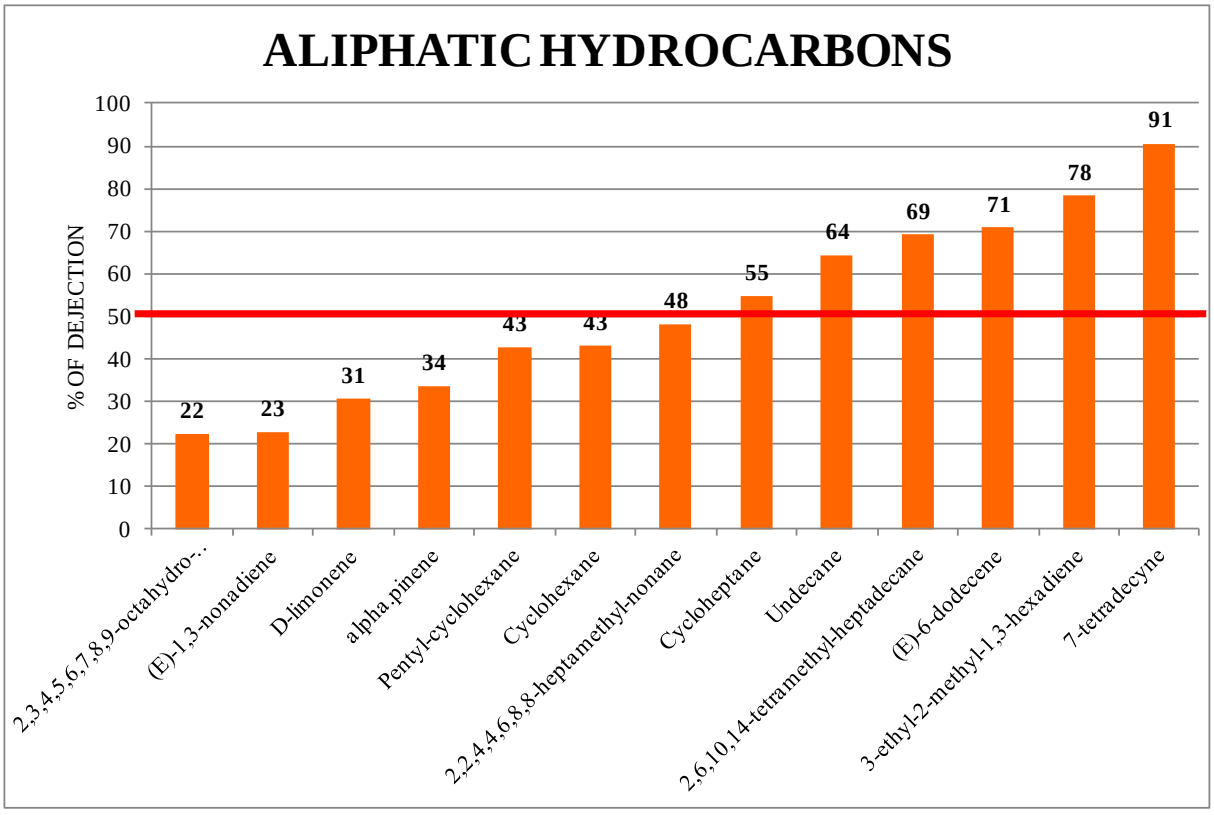
Graph 13. Esters absolute areas before and after passing filter



Graph 14. Dejection percentage after filter passage for esters compounds



Graph 15. Aliphatic hydrocarbons absolute areas before and after passing filter

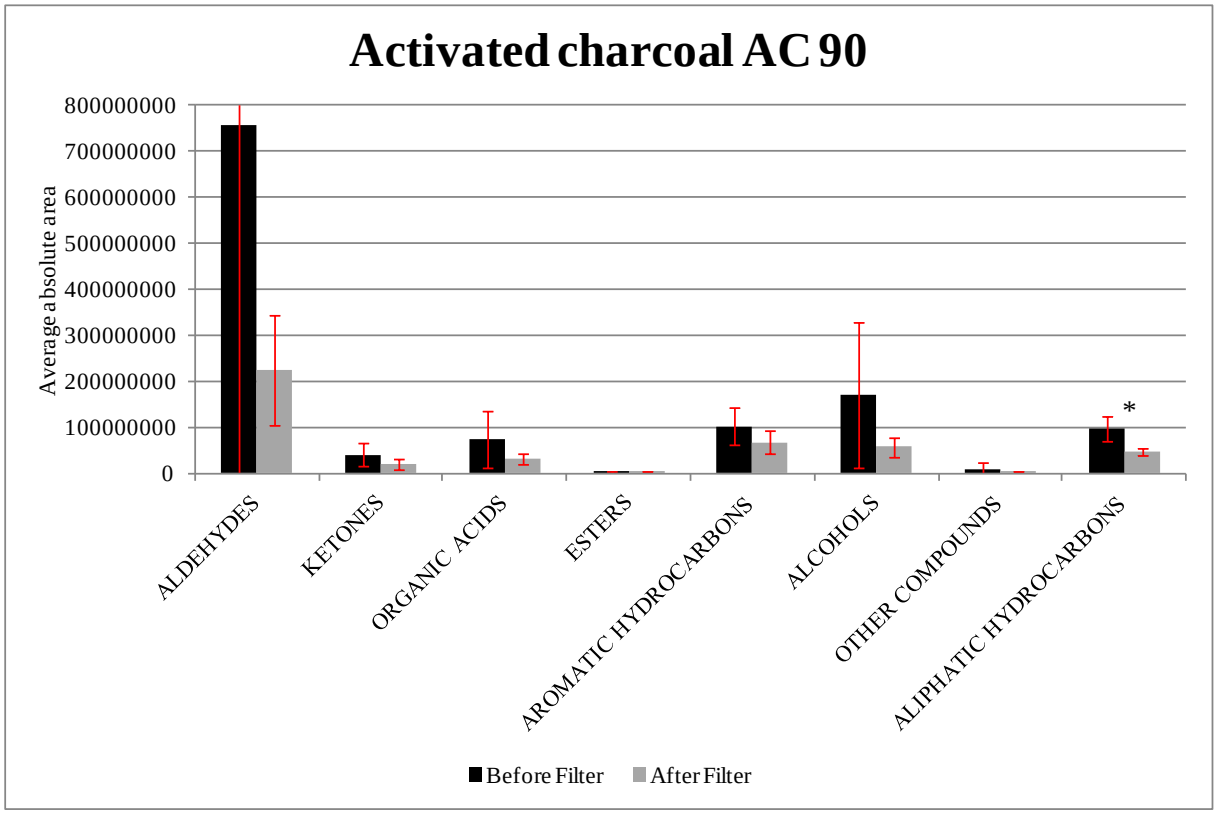


Graph 16. Dejection percentage after filter passage for aliphatic hydrocarbons compounds

Comparing the absolute areas obtained for compounds detected in the air before passing the filter and for the same compounds in the air after passing the filter for each class of compounds (Graph 1, Graph 3, Graph 5, Graph 7, Graph 9, Graph 11, Graph 13 and Graph 15), a decrease of these analytes was noted. In all classes of compounds, the analytes undergo a clear dejection by passing through the filter (Graph 2, Graph 4, Graph 6, Graph 8, Graph 10, Graph 12, Graph 14 and Graph 16). More in detail, as regards aldehyde compounds, it is possible to see an evident decrease of these molecules, in particular of longer carbon chain aldehydes; confirmed also by the percentage of dejection that range from 73% to 93%. On the contrary, in the case of aromatic hydrocarbons, this percentage is always less than 50%, except for only three compounds (2-pentyl-furan, 4-(1-methylpropyl)-phenol and 2-pentyl-pyridine) that shown respectively 73%, 83% and 96% of dejection. Considering also ketone and alcohol categories, a clear decrease was noticed; most of these molecules were dejected with a percentage greater than 50% and this abatement is particularly evident for some alcohols that have almost 100% of dejection. Regarding organic acids, esters and other compounds, there was always a decrement of these molecules after passing through the filter, confirmed also by a high value of dejection. Almost all analytes, in fact, result to be dejected with a percentage greater than 50%. Aliphatic hydrocarbons, had undergone a clear abatement passing through the filter, but this reduction is quite variable, because the values range from 22% to 91%.

In particular, considering the average percentage of dejection for each class of compounds (as it is possible to see in Graph 58), this value is more marked for categories like aldehydes, organic acids, aliphatic hydrocarbons, esters and other compounds, with values ranging from 52% to 70%. As regards ketones, alcohols and aromatic hydrocarbons categories, this percentage is always below 50%, as possible to see in Graph 58.

Moreover, as regards this kind of filter, the differences in the extent of dejection are statistically significant ($P < 0.05$) for the aliphatic hydrocarbons category (Graph 17).



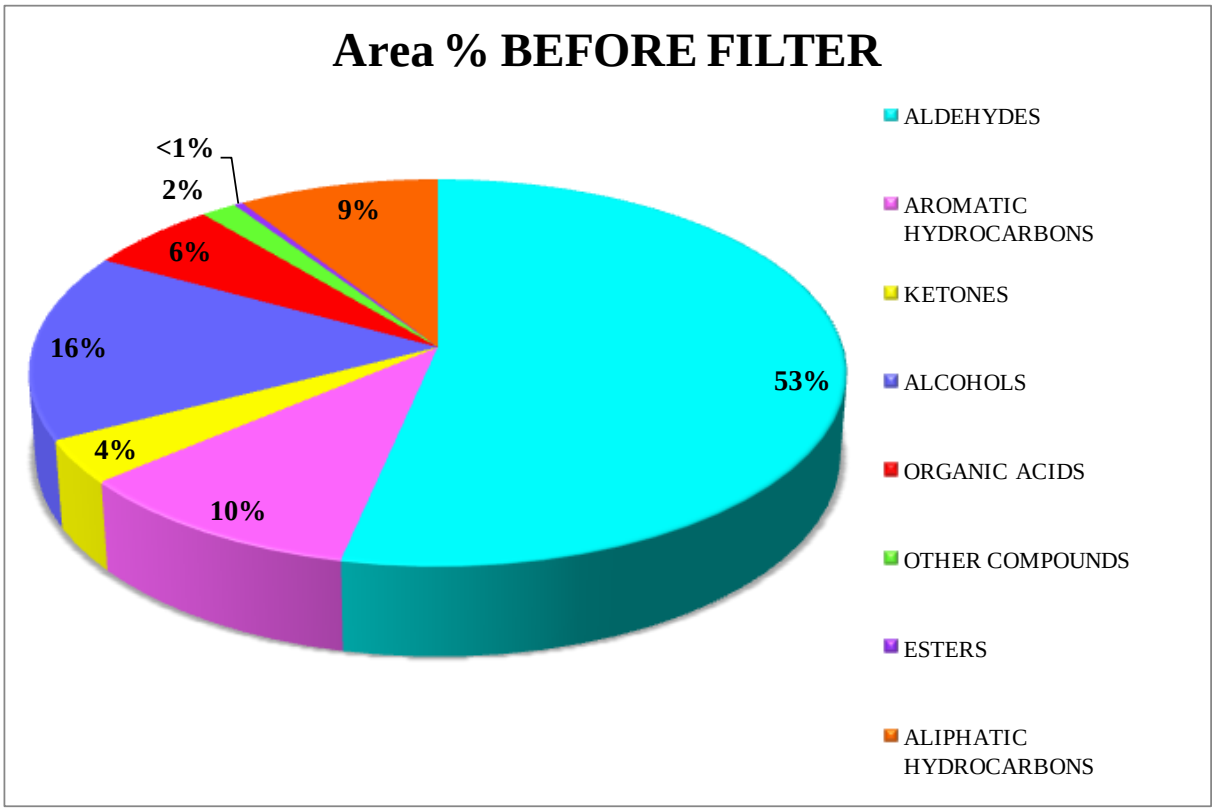
Graph 17. Comparison of the average absolute areas (± standard deviation) of different classes of extracted compounds. Significant differences ($P < 0.05$, one-way ANOVA and Tukey's test for pairwise comparison) are indicated by "*".

From a qualitative point of view, considering that analytes were grouped into aldeydes, aromatic hydrocarbons, ketones, aliphatic hydrocarbons and so on, and assuming that these molecules give a similar analytical response, the area percentage before filter and the area percentage after filter for each class of detected compounds were considered (Table 5). From the Graph 18 and Graph 19, it is possible to see that aldehydes, alcohols and other compounds classes decrease passing through the filter, while organic acids, aliphatic hydrocarbons, ketones and aromatic hydrocarbon compounds increase. Nevertheless, it is important to consider that these results (expressed in percentage) are relative to the sample composition before and after passing filter. In fact, as discussed above, all classes of compounds undergo a reduction passing through the filter.

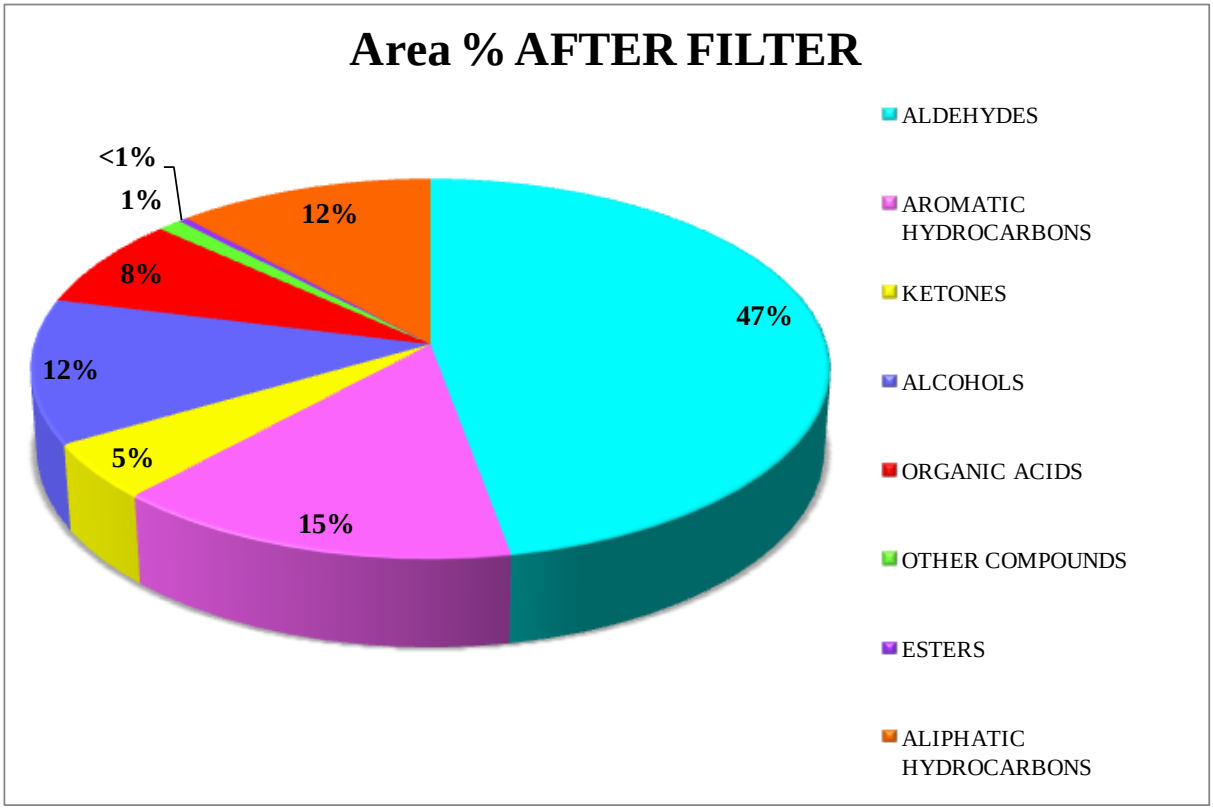
CLASSES OF COMPOUNDS	**BEFORE FILTER**		**AFTER FILTER**	
	Area %	**S.D.**	**Area %**	S.D.
ALDEHYDES	53	15	47	9
AROMATIC HYDROCARBONS	10	4	15	7
KETONES	4	5	5	3
ALCOHOLS	16	4	12	2
ORGANIC ACIDS	6	2	8	1
OTHER COMPOUNDS	2	<1	1	<1
ESTERS	<1	<1	<1	<1
ALIPHATIC HYDROCARBONS	9	7	12	3

Table 5. Area percentage before and after filter for each class of compounds

Anyway, considering the area percentage composition before filter and after filter, from a statistical point of view, there are not significant differences for each class of detected compounds.



Graph 18. Percentage composition of air before filter



Graph 19. Percentage composition of air after filter

1.3.7 Washable filter 1100-6 1400-11

In this section the results obtained with washable filter 1100-6 1400-11, are reported. In the figures below, an example of chromatogram obtained for air before filter (Figure 33) and air after filter (Figure 34), are shown.

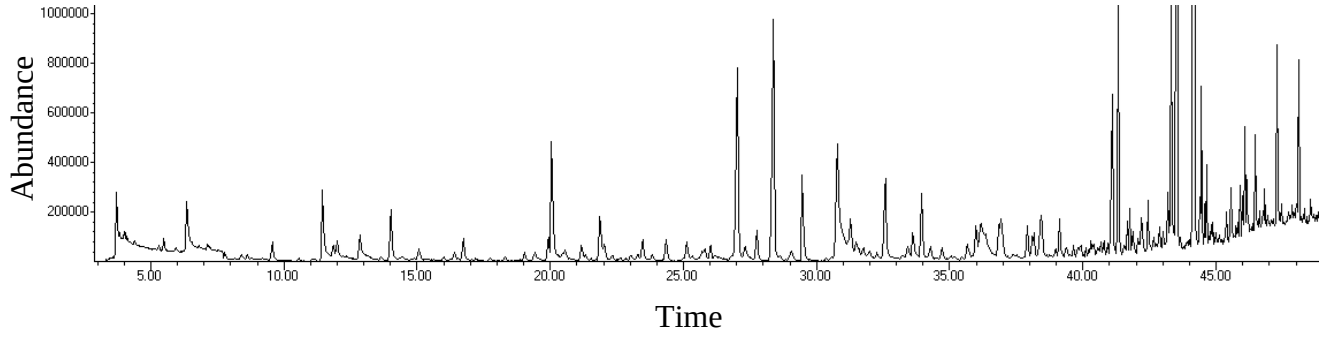


Figure 33. Chromatogram obtained for air before filter

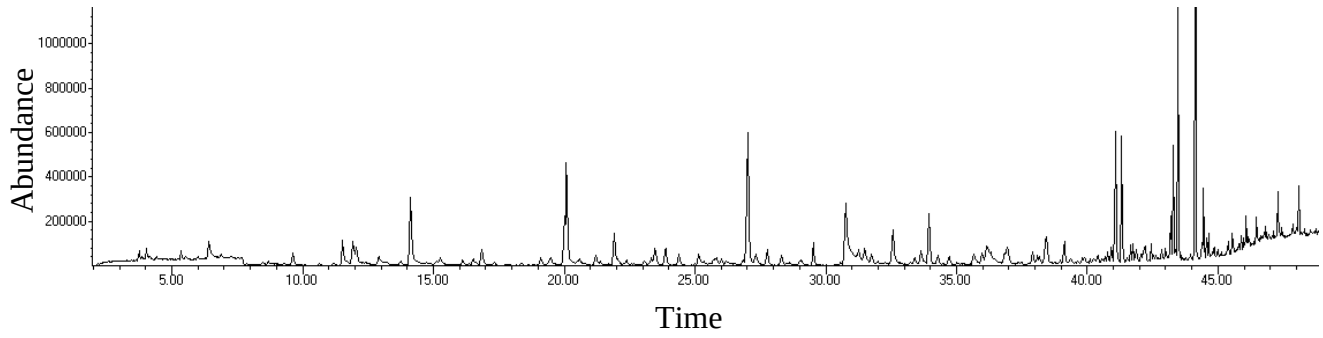
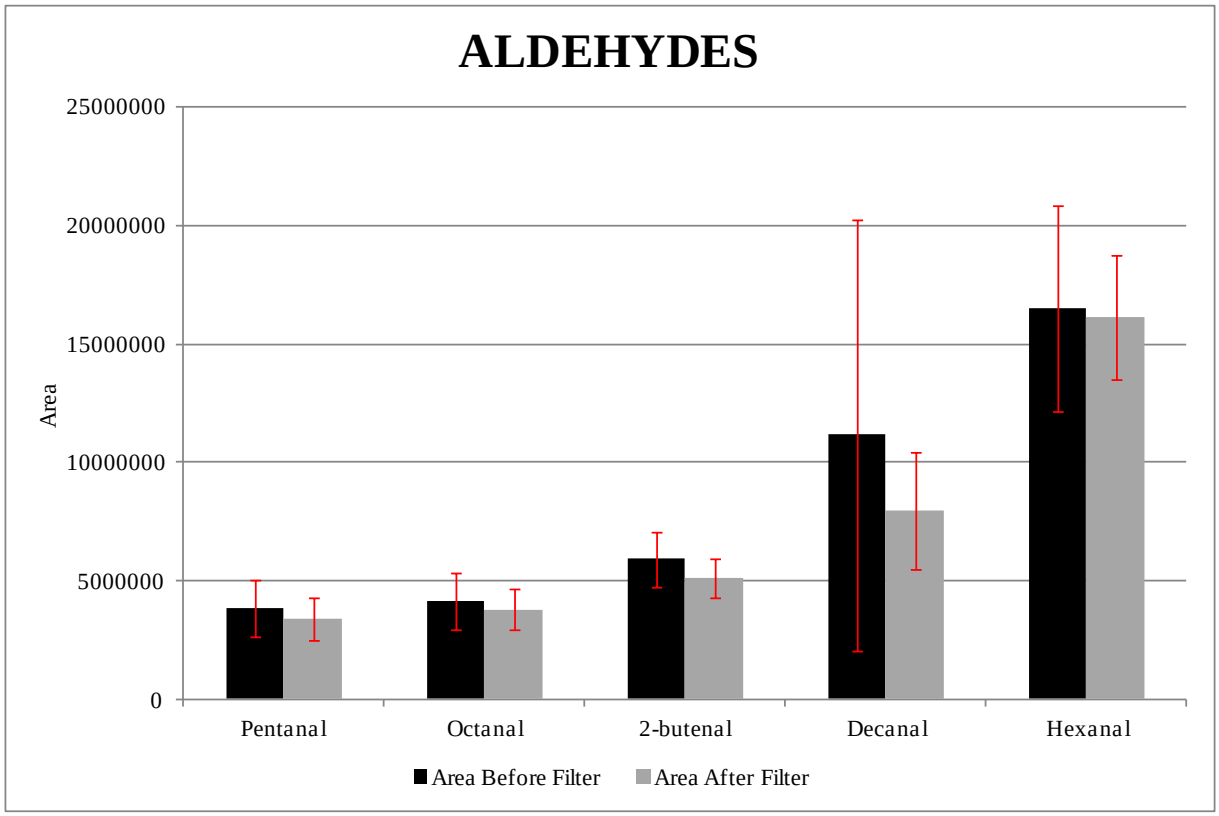


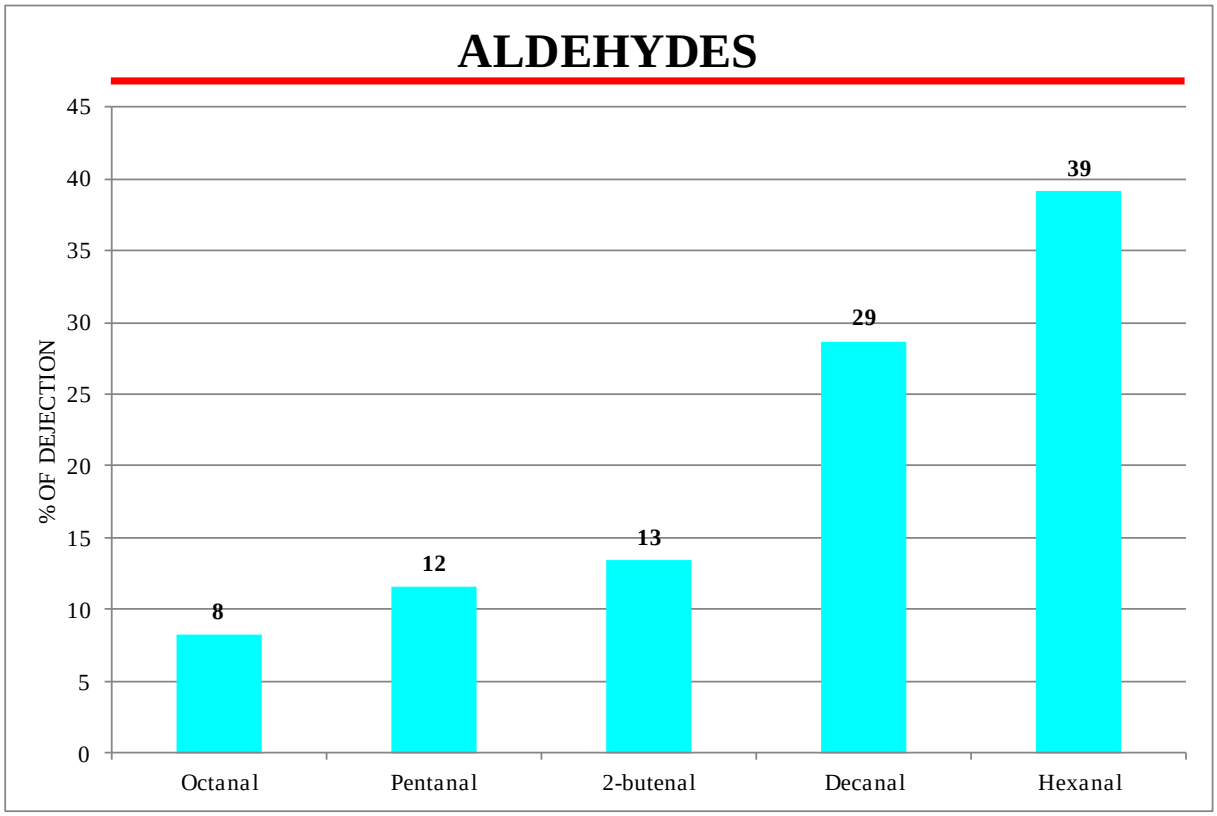
Figure 34. Chromatogram obtained for air after filter

Also in this case, the detected compounds were divided into different classes and for each molecule a comparison between the absolute area before and after passing through the filter and the dejection percentage were reported.

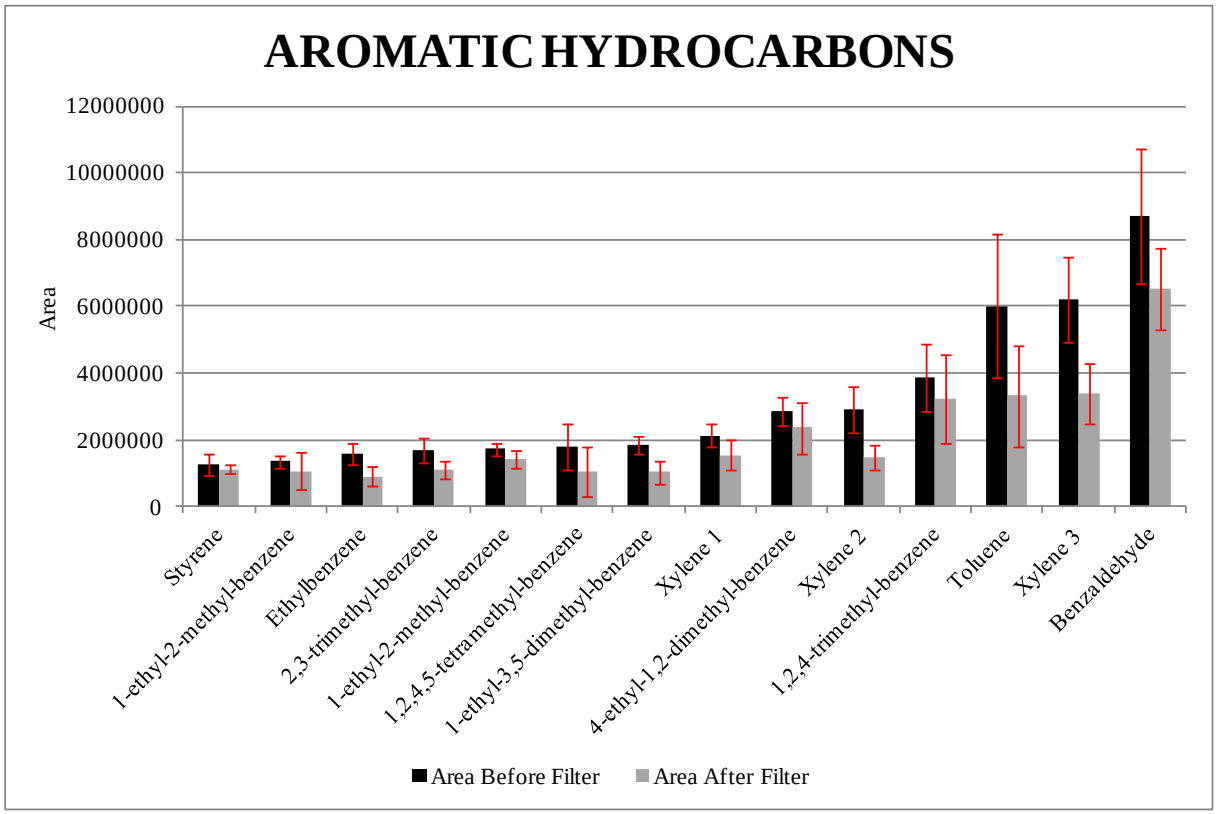
This type of filter is more expensive than the activated charcoal AC 90 filter, it is washable and regenerable at high temperature and, in terms of filtering, it should provide better performance than the previous one (activated charcoal AC 90).



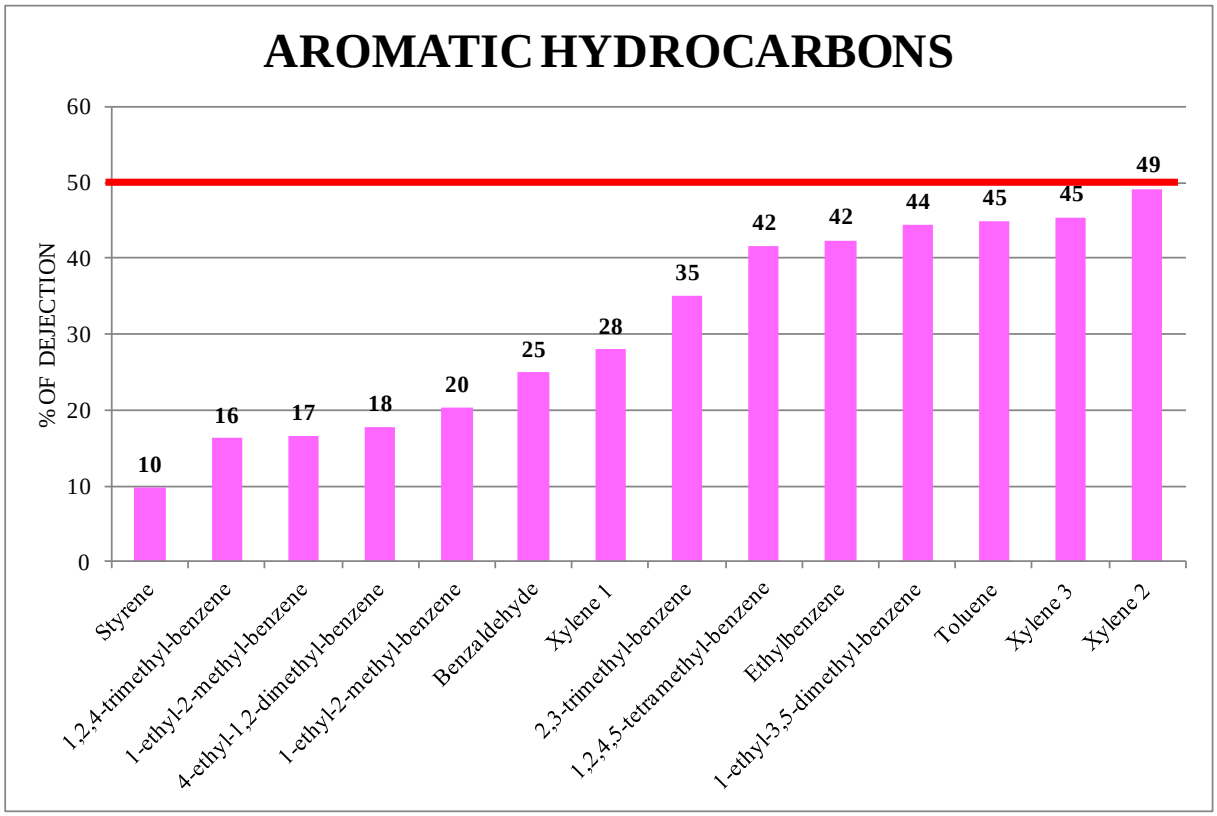
Graph 20. Aldehydes absolute areas before and after passing filter



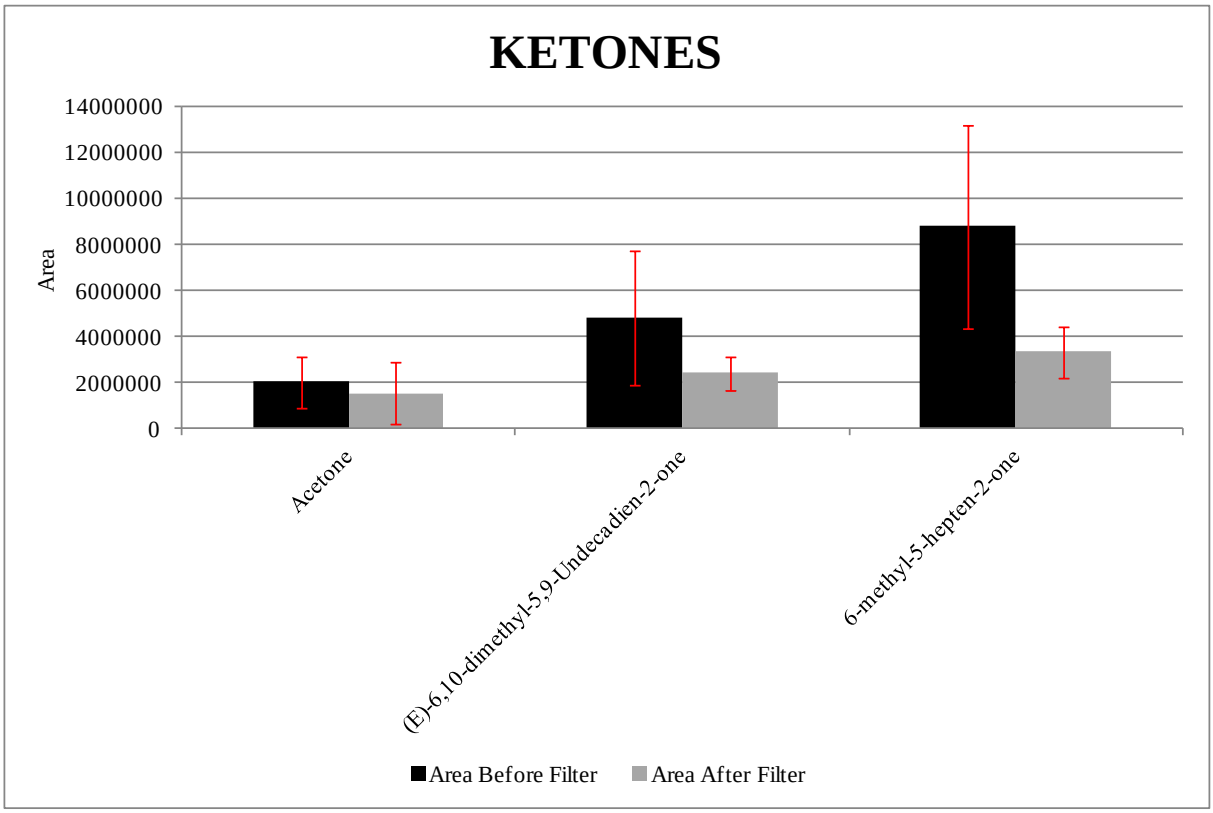
Graph 21. Dejection percentage after filter passage for aldehydes compounds



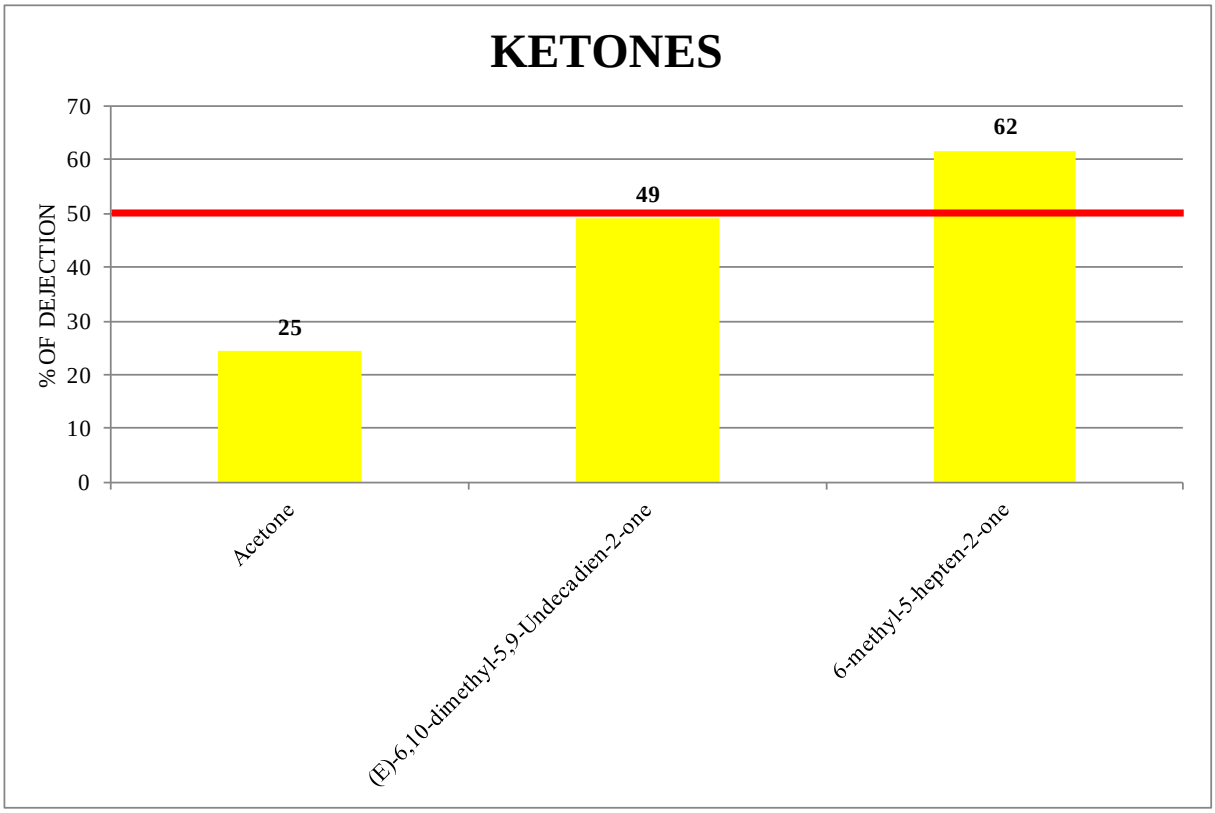
Graph 22. Aromatic hydrocarbons absolute areas before and after passing filter



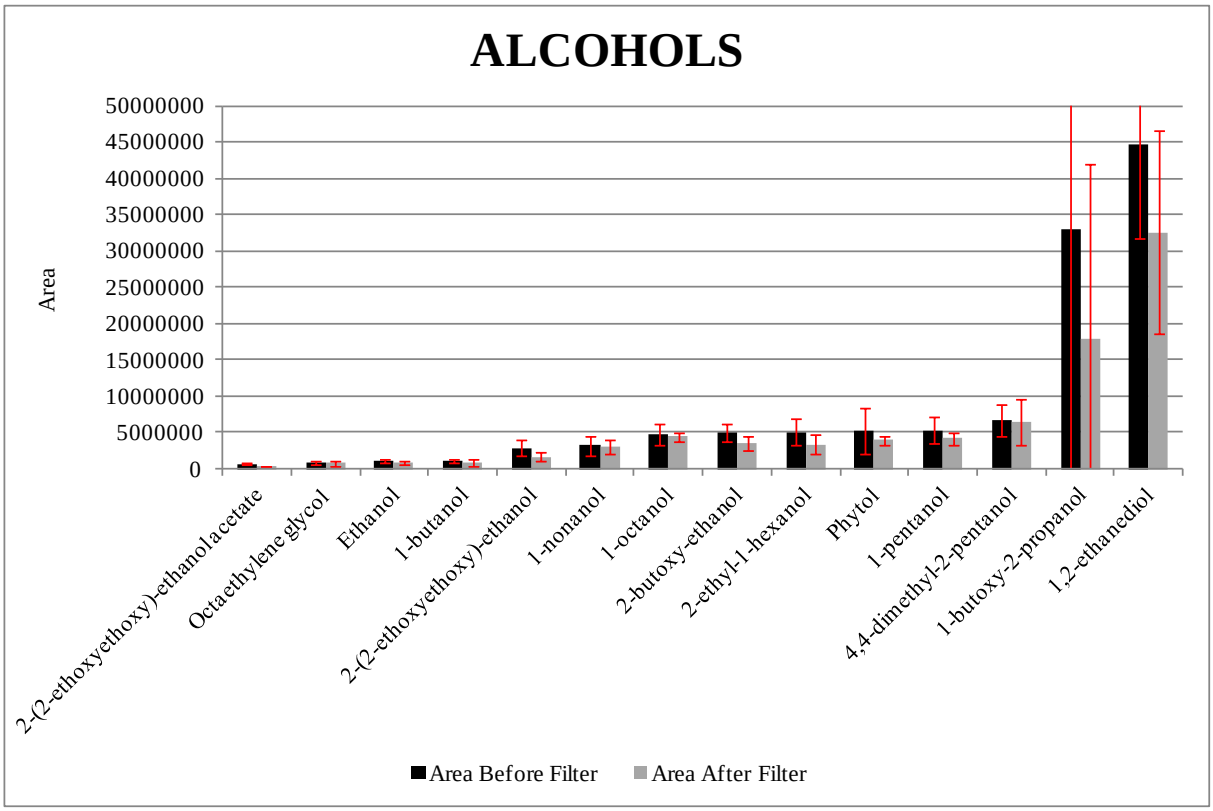
Graph 23. Dejection percentage after filter passage for aromatic hydrocarbons compounds



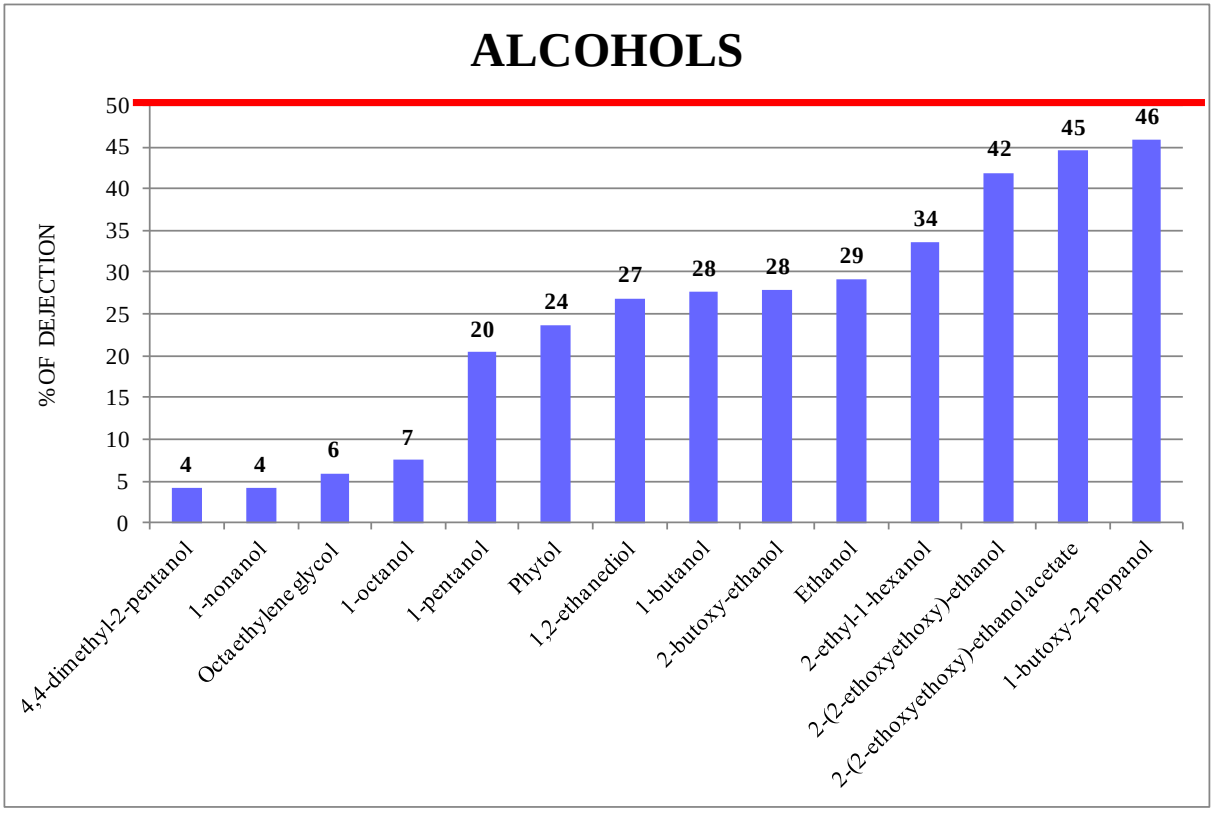
Graph 24. Ketones absolute areas before and after passing filter



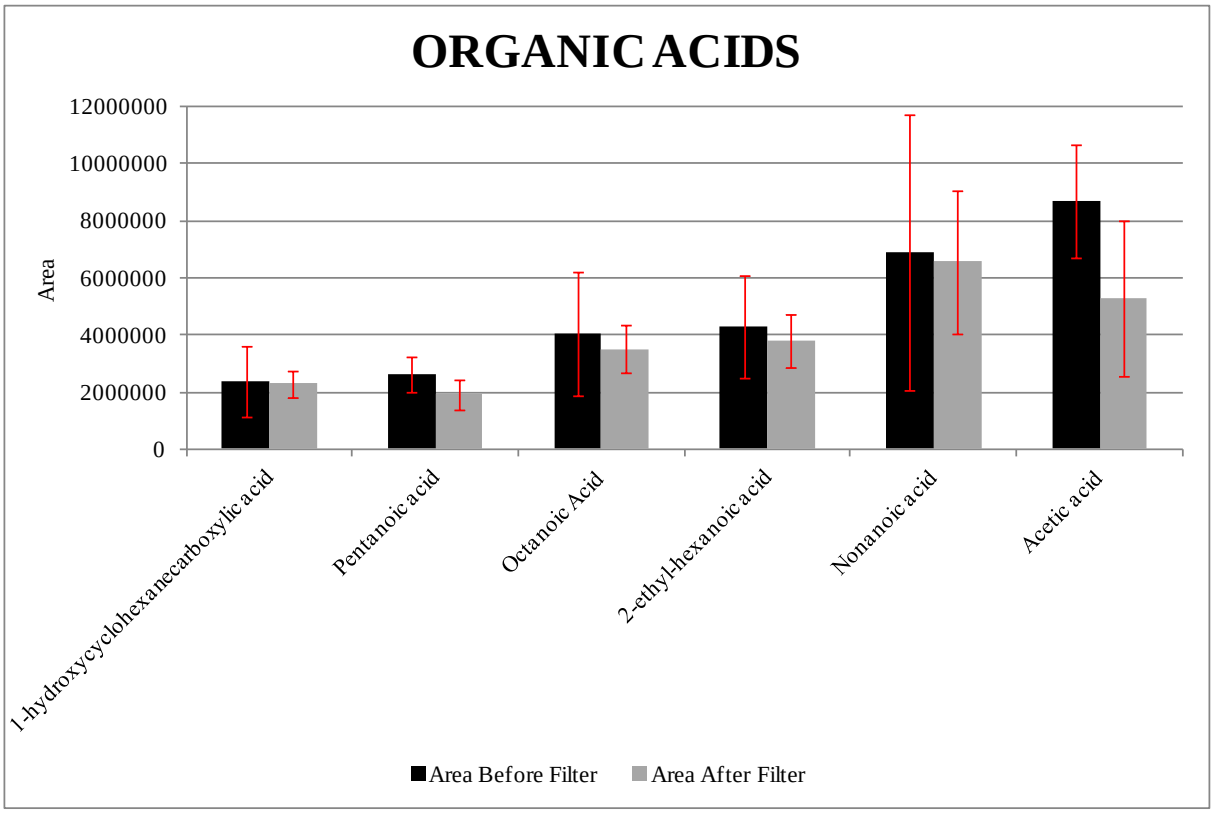
Graph 25. Dejection percentage after filter passage for ketones compounds



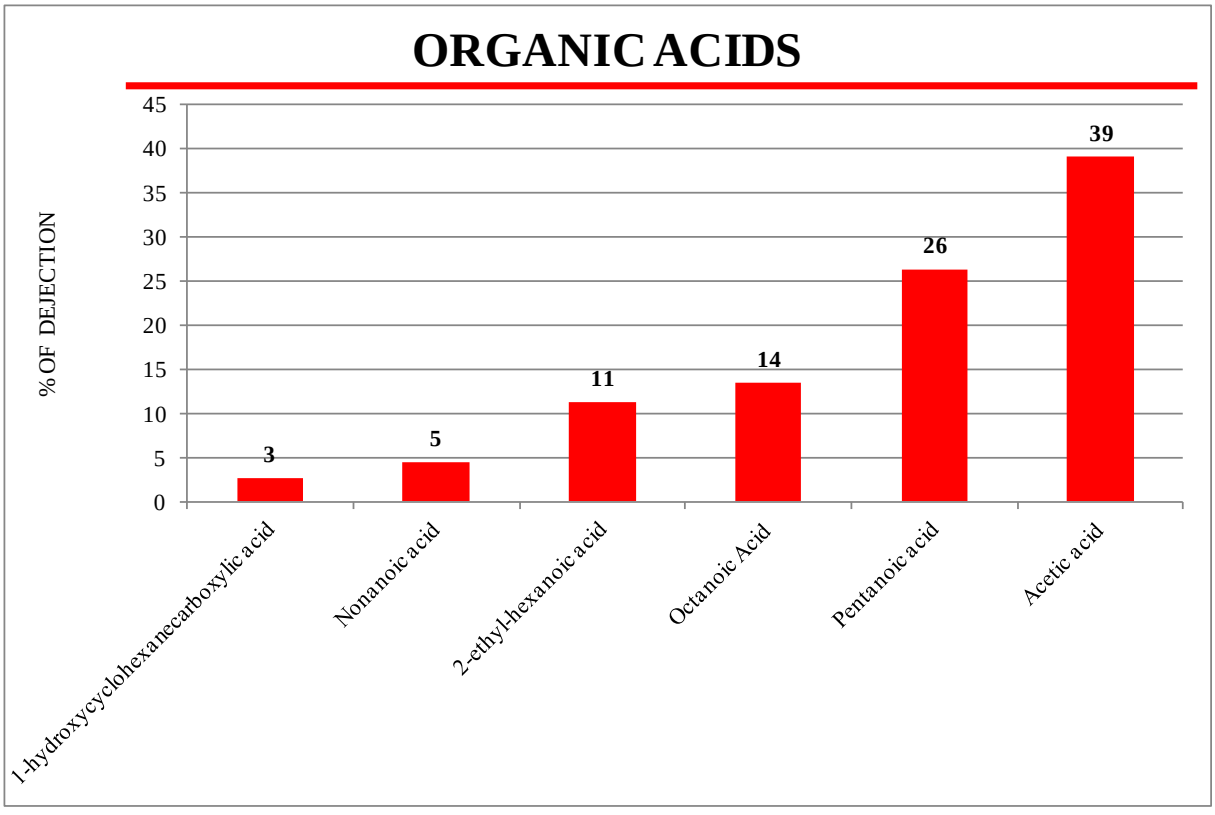
Graph 26. Alcohols absolute areas before and after passing filter



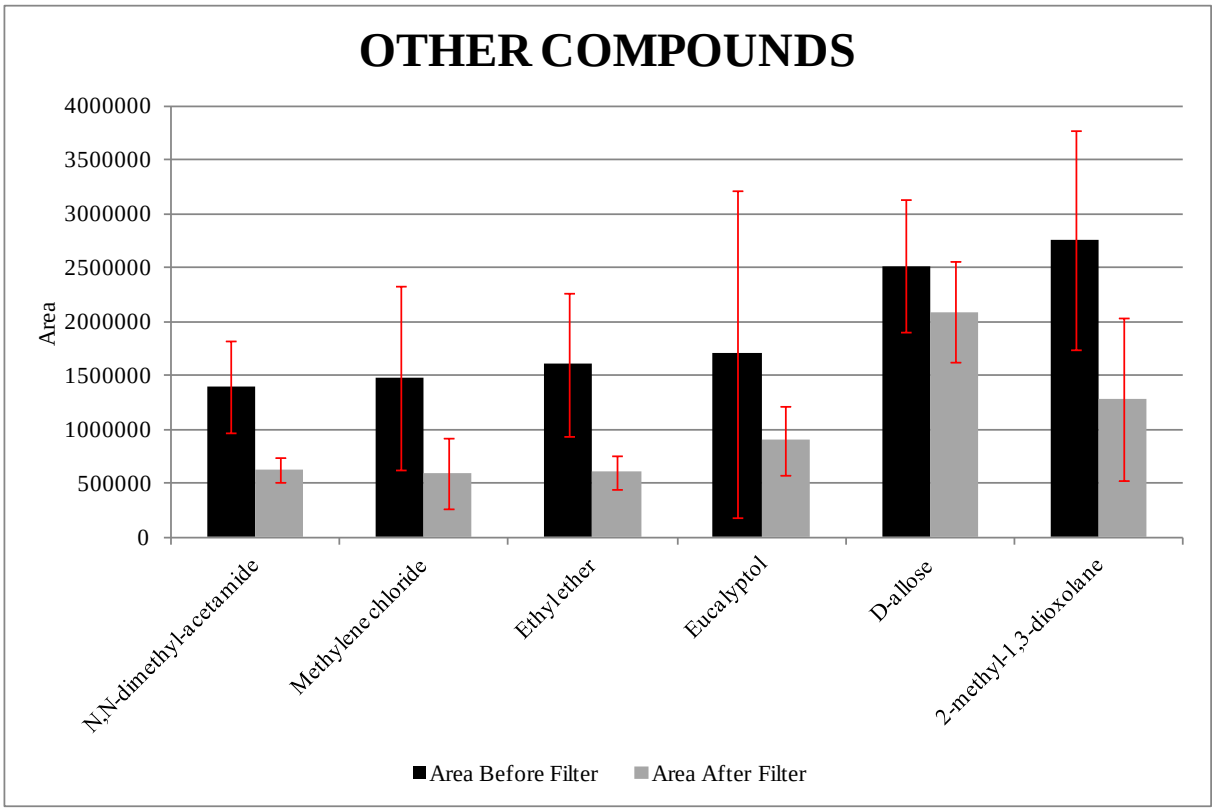
Graph 27. Dejection percentage after filter passage for alcohols compounds



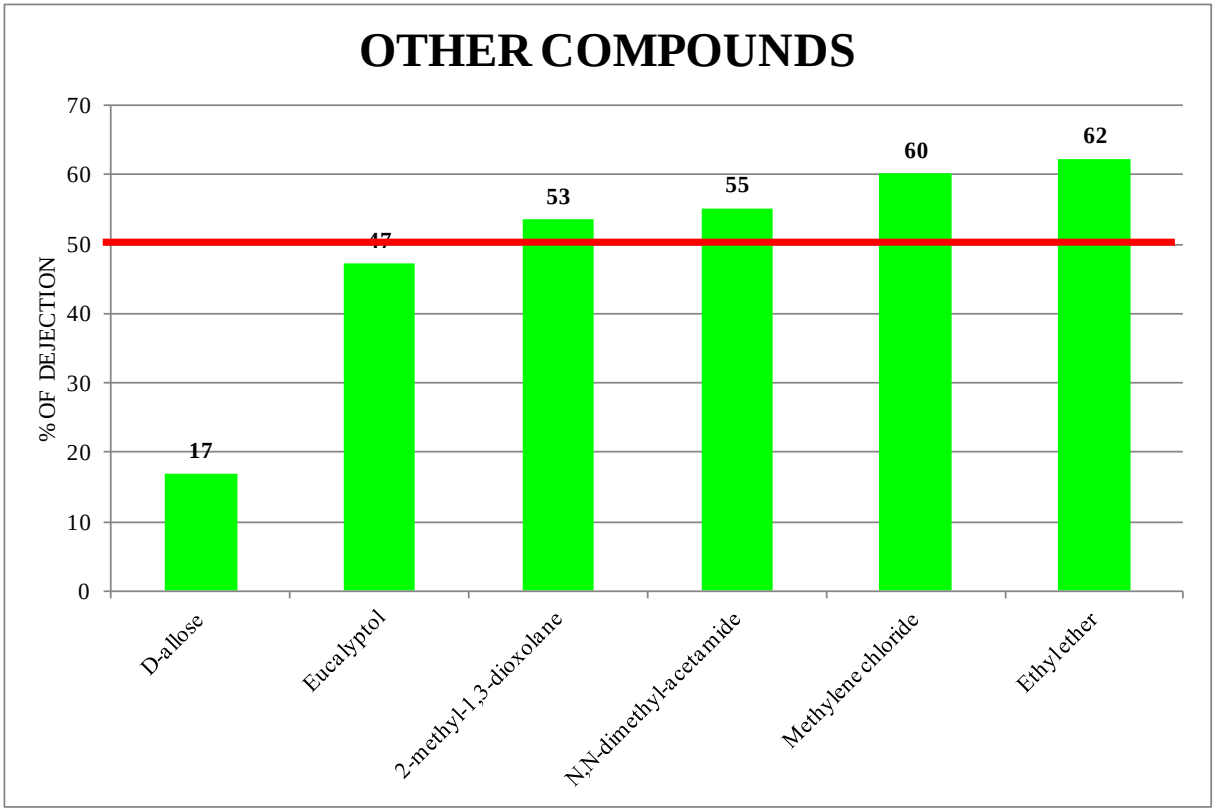
Graph 28. Organic acids absolute areas before and after passing filter



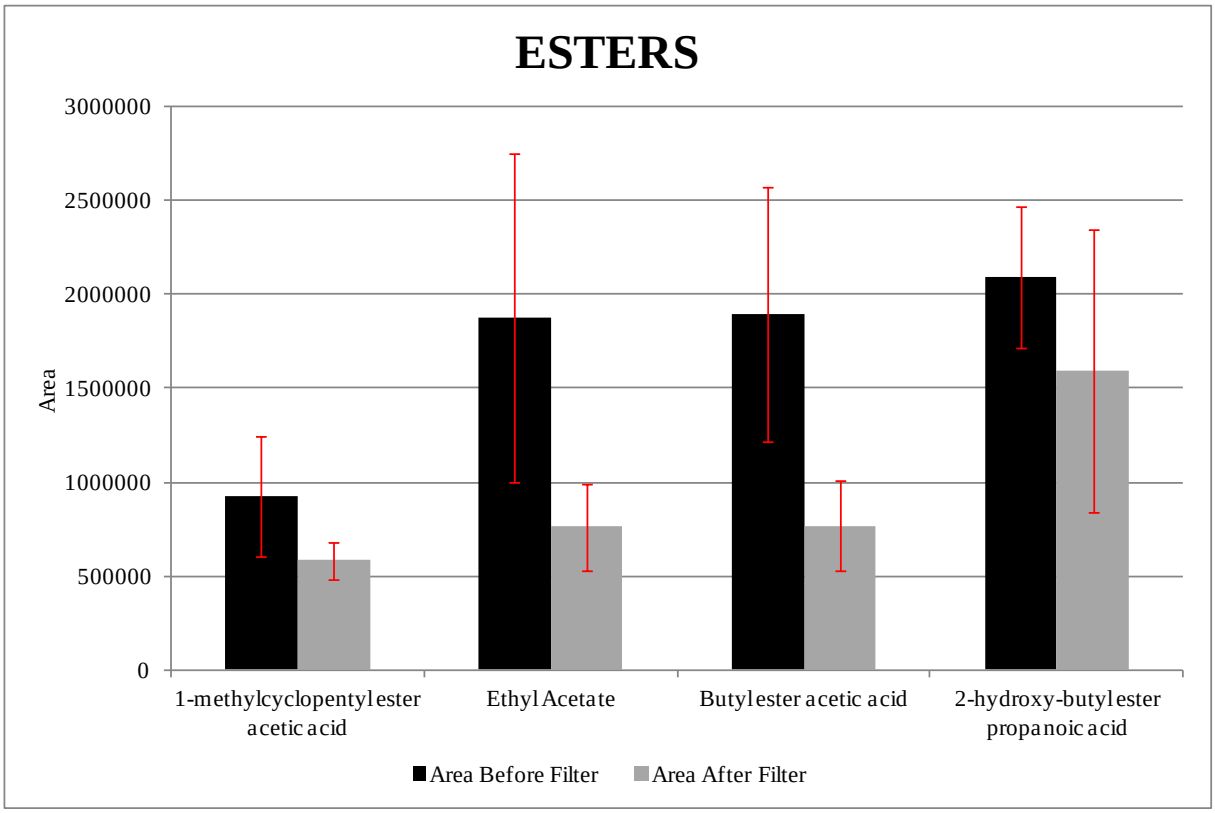
Graph 29. Dejection percentage after filter passage for organic acids compounds



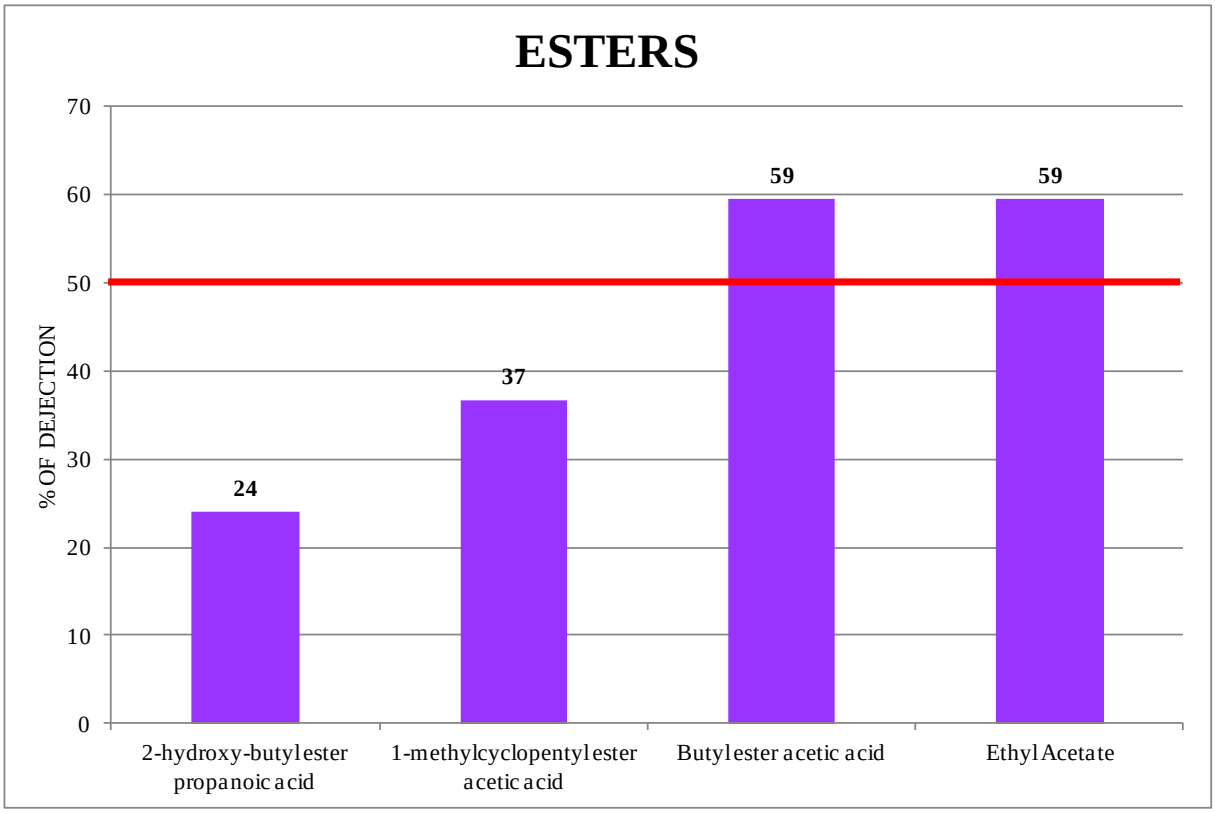
Graph 30. Other compounds absolute areas before and after passing filter



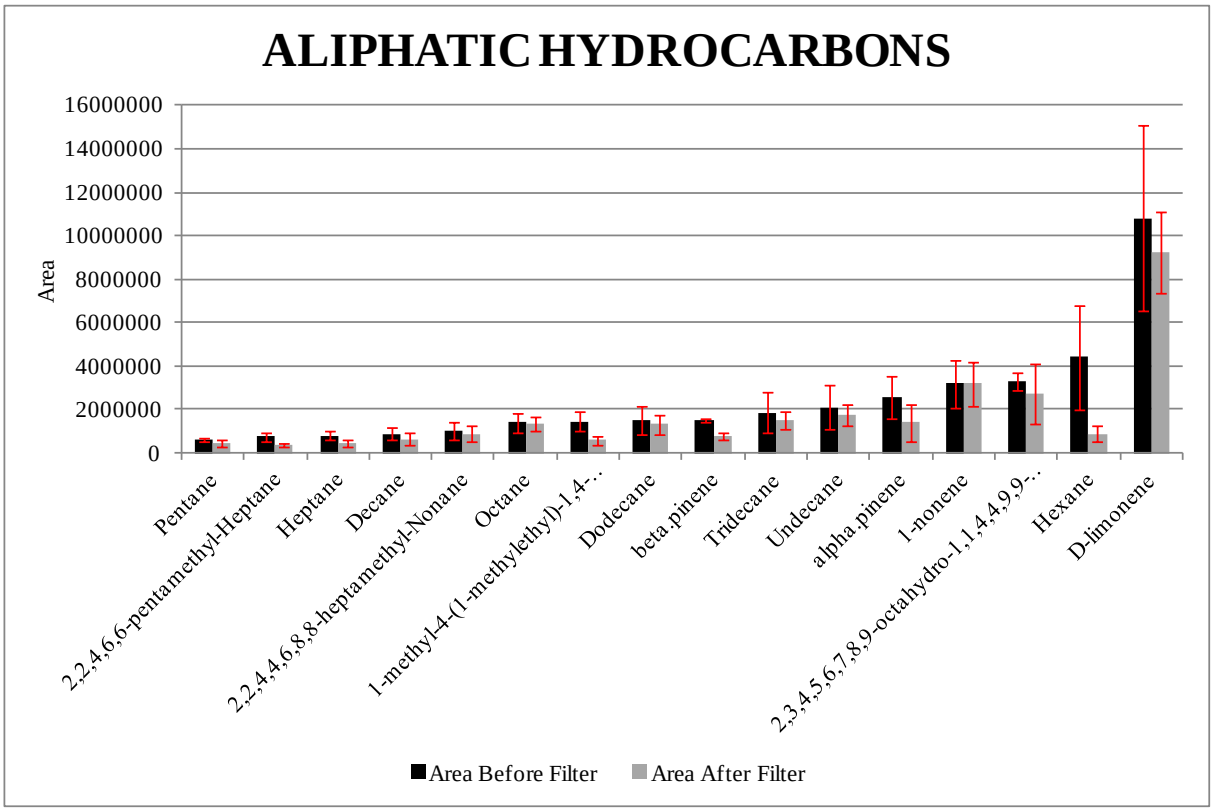
Graph 31. Dejection percentage after filter passage for other compounds



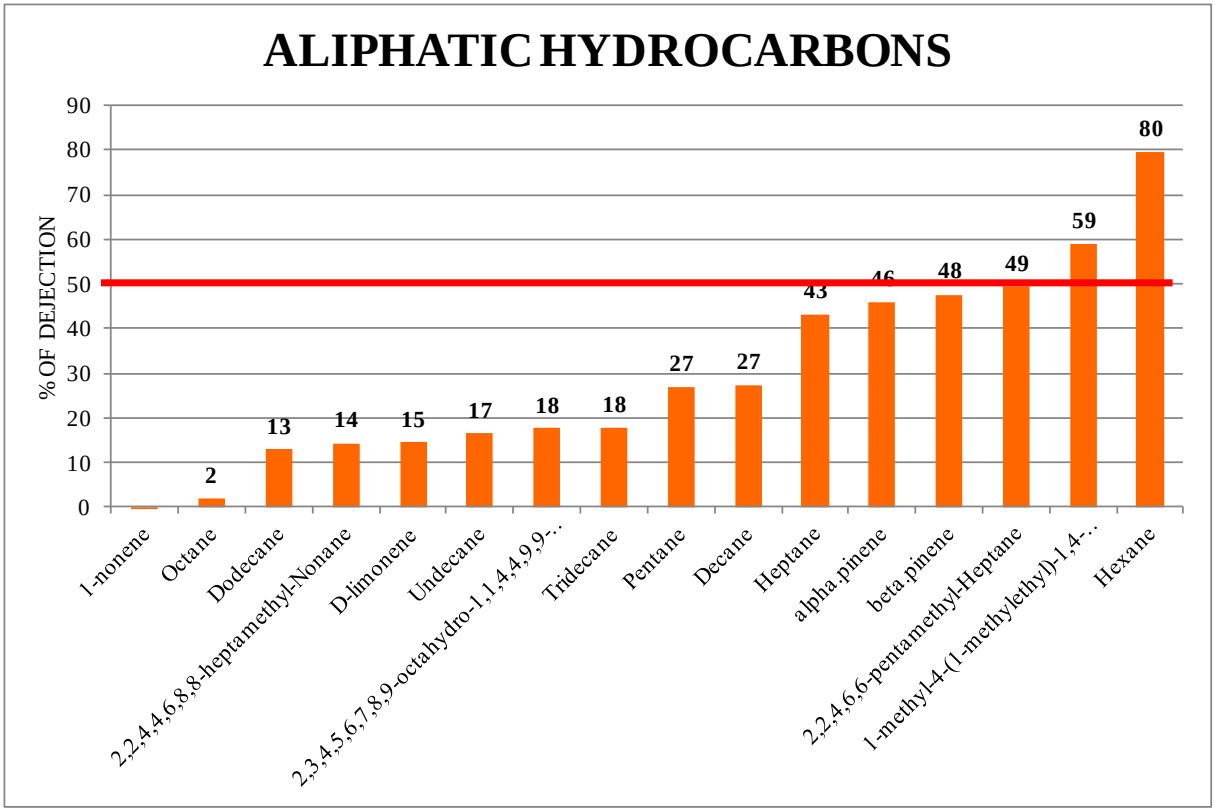
Graph 32. Esters absolute areas before and after passing filter



Graph 33. Dejection percentage after filter passage for esters compounds



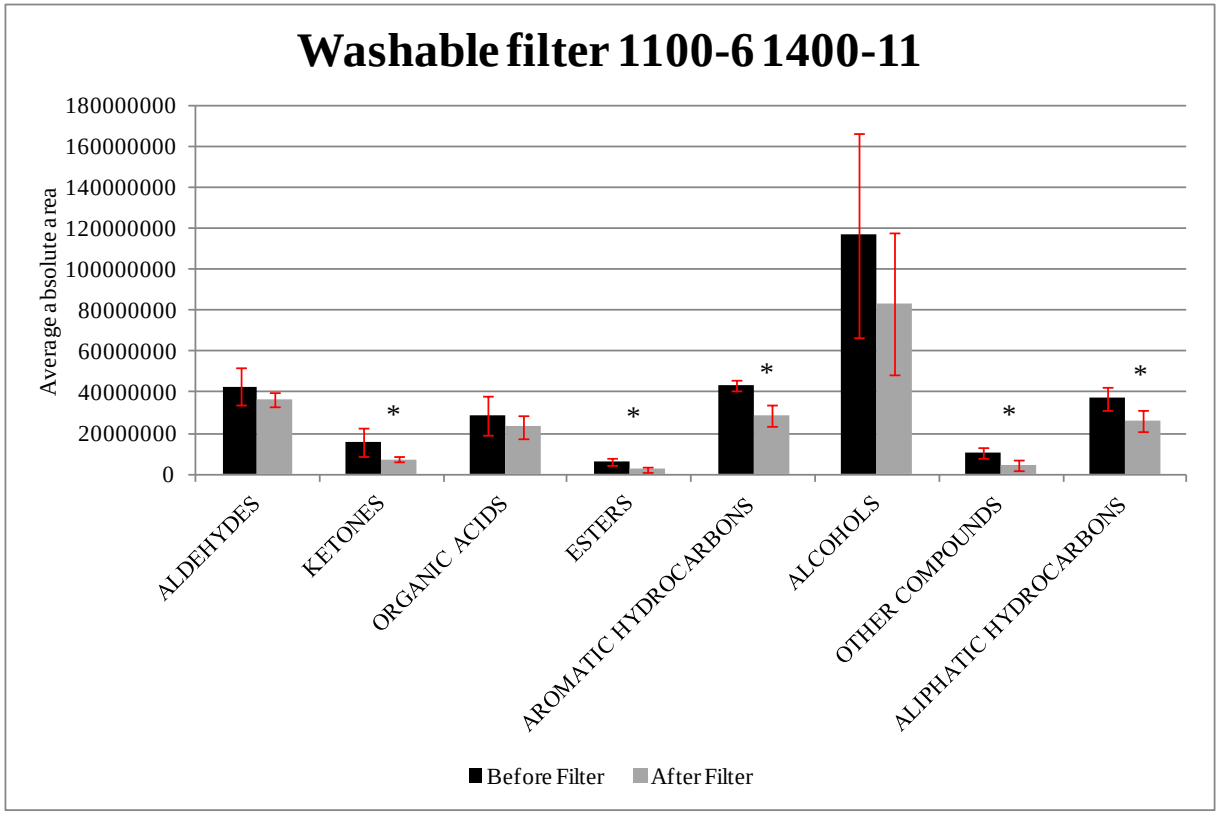
Graph 34. Aliphatic hydrocarbons absolute areas before and after passing filter



Graph 35. Dejection percentage after filter passage for aliphatic hydrocarbons compounds

The results obtained from this second type of filter (washable filter 1100-6 1400-11) are completely different. Also in this case, comparing the absolute areas obtained before filter and after filter, all the analytes decrease by passing through the filter, regardless of their category (Graph 20, Graph 22, Graph 24, Graph 26, Graph 28, Graph 30, Graph 32 and Graph 34). Anyway this reduction is always below 50% for each class of compounds considered (Graph 59). More specifically, aldehydes were dejected with a range from 2% to 39% (Graph 21), aromatic hydrocarbons were shot down with a range from 10% to 49% (Graph 23), alcohols and organic acids classes were respectively dejected with a range 4% - 46% (Graph 27) and 3% - 39% (Graph 29). As regards classes of ketones, esters and other compounds, some of these molecules were dejected with a percentage greater than 50% (Graph 25, Graph 33 and Graph 31) in which the following range of dejection is observed: 25% - 62% for ketones, 17% - 62% for other compounds and 24% - 59% for esters. Nevertheless, an average percentage of dejection lower than 50% is recorded: 45% for ketones, 49% for other compounds and 45% for esters (see Graph 59). In the case of aliphatic hydrocarbon compounds (Graph 35), they were dejected below 50%, except for 1-methyl-4-(1-methylethyl)-1,4-cyclohexadiene (percentage of dejection of 59%) and hexane (percentage of dejection of 80%). Anyway, an average percentage of dejection of 30% is obtained for this class of compounds.

For this kind of filter, the differences in the extent of dejection are statistically significant ($P < 0.05$) for the categories of ketones, esters, aromatic hydrocarbons, aliphatic hydrocarbons and of "other compounds" (Graph 36).



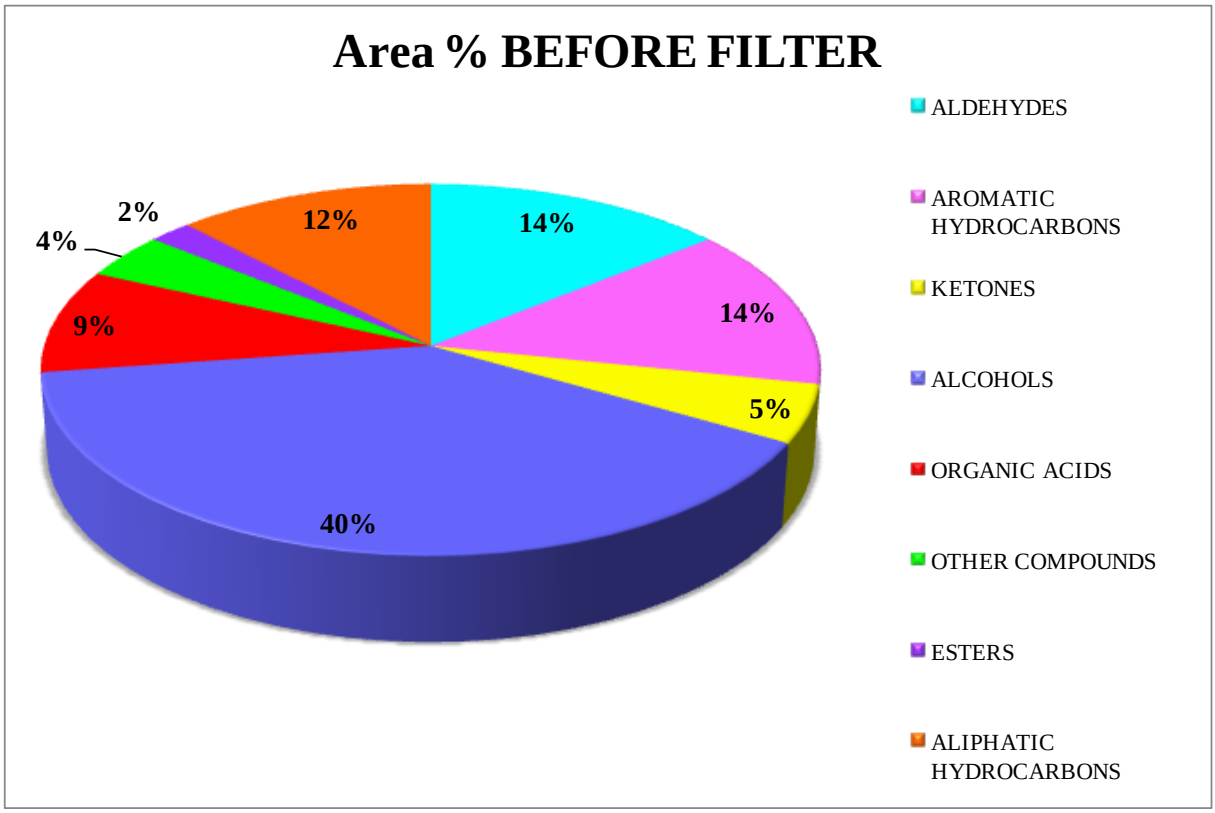
Graph 36. Comparison of the average absolute areas (± standard deviation) of different classes of extracted compounds. Significant differences ($P < 0.05$, one-way ANOVA and Tukey's test for pairwise comparison) are indicated by "*".

Considering the qualitative aspect, also in this case, the area percentage before filter and after filter for each class of detected compounds were calculated, as it is possible to see in the table below (Table 6). The air composition before filter and after filter is reported in the graph below (Graph 37 and Graph 38).

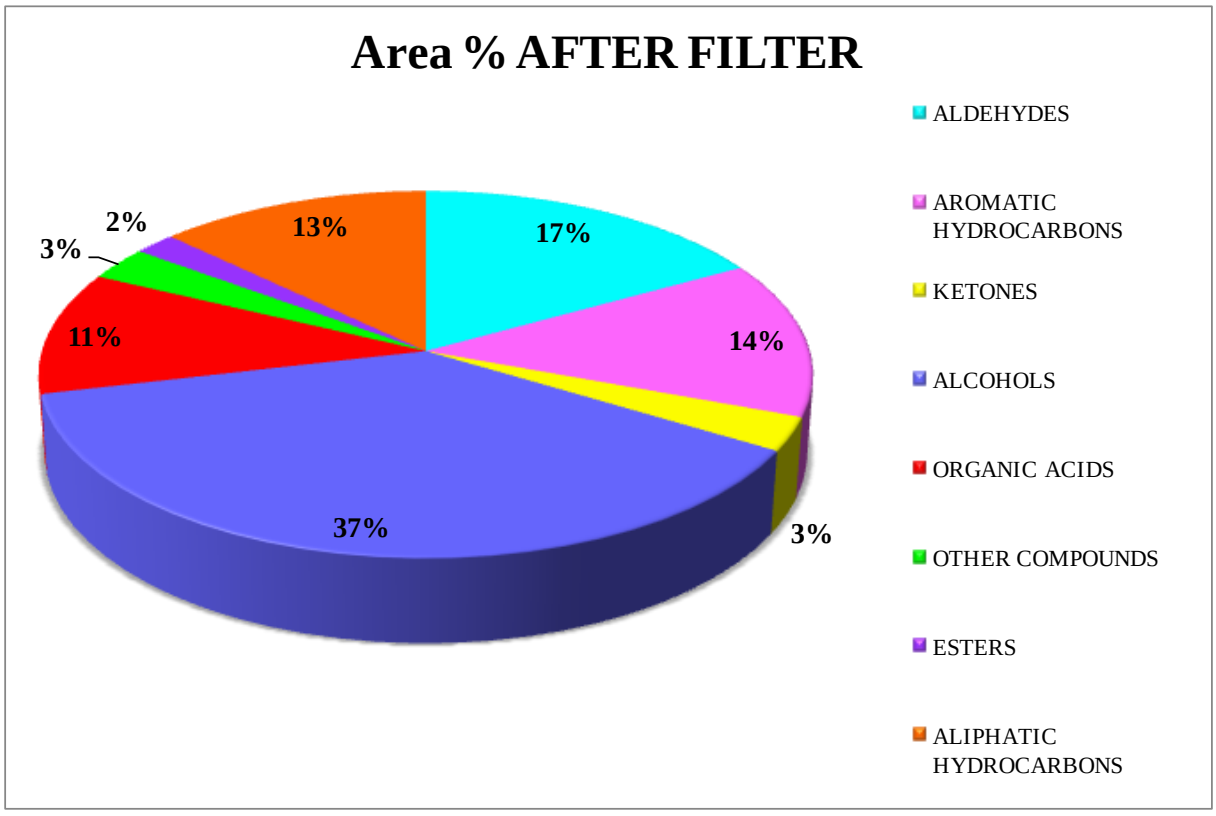
CLASSES OF COMPOUNDS	BEFORE FILTER		AFTER FILTER	
	Area %	S.D.	Area %	S.D.
ALDEHYDES	14	3	17	4
AROMATIC HYDROCARBONS	14	3	14	3
KETONES	5	3	3	1
ALCOHOLS	39	10	38	8
ORGANIC ACIDS	9	3	11	1
OTHER COMPOUNDS *	4	1	3	1
ESTERS *	2	1	2	1
ALIPHATIC HYDROCARBONS	12	3	13	2

Table 6. Area percentage before and after filter for each class of compounds

Considering the area percentage composition before filter and after filter, from a statistical point of view, there are significant differences ($P < 0.05$, one-way ANOVA and Tukey's test for pairwise comparison) for the categories of esters and “other compounds”, indicated in the table with “*”.



Graph 37. Percentage composition of air before filter



Graph 38. Percentage composition of air after filter

1.3.8 Helsa-Sorbexx-CS filter

The Helsa-Sorbexx-CS filter results are reported in this section. In the figures below, an example of chromatogram obtained for air before filter (Figure 35) and air after filter (Figure 36), are shown.

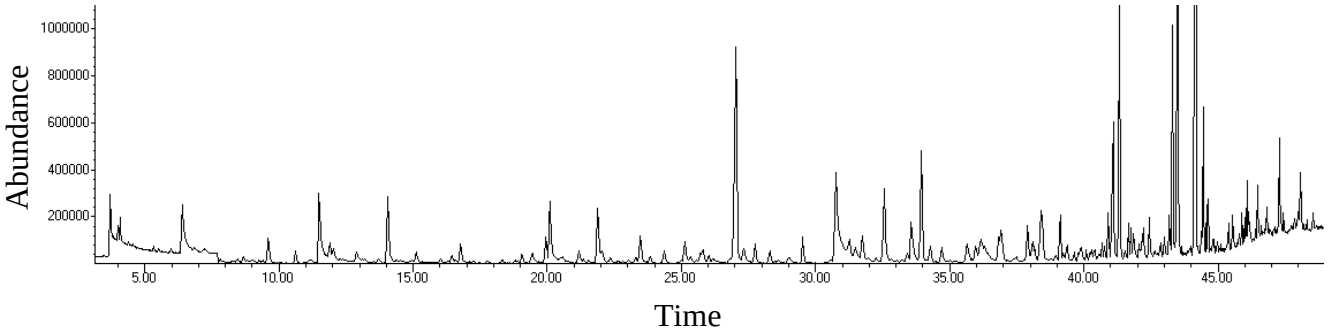


Figure 35. Chromatogram obtained for air before filter

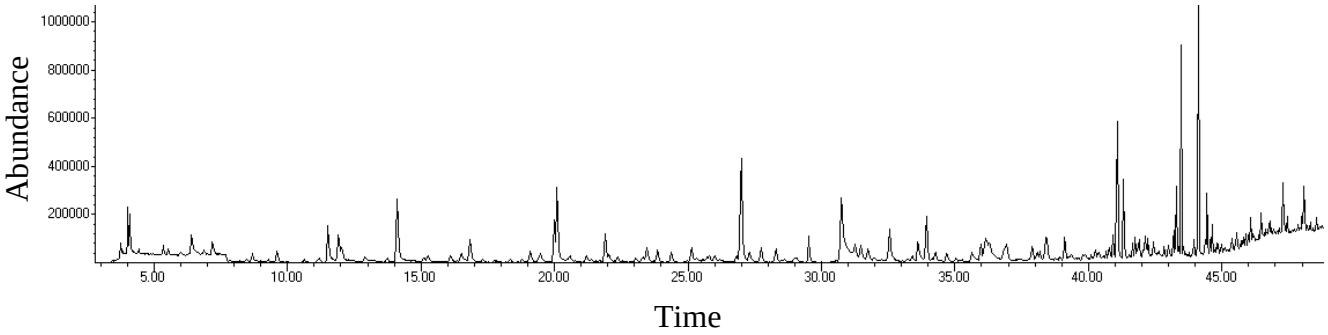
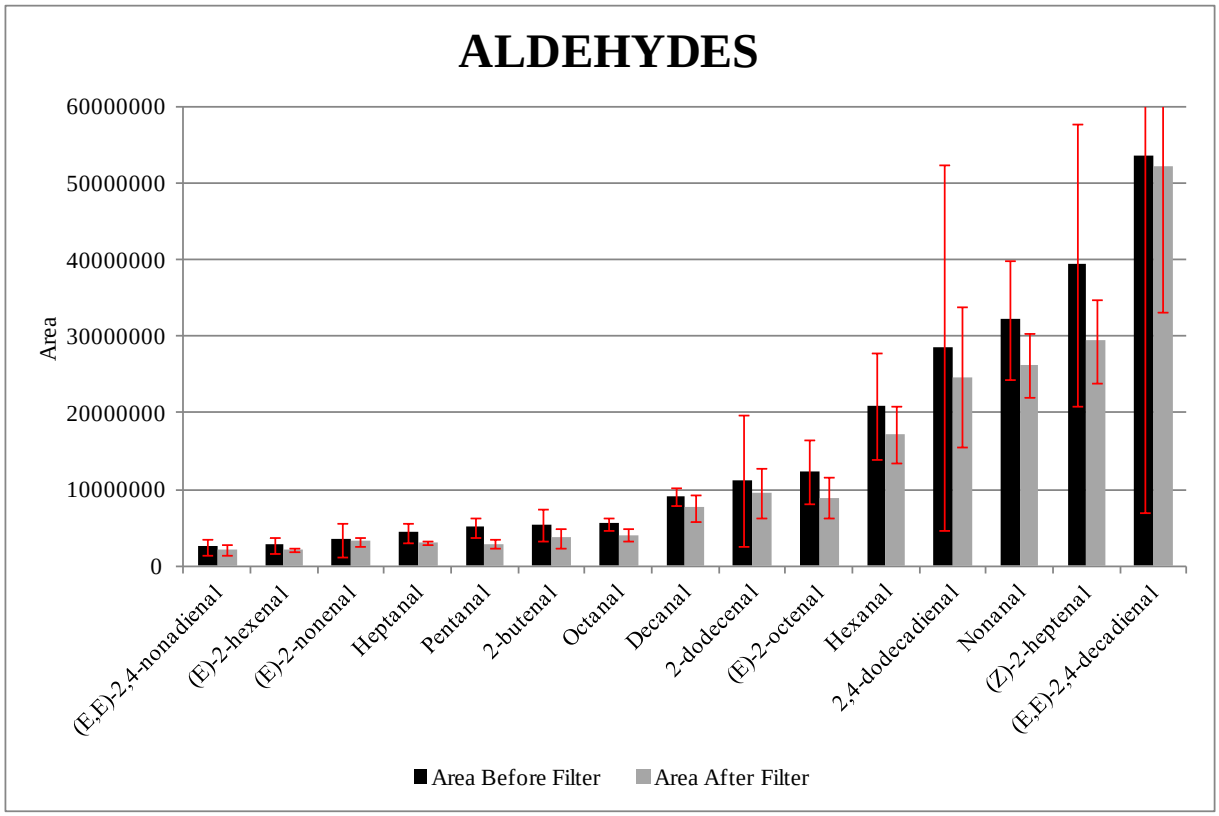


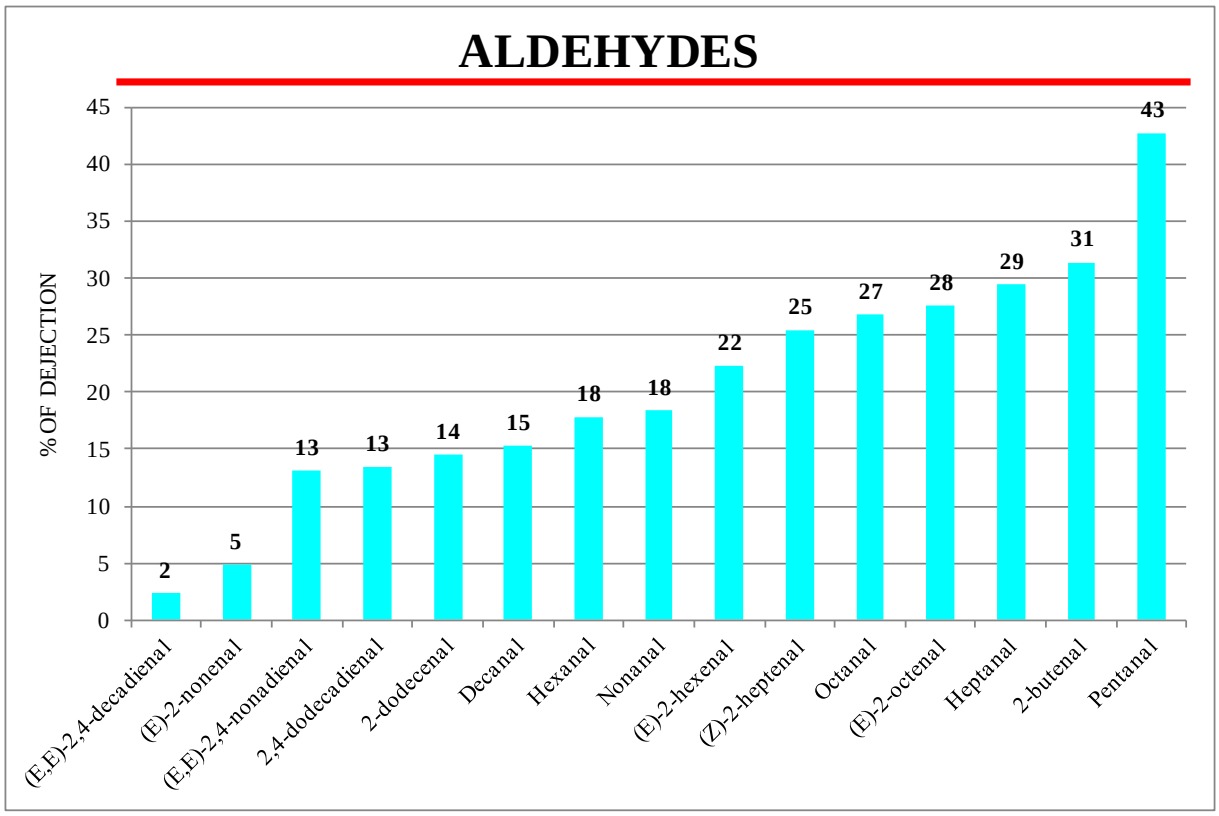
Figure 36. Chromatogram obtained for air after filter

Also for this filter, the detected compounds were divided into different classes and for each substance a comparison between the absolute area before and after passing through the filter and the dejection percentage were reported.

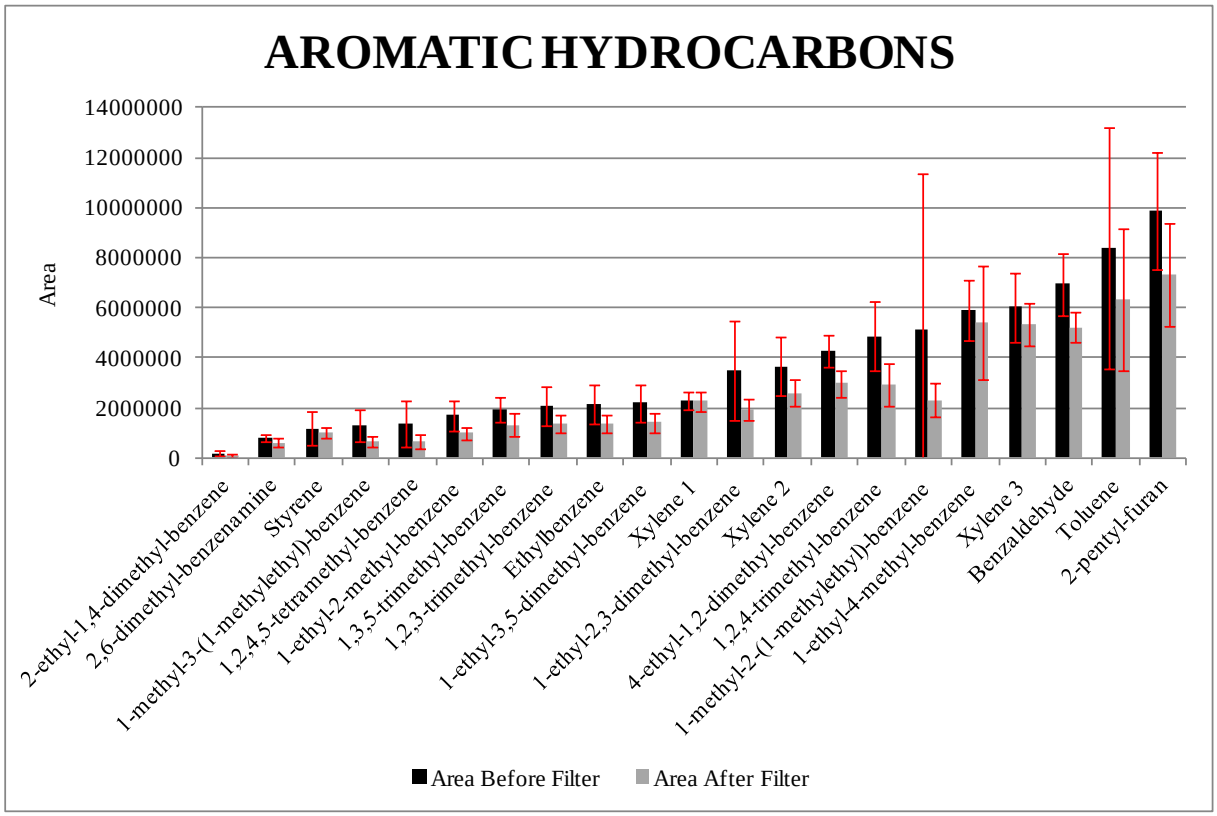
Helsa-Sorbexx-CS filter is the best filter respect to the others. It has a high cleaning performance, huge capacity, low energy consumption, low noise level during operation and an high ability to regenerate at high temperatures. It is highly customizable but it is more expensive than the activated charcoal AC 90 filter and Washable filter 1100-6 1400-11.



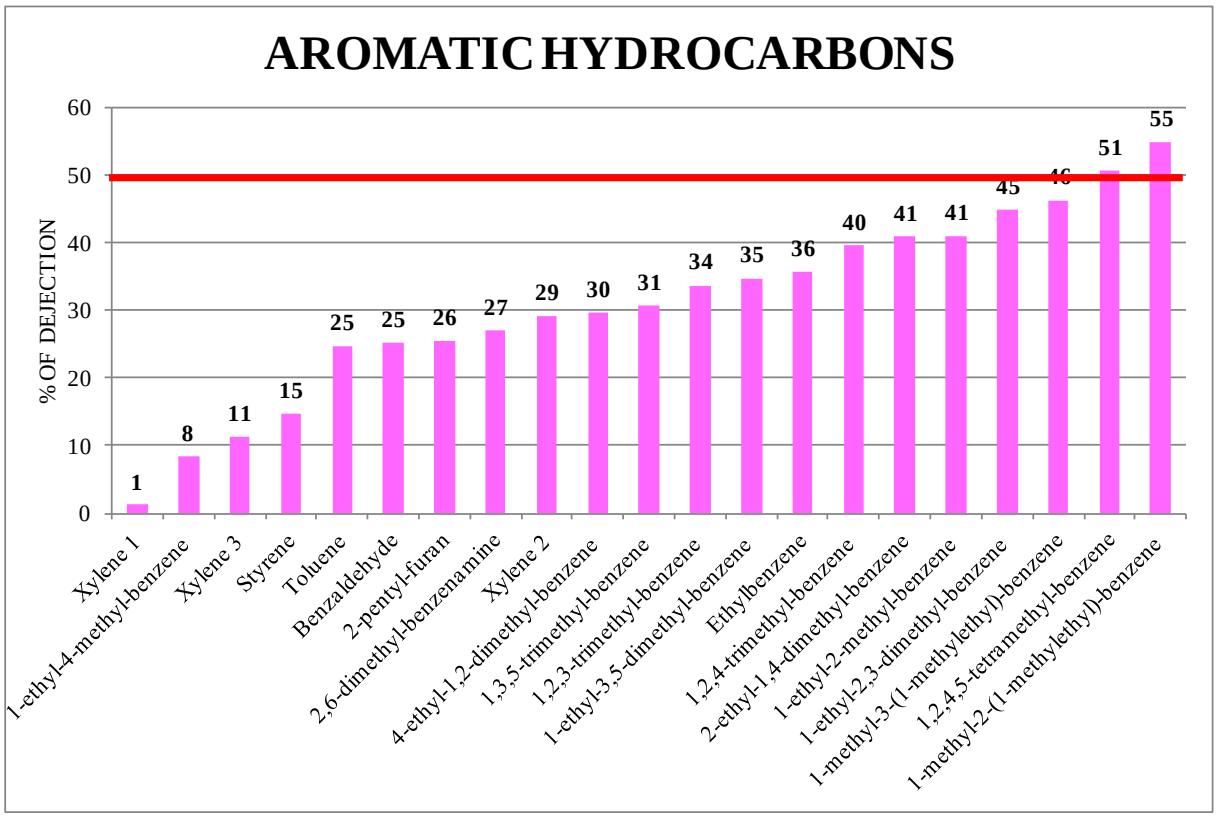
Graph 39. Aldehydes absolute areas before and after passing filter



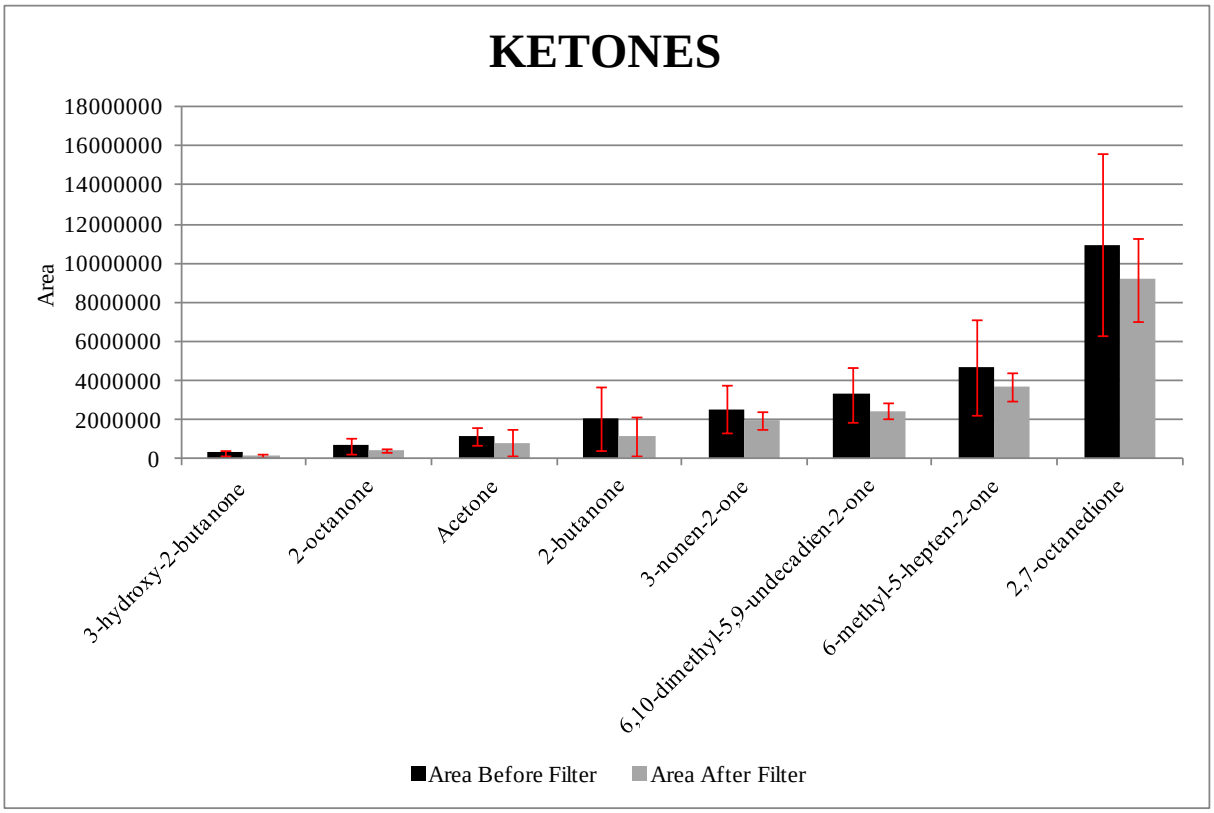
Graph 40. Dejection percentage after filter passage for aldehydes compounds



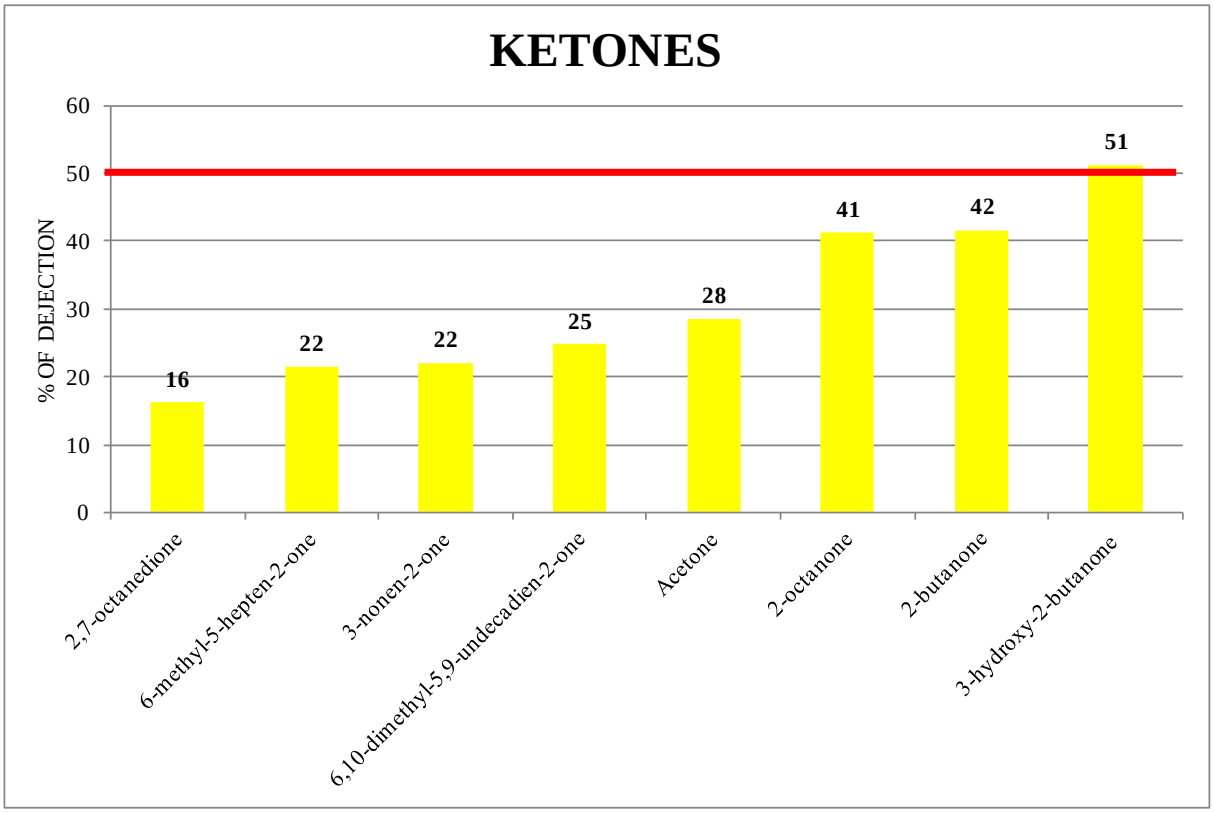
Graph 41. Aromatic hydrocarbons absolute areas before and after passing filter



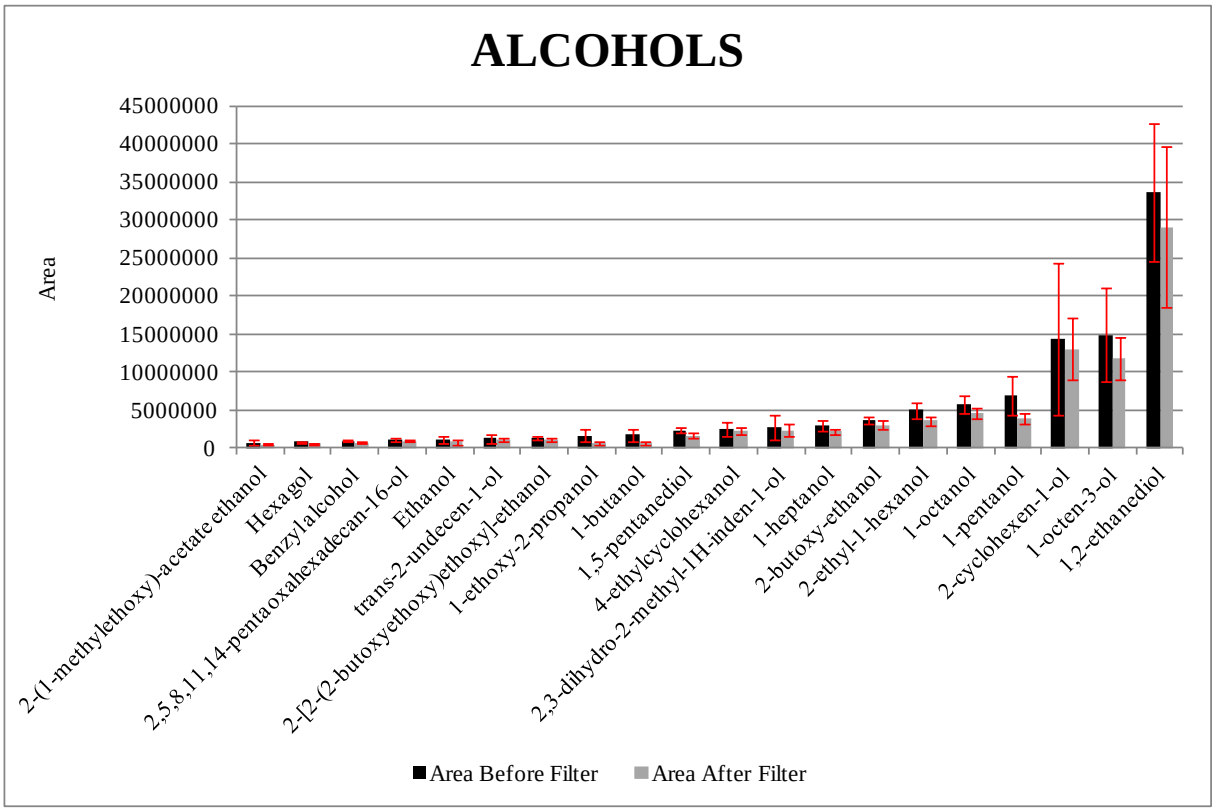
Graph 42. Dejection percentage after filter passage for aromatic hydrocarbons compounds



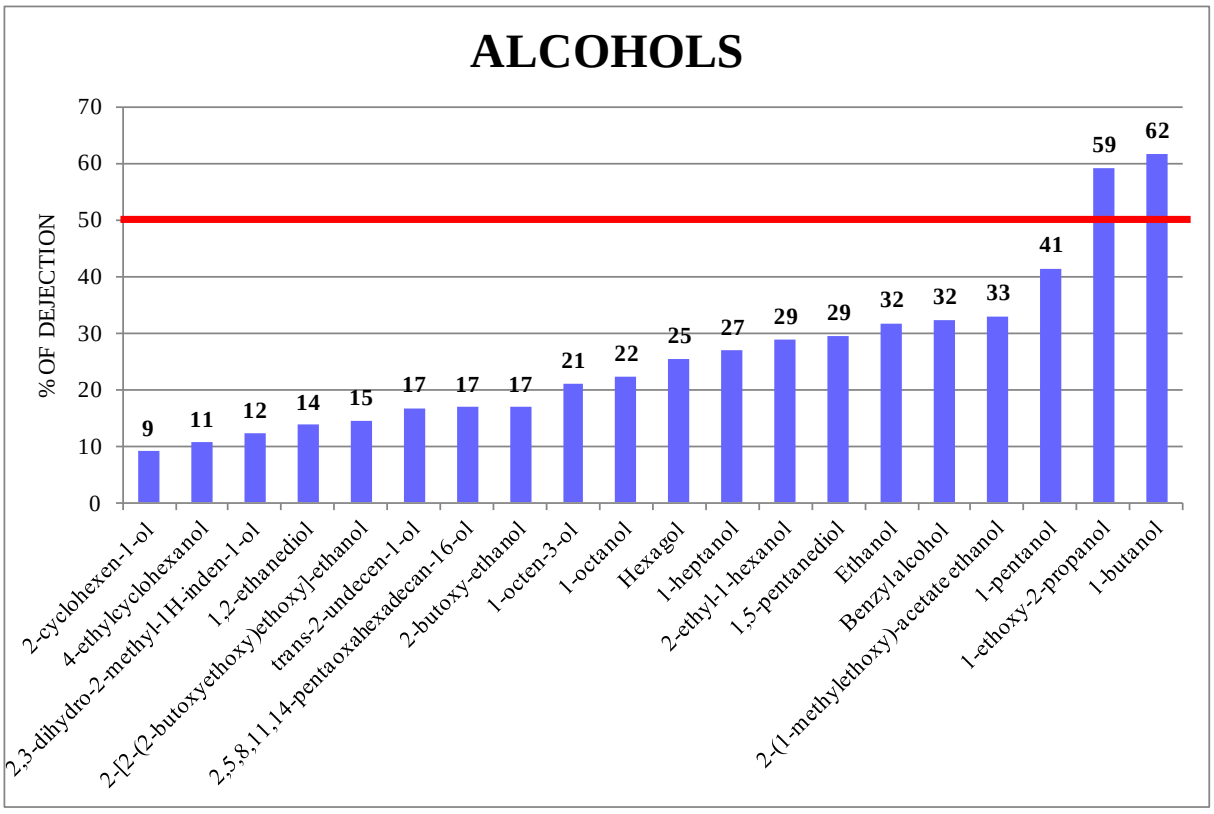
Graph 43. Ketones absolute areas before and after passing filter



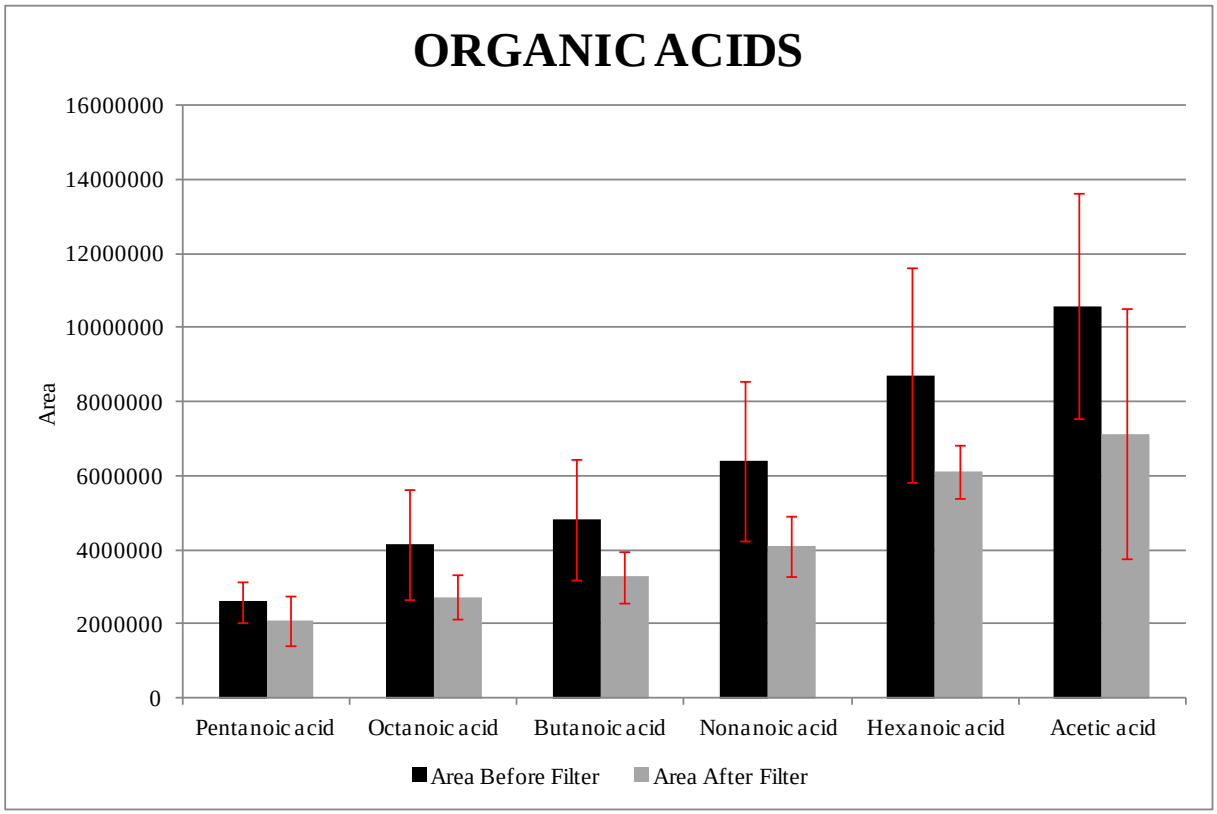
Graph 44. Dejection percentage after filter passage for ketones compounds



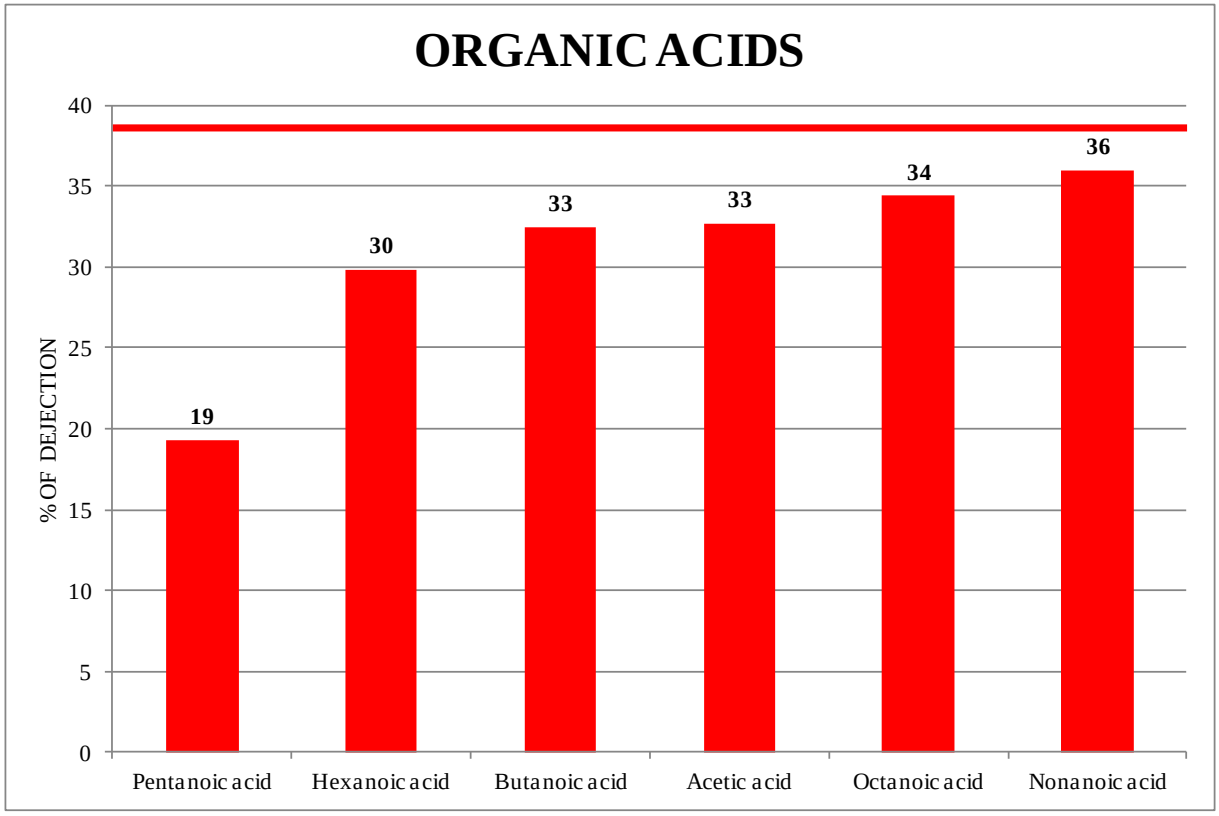
Graph 45. Alcohols absolute areas before and after passing filter



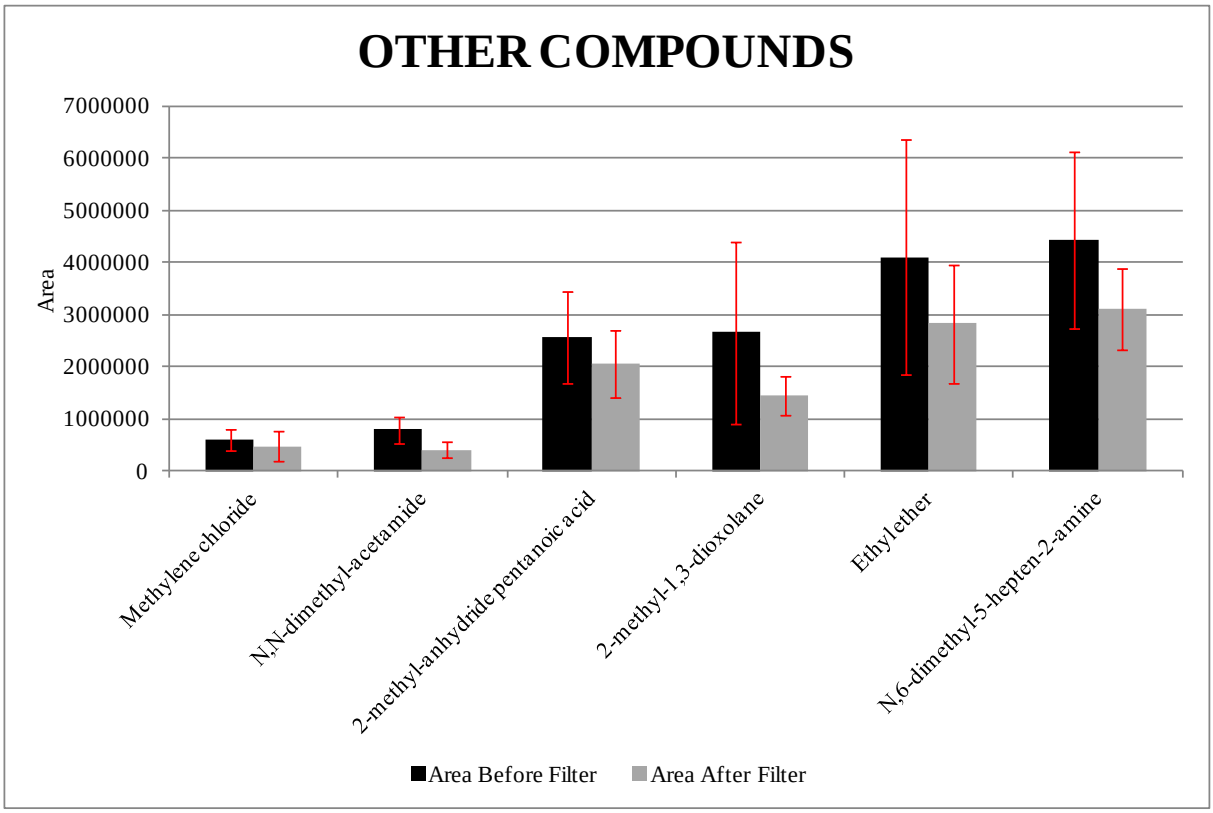
Graph 46. Dejection percentage after filter passage for alcohols compounds



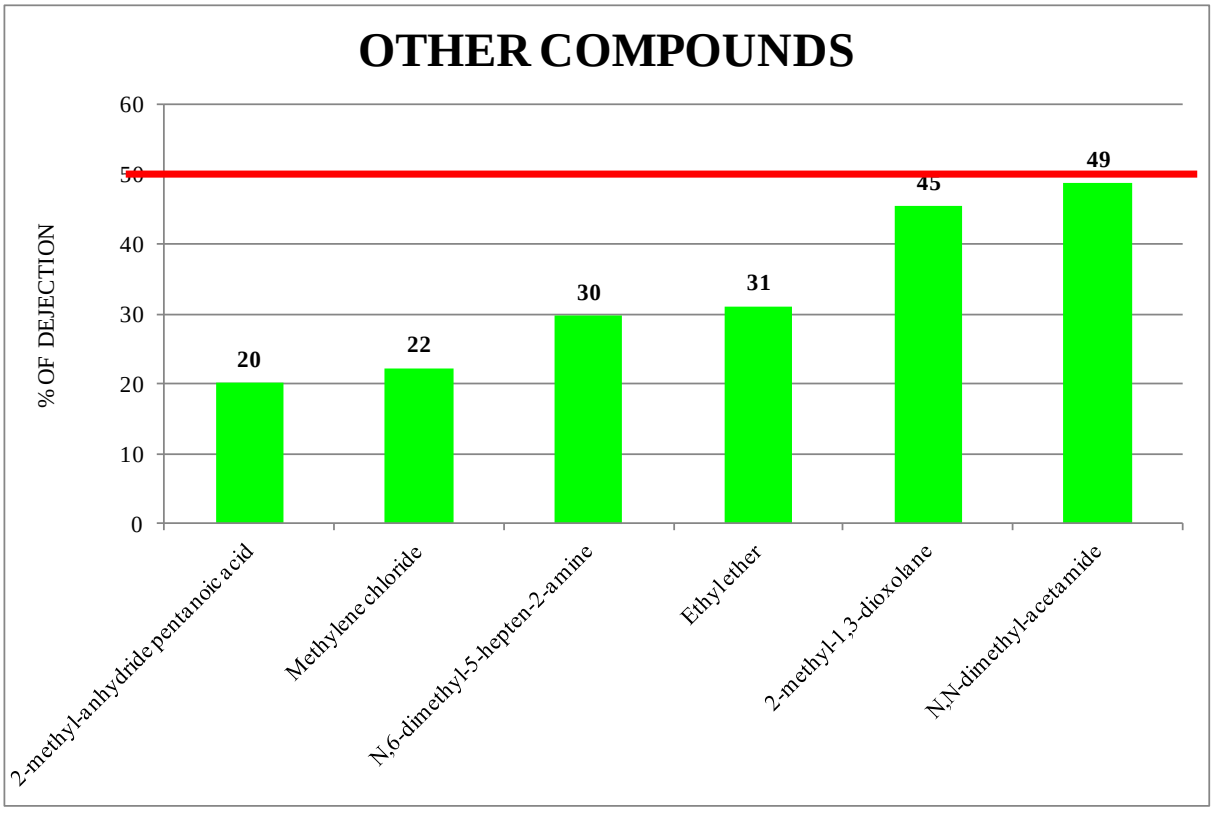
Graph 47. Organic acids absolute areas before and after passing filter



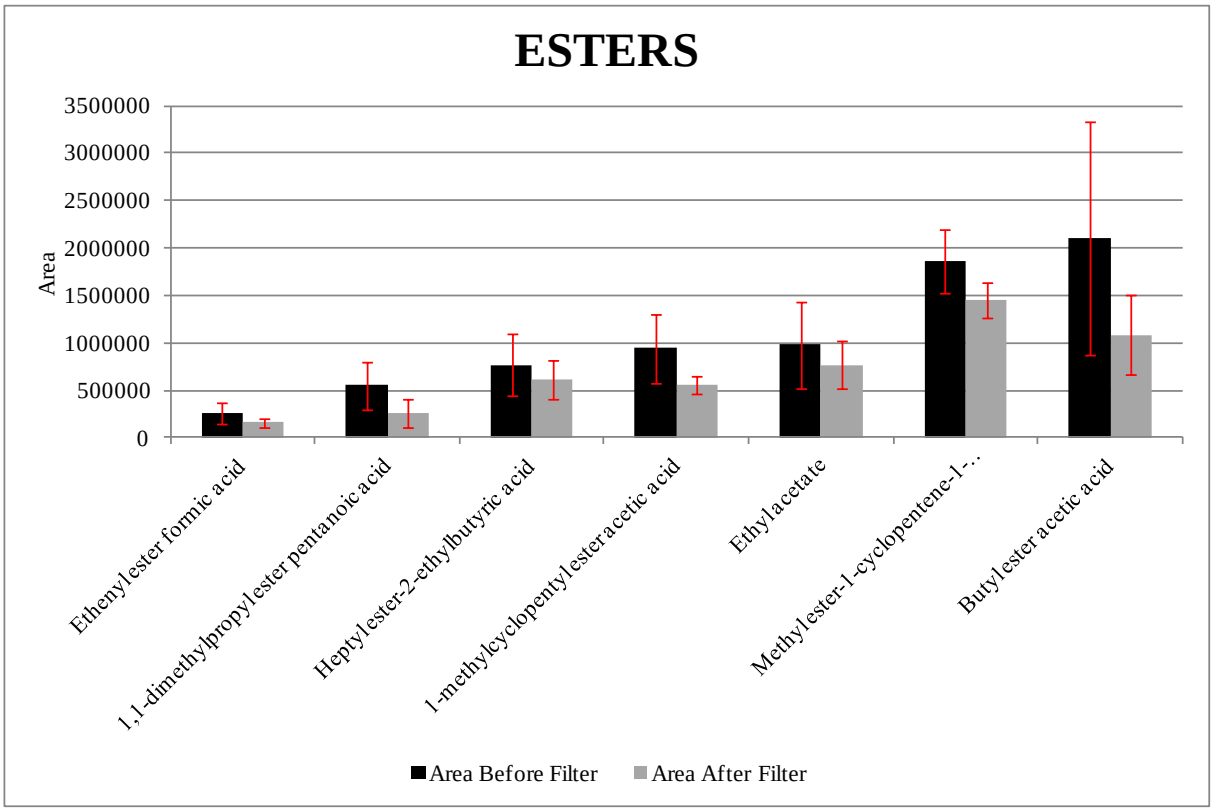
Graph 48. Dejection percentage after filter passage for organic acids compounds



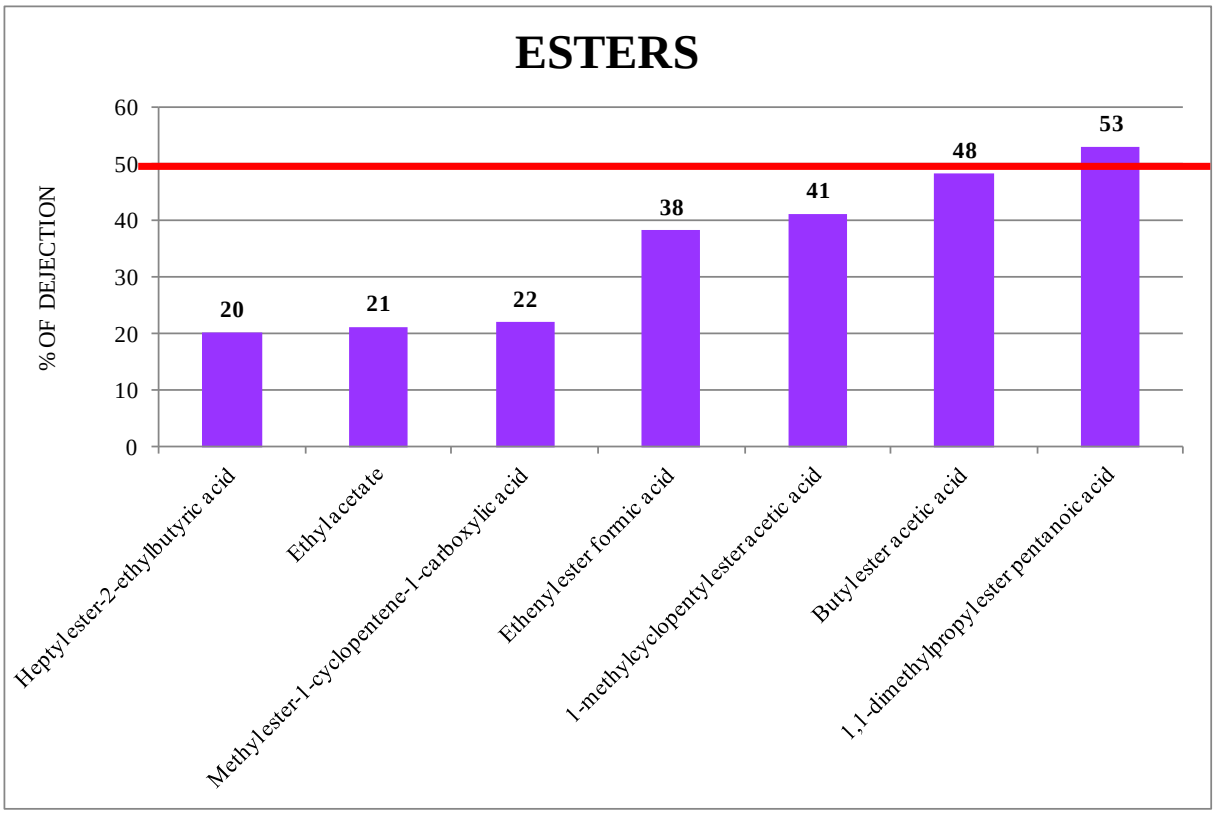
Graph 49. Other compounds absolute areas before and after passing filter



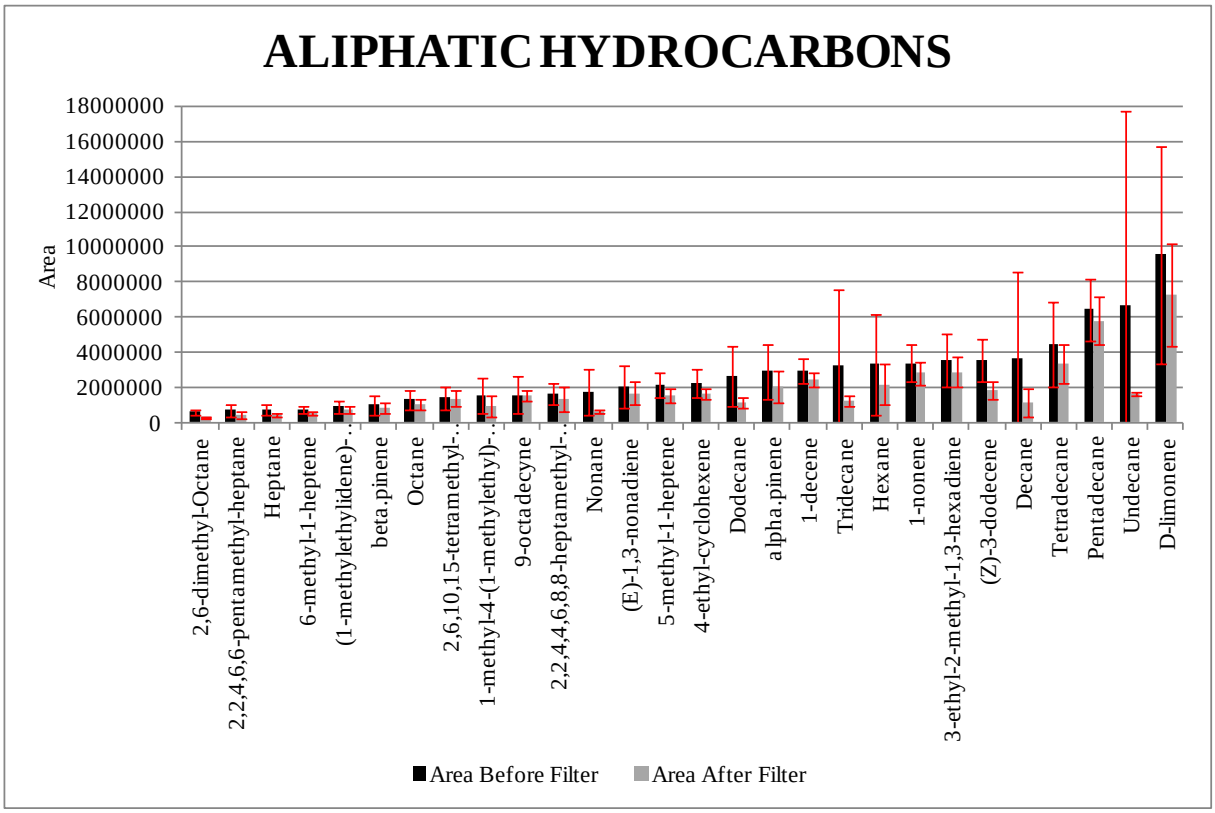
Graph 50. Dejection percentage after filter passage for other compounds



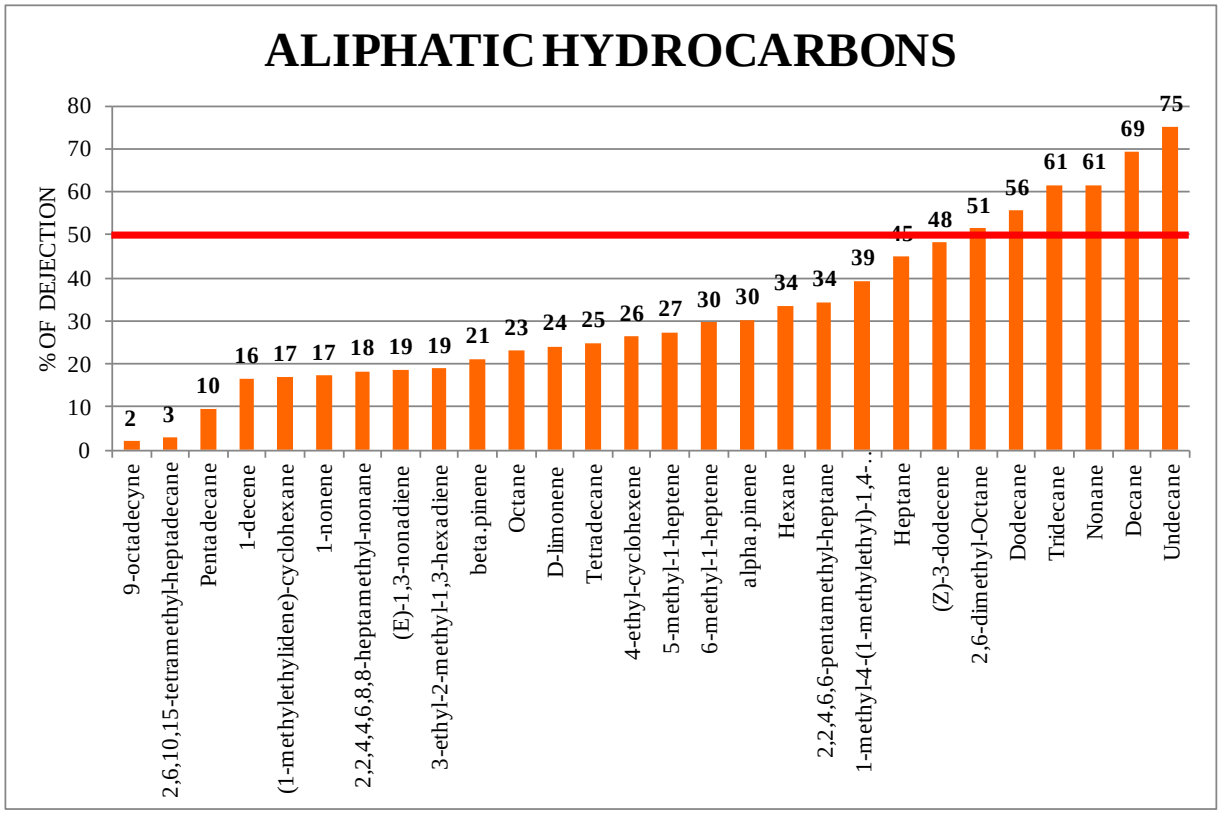
Graph 51. Esters absolute areas before and after passing filter



Graph 52. Dejection percentage after filter passage for esters compounds



Graph 53. Aliphatic hydrocarbons absolute areas before and after passing filter

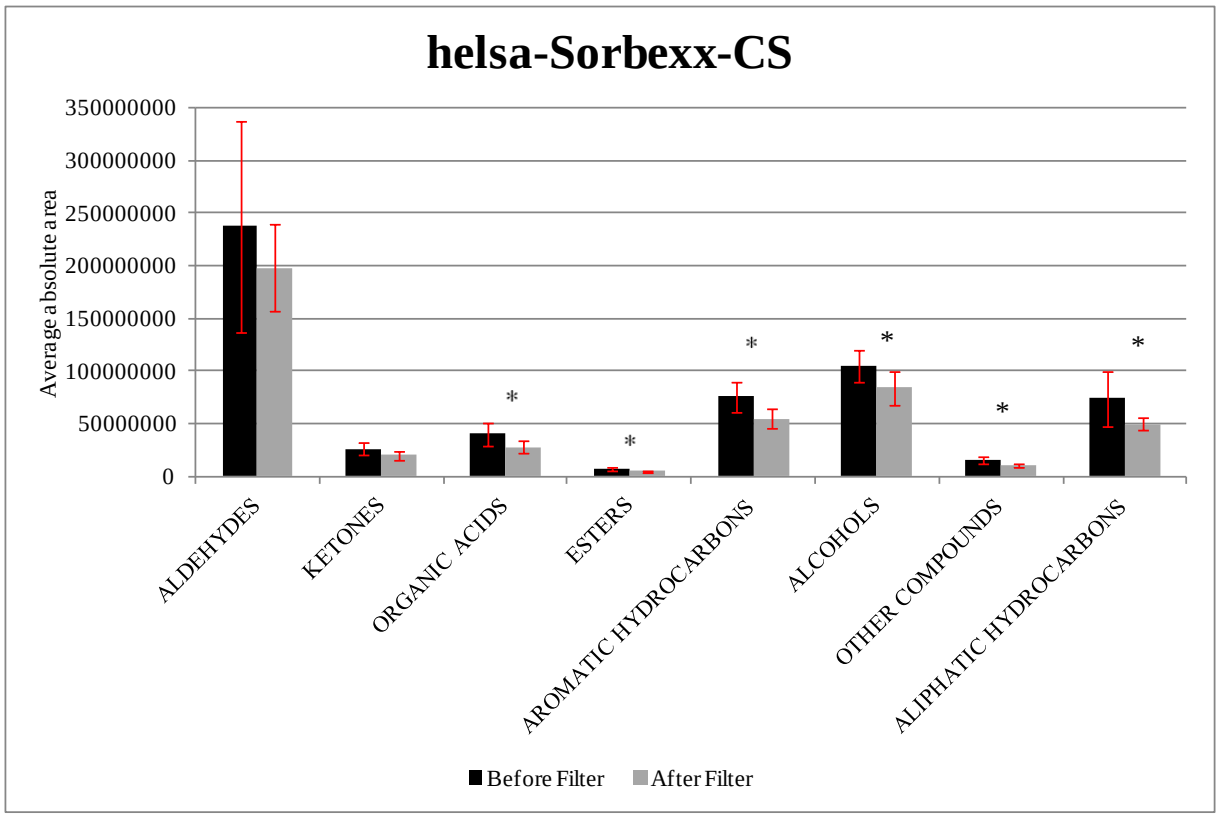


Graph 54. Dejection percentage after filter passage for aliphatic hydrocarbons compounds

From the results obtained with helsa-Sorbexx-CS filter, it was seen that all analytes detected, independently of their class, are kept by passing through the filter (Graph 39, Graph 41, Graph 43, Graph 45, Graph 47, Graph 49, Graph 51 and Graph 53), in which the absolute areas before filter are in black and the absolute areas after filter are in grey. More in details, all the aldehydes, organic acids and other compounds classes, were dejected below 50%: range 2% - 43% for aldehydes (Graph 40), range 19% - 36% for organic acids (Graph 48) and range 20% - 49% for other compounds (Graph 50). In the case of aromatic hydrocarbons (Graph 42) and alcohols (Graph 46), only two molecules were dejected with a percentage greater than 50%: 1,2,4,5-tetramethyl-benzene (51% of dejection) and 1-methyl-2-(1-methylethyl)-benzene (55% of dejection) for aromatic hydrocarbons, while 1-ethoxy-2-propanol (59% of dejection) and 1-butanol (62% of dejection as regard alcohol compounds. Considering ketones (Graph 44) and esters (Graph 52), only one molecule for each of these classes of compounds was dejected with a percentage greater than 50%: 3-hydroxy-2-butanone (51% of dejection) for ketones and 1,1-dimethylpropyl ester pentanoic acid (53% of dejection) for esters. Finally, for the aliphatic hydrocarbon category (Graph 54), a range of dejection from 2% to 75% was observed. In particular, it is possible to see a major abatement for the alkanes with a long linear carbon chain, as for example dodecane, tridecane, nonane, decane and undecane, that show the following percentages of dejection: 56%, 61%, 61%, 69% and 75%.

However also for this filter, the reduction is always lower than 50% for each class of compounds considered (Graph 60).

Moreover, for this kind of filter, the differences in the extent of dejection are statistically significant ($P < 0.05$) for the categories of organic acids, esters, aromatic hydrocarbons, alcohols, aliphatic hydrocarbons and of "other compounds" (Graph 55).



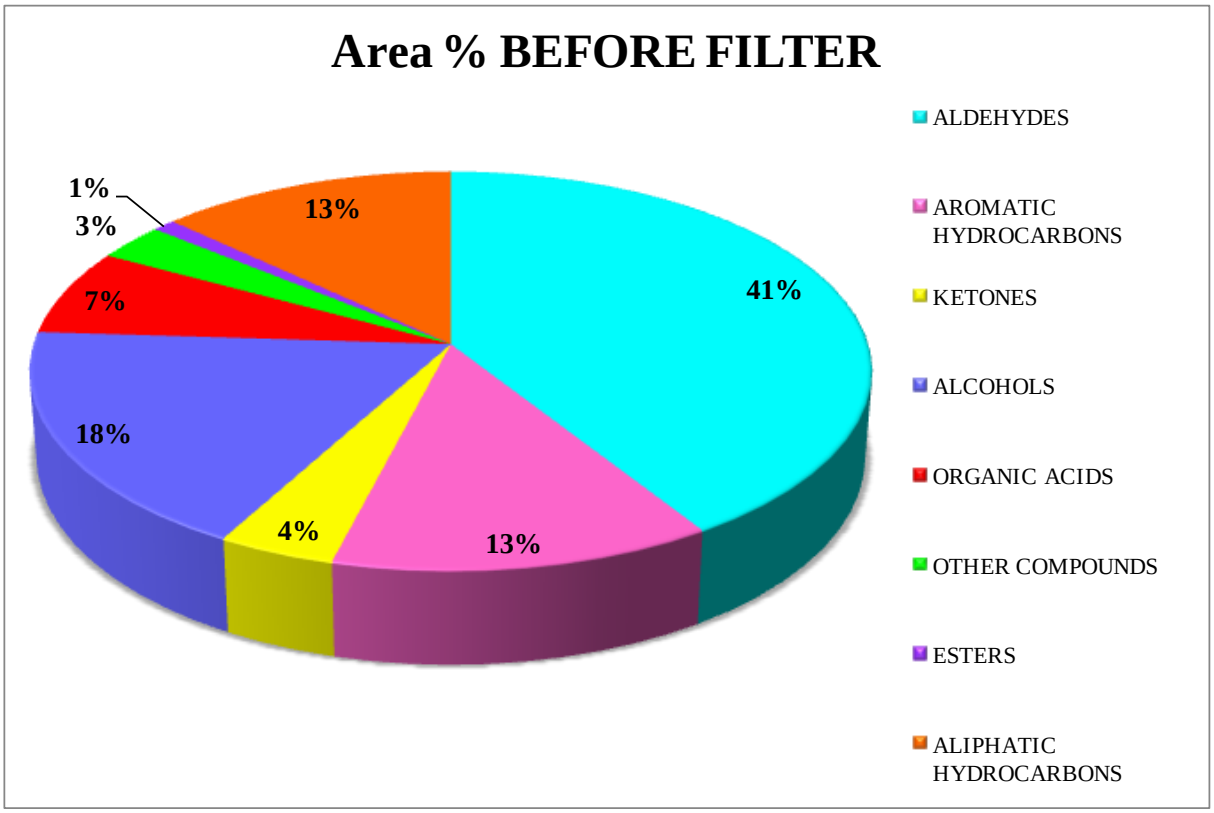
Graph 55. Comparison of the average absolute areas (± standard deviation) of different classes of extracted compounds. Significant differences ($P < 0.05$, one-way ANOVA and Tukey's test for pairwise comparison) are indicated by "*".

Also for this filter, the area percentage before filter and after filter for each class of detected compounds were calculated, as shown in the table below (Table 7). The air composition before filter and after filter is reported in the graph below (Graph 56 and Graph 57).

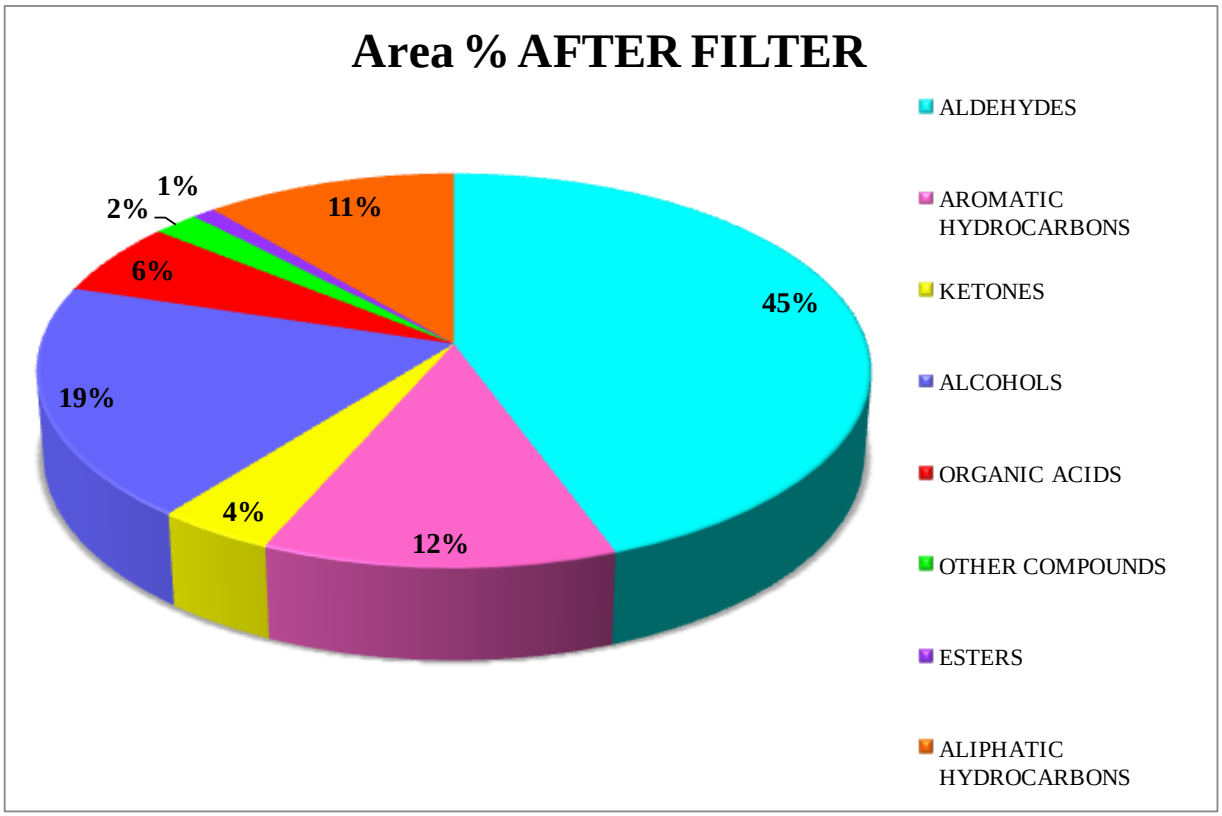
CLASSES OF COMPOUNDS	BEFORE FILTER		AFTER FILTER	
	Area %	S.D.	Area %	S.D.
ALDEHYDES	41	9	44	3
AROMATIC HYDROCARBONS	13	5	12	1
KETONES	4	1	4	1
ALCOHOLS	18	3	19	2
ORGANIC ACIDS	7	2	6	1
OTHER COMPOUNDS	3	1	2	<1
ESTERS	1	<1	1	<1
ALIPHATIC HYDROCARBONS	13	4	11	1

Table 7. Area percentage before and after filter for each class of compounds

Considering the area percentage composition before filter and after filter, from a statistical point of view, there are not significant differences for each class of detected compounds.



Graph 56. Percentage composition of air before filter

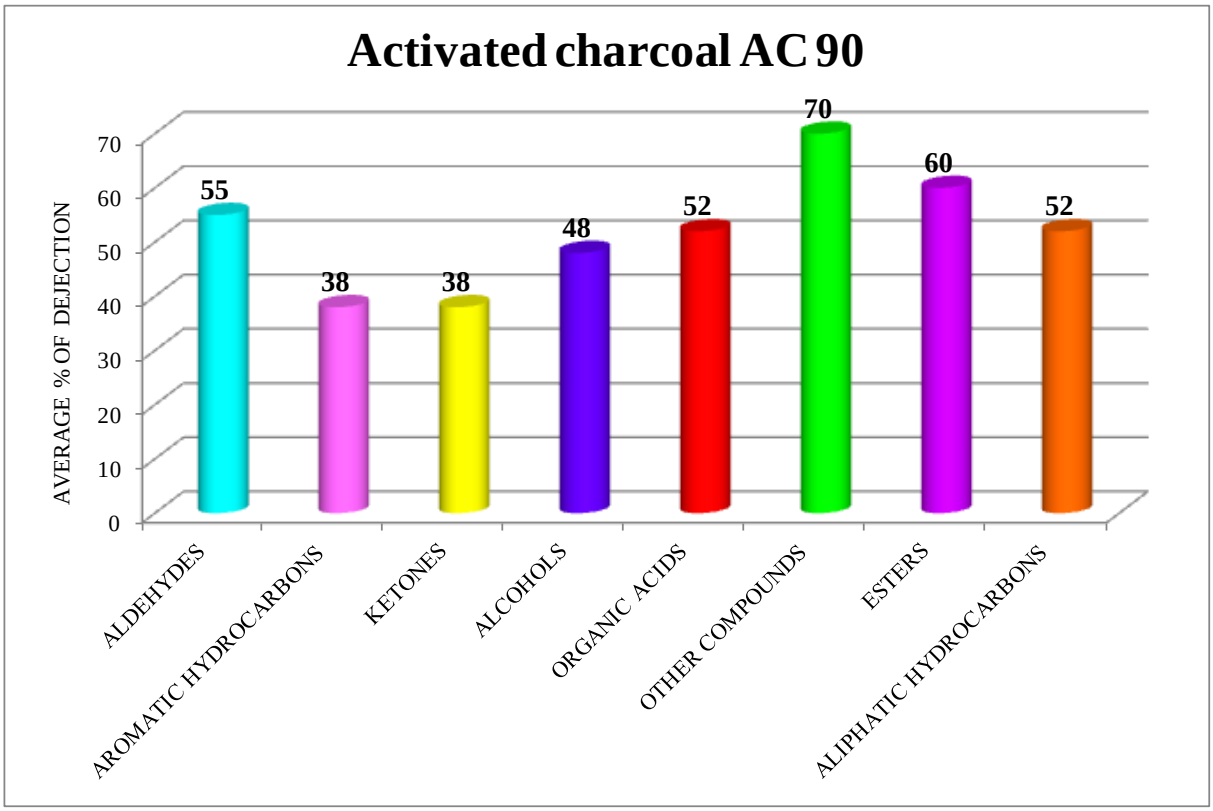


Graph 57. Percentage composition of air after filter

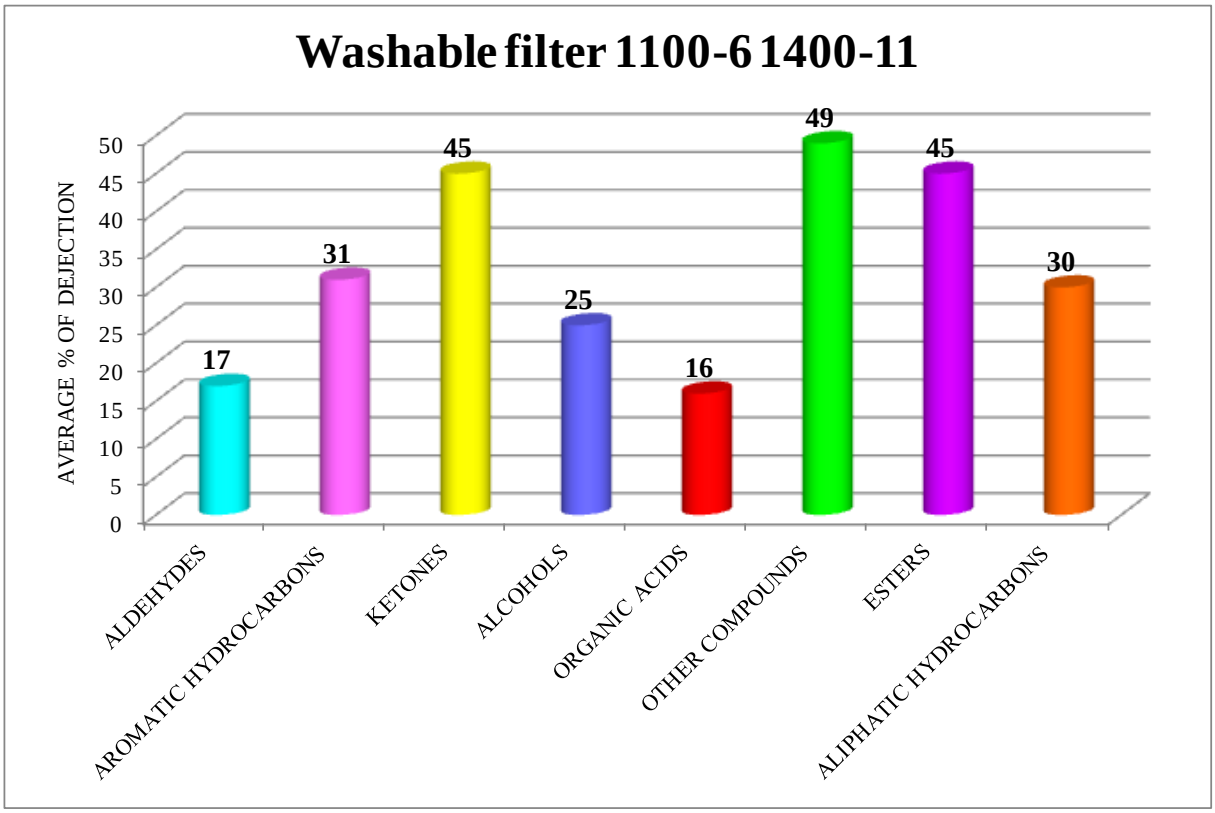
Therefore, comparing only the average percentage of dejection for each class of compounds, we can say that each filter has a particular selectivity for certain compounds rather than others. As it is possible to see in the table below (Table 8) and in histograms below (Graph 58, Graph 59 and Graph 60), for example, activated charcoal AC 90 filter (Graph 58) exhibits greater selectivity for molecules belonging to the category of esters and other compounds and it is less efficient with substances such as aromatic hydrocarbons or ketones (with an average percentage of dejection respectively of 60%, 70% and 38%). On the contrary washable filter 1100-6 1400-11 (Graph 59), acts more on compounds such as other compounds with an average percentage of dejection of 49% and less on categories such as aldehydes and organic acids with an average percentage of dejection respectively of 17% and 16%. As regards the third filter (Graph 60), helsa-Sorbexx-CS filter has a higher retention effect for esters (average percentage of dejection of 35%) or aliphatic hydrocarbons (average percentage of dejection of 32%) rather than for compounds such as aldehydes (average percentage of dejection of 21%). Anyway, considering the results from statistical analysis, washable filter 1100-6 1400-11 and helsa-Sorbexx-CS filter would seem to provide better filtering performance than the activated charcoal AC 90 filter, according also with their market price.

	AVERAGE % OF DEJECTION		
CLASSES OF COMPOUNDS	**Activated charcoal AC 90**	**Washable filter 1100-6 1400-11**	**helsa-Sorbexx-CS**
ALDEHYDES	55	17	21
AROMATIC HYDROCARBONS	38	31*	31*
KETONES	38	45*	31
ALCOHOLS	48	25	26*
ORGANIC ACIDS	52	16	31*
OTHER COMPOUNDS	70	49*	33*
ESTERS	60	45*	35*
ALIPHATIC HYDROCARBONS	52*	30*	32*

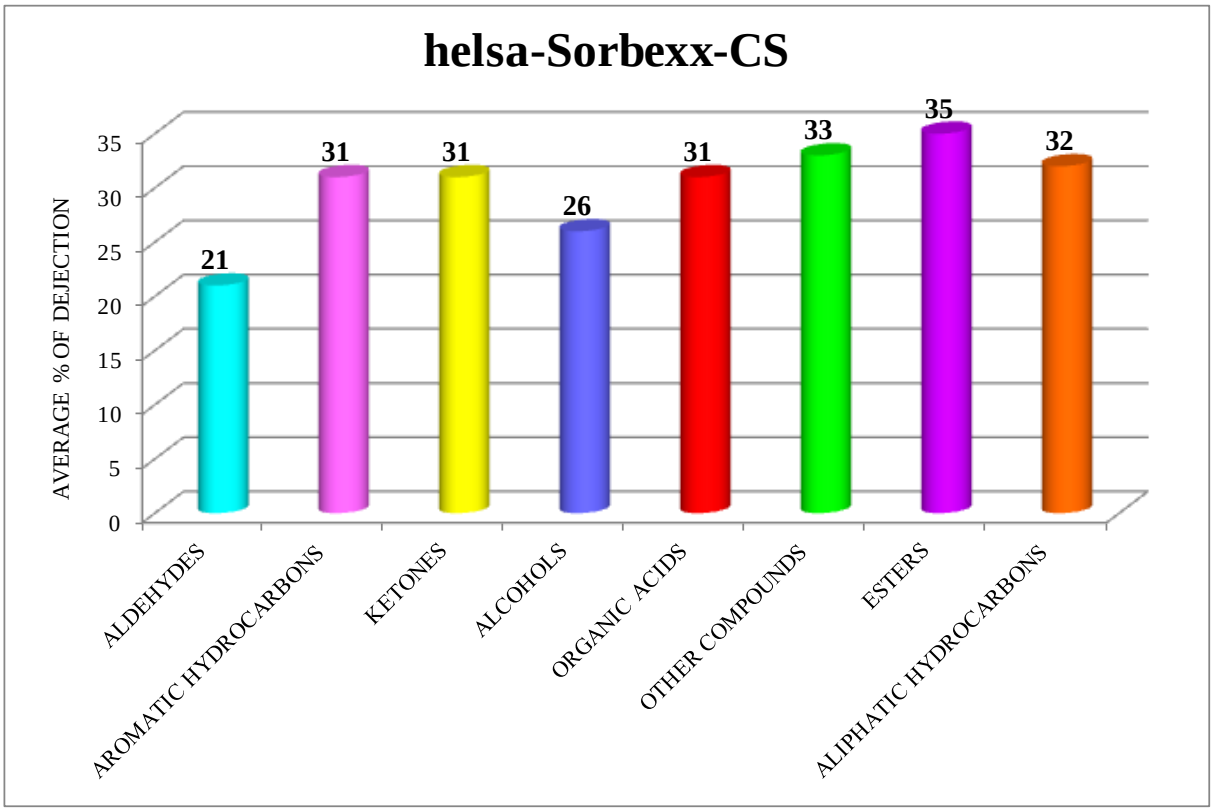
Table 8. Average percentage of dejection for each class of compounds for the three filters. Significant differences ($P < 0.05$, one-way ANOVA and Tukey's test for pairwise comparison) are indicated by "*".



Graph 58. Average % of dejection for activated charcoal AC 90 filter

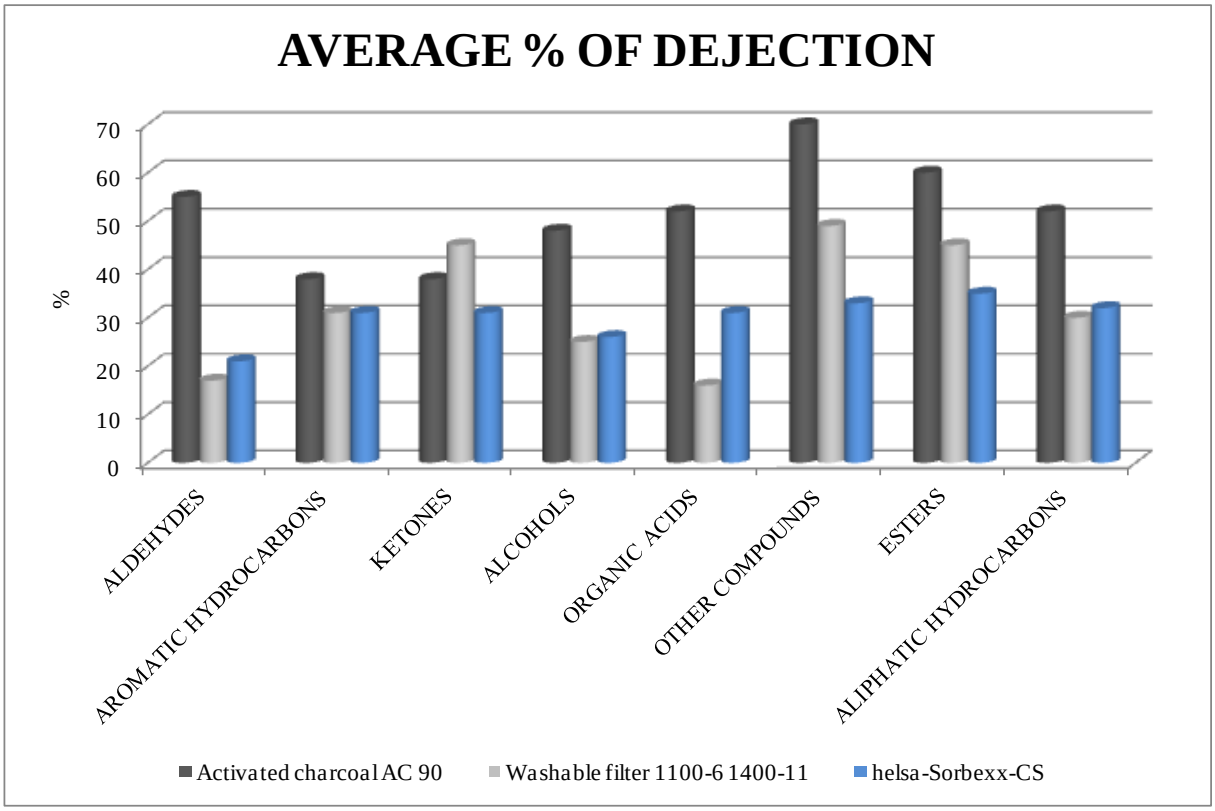


Graph 59. Average % of dejection for washable filter 1100-6 1400-11



Graph 60. Average % of dejection for helsa-Sorbexx-CS filter

Finally, comparing the average percentage of dejection for each category of detected compounds in relation to each filter examined (Graph 61), is possible to see that activated charcoal AC 90 filter (in black) has, generally, a filtering effect greater than the other two filters for all the categories of compounds, except for ketones in which there is a better performance of washable filter 1100-6 1400-11. Comparing the other two filters, washable filter 1100-6 1400-11 (in grey) and helsa-Sorbexx-CS filter (in blue), we can see the best efficacy of the first filter for categories such as ketones, esters and other compounds; on the contrary there is a better performance of the helsa-Sorbexx-CS filter for classes of compounds such as aldehydes, organic acids and aliphatic hydrocarbons. For alcohols category, both the filters have the same filtering effect; the average percentages of dejection are 25% for washable filter 1100-6 1400-11 and 26% for helsa-Sorbexx-CS filter. Regarding aromatic hydrocarbons, the situation is almost similar among the three filters: in particular, washable filter 1100-6 1400-11 and helsa-Sorbexx-CS filter show the same behavior with an average percentage of dejection of 31%, while activated charcoal AC 90 shows an average percentage of dejection of 38%.



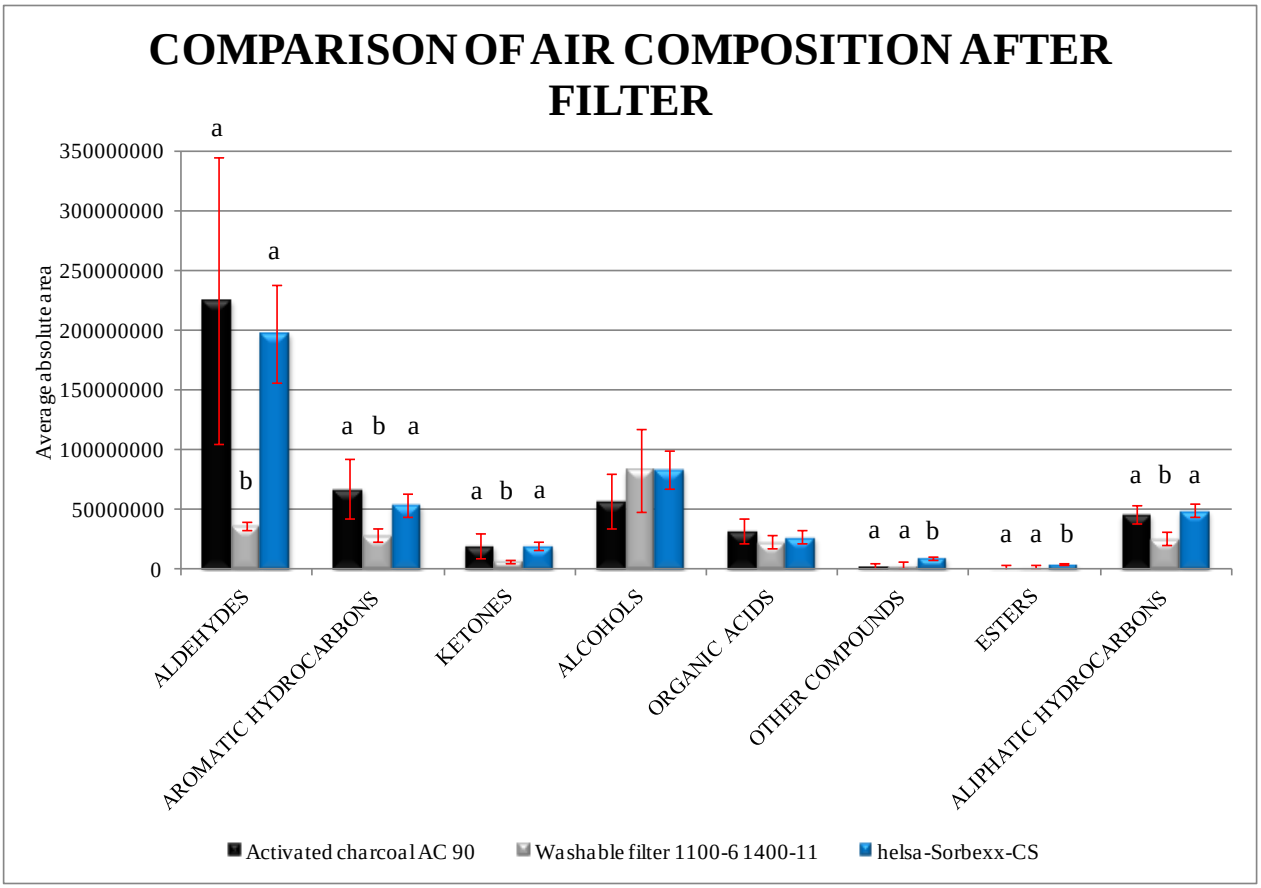
Graph 61. Comparison of the average % value of dejection of the three filters for each class of compounds

Anyway, to better understand the behavior of the three different filters, it is important to consider also the absolute area after filter for each category of detected compounds (Table 9).

	Activated charcoal AC 90			Washable filter 1100-6 1400-11			helsa-Sorbexx-CS		
CLASSES OF COMPOUNDS	**AVERAGE ABSOLUTE AREA**	**S.D.**	**R.S.D.%**	**AVERAGE ABSOLUTE AREA**	**S.D.**	**R.S.D.%**	**AVERAGE ABSOLUTE AREA**	**S.D.**	**R.S.D.%**
ALDEHYDES	2.25E+08	1.E+08	53	3.68E+07	3.33E+06	9	1.98E+08	4.11E+07	21
AROMATIC HYDROCARBONS	6.77E+07	3.E+07	37	2.89E+07	5.16E+06	18	5.42E+07	9.34E+06	17
KETONES	2.00E+07	1.E+07	54	7.37E+06	1.35E+06	18	1.99E+07	3.98E+06	20
ALCOHOLS	5.74E+07	2.E+07	39	8.33E+07	3.50E+07	42	8.40E+07	1.58E+07	19
ORGANIC ACIDS	3.24E+07	1.E+07	33	2.34E+07	5.62E+06	24	2.74E+07	5.69E+06	21
OTHER COMPOUNDS	3.45E+06	2.E+06	50	4.04E+06	2.63E+06	65	1.02E+07	1.43E+06	14
ESTERS	1.23E+06	2.E+06	172	2.39E+06	1.47E+06	61	4.72E+06	4.32E+05	9
ALIPHATIC HYDROCARBONS	4.65E+07	7.E+06	16	2.62E+07	5.18E+06	20	4.97E+07	5.56E+06	11

Table 9. Average absolute area, standard deviation and relative standard deviation percentage for each class of compounds for the three filters

The washable filter 1100-6 1400-11 shows the lowest content (in terms of absolute area) of each category of detected compounds (except for alcohols) than the other two filters (Graph 62). In particular, important differences are obtained for categories like aldehydes, aromatic hydrocarbons, ketones and aliphatic hydrocarbons. These results are also confirmed by the statistical analysis that underlines significant differences ($P < 0.05$) between this filter and the other two for the most classes of compounds. Therefore, it is possible to say that this type of filter provides better performance than the other two examined. Considering instead the activated charcoal AC 90 filter and the helsa-Sorbexx-CS filter, the behavior is similar. There are no significant differences for all classes of detected compounds, with only exception for esters and "other compounds" categories, in which the helsa-Sorbexx-CS filter seems to give more satisfactory results.



Graph 62. Comparison of VOCs content, in terms of absolute peak area, in air after filtration for the three filters. Different letters indicate significant differences between the three filters ($P < 0.05$, one-way ANOVA and Tukey's test for pairwise comparison)

1.4 Conclusions

In conclusion, considering the purpose of this research work, we can summarize:

- ❖ The proposed system, allows to analyze VOCs produced during cooking, making use of olfactometric bags to collect air samples, and SPME-GC-MS to extract and analyse VOCs. This procedure is simple, economic and exploits tools and analytical instrumentation readily available in many laboratories. In addition it allows to analyse samples even collected far from the analytical instrument location to be used for the determination.
- ❖ The analysis provides good results in terms of reproducibility and sensitivity, which can be modulated using different extraction times.
- ❖ The selected SPME extraction time is 24h.
- ❖ There is a significant percentage of reduction for most of compounds detected after air passes the filter of the kitchen hoods.
- ❖ Filters show a certain selectivity for particular classes of compounds.
- ❖ Washable filter 1100-6 1400-11 provides better filtering performance than the other two according with the price market.
- ❖ The behavior of activated charcoal AC 90 filter and of the helsa-Sorbexx-CS filter is similar, even if the second one gives slightly more satisfactory results.

Anyway, further studies are being carried out in order to better understand the behavior of the filters by means of a characterization of the filters morphological composition by scanning electron microscope - energy dispersive X-ray (SEM-EDX) analysis.

References

[1] L. Lucattini, G. Poma, A. Covaci, J. D. Boer, M. H. Lamoree, P. E.G. Leonards, 2018. A review of semi-volatile organic compounds (SVOCs) in the indoor environment: occurrence in consumer products, indoor air and dust. Chemosphere 201, 466-482.

[2] S. Wang, H. M. Ang, M. O. Tade, 2007. Volatile organic compounds in indoor environment and photocatalytic oxidation: State of the art. Environment International 33, 694-705.

[3] K. W. Tham, 2016. Indoor air quality and its effects on humans - A review of challenges and developments in the last 30 years. Energy and Buildings 130, 637-650.

[4] S. A. Abdul-Wahab, S. C. Fah En, A. Elkamel, L. Ahmadi, K. Yetilmezsoy, 2015. A review of standards and guidelines set by international bodies for the parameters of indoor air quality. Atmospheric Pollution Research 6, 751-767.

[5] C. Garcia-Jares, R. Barro, M. Liompart, 2012. Comprehensive Sampling and Sample Preparation. Analytical Chemistry 1, 125-161.

[6] K. H. Kima, S. K. Pandeya, E. Kabira, J. Susaya, R. J. C. Brown, 2011. The modern paradox of unregulated cooking activities and indoor air quality. Journal of Hazardous Materials 195, 1-10.

[7] E. Kabir, K. H. Kim, 2011. An investigation on hazardous and odorous pollutant emission during cooking activities. Journal of Hazardous Materials 188, 443-454.

[8] H. H. Cheng, C. C. Hsieh, 2010. Integration of chemical scrubber with sodium hypochlorite and surfactant for removal of hydrocarbons in cooking oil fume. Journal of Hazardous Materials 182, 39-44.

[9] L. Y. He, M. Hu, X. F. Huang, B. D. Yu, Y. H. Zhang, D. Q. Liu, 2004. Measurement of emissions of fine particulate organic matter from Chinese cooking. Atmospheric Environment 38, 6557-6564.

[10] K. P. Yu, K. R. Yang, Y. C. Chen, J. Y. Gong, Y. P. Chen, H. C. Shih, S. C. Candice Lung, 2015. Indoor air pollution from gas cooking in five Taiwanese families. Building and Environment 93, 258-266.

[11] K. Umano, T. Shibamoto, 1987. Analysis of acrolein from heated cooking oils and beef fat. Journal of Agricultural and Food Chemistry 35, 909-912.

[12] G. Blanda, L. Cerretani, P. Comandini, T. Gallina Toschi, G. Lercker, 2010. Investigation of off-odour and off-flavour development in boiled potatoes. Food Chemistry 118, 282-290.

[13] L. Sghaier, C. B. Y. Cordella, D. N. Rutledge, M. Watiez, S. Breton, A. Kopczuk, P. Sassiat, D. Thiebaut, J. Vial, 2015. Comprehensive two-dimensional gas chromatography for analysis of

the volatile compounds and fishy odor off-flavors from heated repeseed oil. Chromatographia 78, 805-817.

[14] National Air Filtration Association, 2014. NAFA Guide to Air Filtration, Fifth Edition.

[15] M. Estévez, D. Morcuende, S. Ventanas, R. Cava, 2003. Analysis of volatiles in meat from Iberian pigs and lean pigs after refrigeration and cooking by using SPME-GC-MS. Journal of Agricultural and Food Chemistry 51, 3429-3435.

[16] A. Sanches-Silvia, J. Lopez-Hernàndez, P.Paseiro-Losada, 2005. Profiling flavor compounds of potato crisps during storage using solid-phase microextraction. Journal of Chromatography A 1064, 239-245.

[17] N. P. Brunton, D. A. Cronin, F. J. Monahan, 2002. Volatile components associated with freshly cooked and oxidized off-flavours in turkey breast meat. Flavour and Fragrance Journal 17, 327-334.

[18] R. Munoz, E. C. Sivret, G. Parcsi, R. Lebrero, X. Wang, I. H. (Mel) Suffet, R. M. Stuetz, 2010. Monitoring techniques for odour abatement assessment. Water Research 44, 5129-5149.

[19] F. Augusto, A. Leite e Lopes, C. A. Zini, 2003. Sampling and sample preparation for analysis of aromas and fragrances. Trends in Analytical Chemistry, 160-169.

[20] M. Ajhar, B. Wens, K. H. Stollenwerk, G. Spalding, S. Yuce, T. Melin, 2010. Suitability of Tedlar gas sampling bags for siloxane quantification in landfill gas. Talanta 82, 92-98.

[21] E. Kabir, K.-H. Kim, J.-W. Ahn, O.-F. Hong, J. R. Sohn, 2010. Barbecue charcoal combustion as a potential source of aromatic volatile organic compounds and carbonyls. Journal of Hazardous Materials 174, 492-499.

[22] M. A. Iqbal, K.-H. Kim, 2016. Sampling, pretreatment, and analysis of particulate matter and trace metals emitted through charcoal combustion in cooking activities. Trends in Analytical Chemistry 76, 52-59.

[23] K. Curran, M. Underhill, L. T. Gibson, M. Strlic, 2016. The development of a SPME-GC/MS method for the analysis of VOC emissions from historic plastic and rubber materials. Microchemical Journal 124, 909-918.

[24] A. T. Nielsen, S. Jonsson, 2002. Quantification of volatile sulfur compounds in complex gaseous matrices by solid-phase microextraction. Journal of Chromatography A 963, 57-64.

[25] P. Mochalski, B. Wzorek, I. Sliwka, A. Amann, 2009. Suitability of different polymer bags for storage of volatile sulphur compounds relevant to breath analysis. Journal of Chromatography B 877, 189-196.

[26] E. M. Gaspar, A. F. Lucena, J. Duro da Costa, H. Chaves das Neves, 2009. Organic metabolites in exhaled human breath – A multivariate approach for identification of biomarkers in lung disorders. Journal of Chromatography A 1216, 2749-2756.

[27] M. Alonso, J. M. Sanchez, 2013. Analytical challenges in breath analysis and its application to exposure monitoring. Trends in Analytical Chemistry, Vol. 44.

[28] C. Deng, J. Zhang, X. Yu, W. Zhang, X. Zhang, 2004. Determination of acetone in human breath by gas chromatography-mass spectrometry and solid-phase microextraction with on-fiber derivatization. Journal of Chromatography B 810, 269-275.

[29] R. Hyspler, S. Crhova, J. Gasparic, Z. Zadak, M. Cizkova, V. Balasova, 2000. Determination of isoprene in human empire breath using solid-phase microextraction and gas-chromatography-mass spectrometry. Journal of Chromatography B 739, 183-190.

[30] F. Hammer, D. A. T. Harper, P. D. Ryan, 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4, 1-9.

[31] P. Gillatt, 2001. Frying Improving Quality: Flavour and aroma development in frying and fried food. Woodhead Publishing Series in Food Science, Technology and Nutrition, 266-336.

[32] M. D. Guillen, P. S. Uriarte, 2012. Aldehydes contained in edible oils of a very different nature after prolonged heating at frying temperature: Presence of toxic oxygenated α,β unsaturated aldehydes. Food Chemistry 131, 915-926.

[33] H.-H. Cheng, C.-C. Hsieh, 2010. Integration of chemical scrubber with sodium hypochlorite ansd surfactant for removal of hydrocarbons in cooking oil fume. Journal of Hazardous Materials 182, 39-44.

[34] E. Choe, D. B. Min, 2007. Chemistry of Deep-Fat Frying Oils. Journal of Food Science, R1-R10.

[35] H. R. Katragadda, A. Fullana, S. Sidhu, A. A. Carbonell-Barrachina, 2010. Emission of volatile aldehydes from heated cooking oils. Food Chemistry 120, 59-65.

[36] T. Y. Chung, J. P. Eiserich, T. Shibamoto, 1993. Volatile compounds identified in headspace samples of peanut oil heated under temperatures ranging from 50 to 200°C. Journal of Agricultural and Food Chemistry 41, 1467-1470.

[37] J. J. Schauer, M. J. Kleeman, G. R. Cass, B. R. T. Simoneit, 2002. Measurement of emissions from air pollution sources. C_1 - C_{27} organic compounds from cooking with seed oils. Environmental Science and Technology 36, 567-575.

[38] R. A. Murray, 2001. Limitations to the Use of Solid-Phase Microextraction for Quantitation of Mixtures of Volatile Organic Sulfur Compounds. Analytical Chemistry 73, 1646-1649.

[39] J. Beauchamp, J. Herbig, R. Gutmann, A. Hansel, 2008. On the use of Tedlar R bags for breath-gas sampling and analysis. Journal of Breath Research 2.

Abstract

This study draws the attention on the characterization of high quality monovarietal extra virgin olive oils (EVOOs) produced in Marche Region (Italy), in order to find new possible markers of quality. Five different cultivars were selected and investigated: Ascolana Tenera, Coroncina, Mignola, Piantone di Mogliano and Raggia. The study was developed in two different years (2015 and 2016) to underline possible correlation between the same varieties. Meanwhile, a comparison with EVOOs from the large scale distribution was carried out. Chemical analysis and sensory characterization were performed, paying particular attention to the determination of molecules responsible for the sensory and healthy properties, such as volatile and polyphenols substances.

Chapter 2.

Characterization and authentication of extra virgin olive oils (EVOOs)

2.1 Introduction

2.1.1 Mediterranean diet and extra virgin olive oil (EVOO)

"The Mediterranean Diet is much more than a simple diet. It promotes social interaction, seeing as the communal meal lays the foundations for social customs and festivities shared by a given community, which in turn has given space to a remarkable corpus of knowledge, songs, aphorisms, tales and legends. The Diet is based on respect for its territory and biodiversity, and guarantees the conservation and the development of traditional trades and professions associated with fishing and agriculture in Mediterranean communities" [1]. It is with such motivation that the United Nations Educational, Scientific and Cultural Organization (UNESCO) recognized the Mediterranean Diet (MD) as an Intangible Cultural Heritage of Humanity in November 2010. A heritage that reunites the dietary habits of the communities in the Mediterranean basin (Italy, Spain, Greece, Morocco, Portugal, Croatia and Cyprus), reinforced over centuries and almost still unchanged up until the fifties, it represents so much more than a simple list of foods, but rather a culture of social, traditional and agricultural practices.

The olive tree, *Olea europeae L.*, is one of the most important crops in Mediterranean countries, in particular Italy, Spain and Greece [2]. Olive oil, and especially extra virgin olive oil (EVOO) is the main source of lipids in the MD [3]. As established by the International Olive Council (IOC), it is obtained directly from the olive fruit through mechanical and other physical processes like washing, decantation, centrifugation and filtration. The consumption of EVOO is becoming more important in daily diets due to its nutraceutical and beneficial effects on human health. In fact, it has been demonstrated that EVOO has the ability to prevent important chronic and degenerative syndromes of ageing, different tumors and cardiovascular disease, reducing LDL-cholesterol levels and increasing HDL-cholesterol content on plasma [4]-[5]. A European Food Safety Authority (EFSA)'s review about olive oil polyphenols health claim, states that they maintain normal blood LDL-cholesterol concentrations, normal (fasting) blood concentrations of triglycerides, normal blood HDL-cholesterol concentrations, and normal blood glucose concentrations [6]. These important beneficial effects are also due to the typical EVOO composition that can be divided in two different fractions: the major one, the saponifiable fraction and the minor one, the unsaponifiable fraction. The first part is mainly composed of triacylglycerides (TAGs) containing high percentage of monounsaturated fatty acids (in particular oleic acid) and essential fatty acids as linoleic acid (ω-6) and linolenic acid (ω-3) in much smaller. The unsaponifiable fraction is formed by a broad class of compounds such as

polyphenols, sterols, tocopherols, volatile compounds and pigments, like carotenoids and chlorophylls. Many of these minor components are antioxidants and give an important contribute to the nutritional properties, but also the sensory characteristics and the shelf life of olive oil [3]-[7].

2.1.2 Monovarietal extra virgin olive oils (MEVOOs): an Italian excellence

Beyond their health benefits, EVOOs are particularly appreciated by consumers due to their intense color, taste and aromatic characteristics. The Italian production of olive oil, based mainly on autochthonous varieties, contributes to the maintenance of the vast genetic biodiversity present on our territory. Currently, more than 1250 olive varieties have been described and cataloged worldwide and of these over 42%, which is progressively increasing, is represented by Italian varieties [8]. Considering the importance of EVOO in the market, it is necessary to legally protect this product applying several procedures to chemically determine and guarantee the quality, but also to detect possible adulterations or illegal mixtures with other low quality vegetal oils. In this sense, the chemical composition and sensory evaluation of monovarietal EVOOs, which are obtained from a single variety of olive fruit from a specific production area, is very interesting from both a scientific and commercial points of view. For these reasons, in the last years the interest of producers was focused on monovarietal EVOOs, allowing to distinguish and emphasize the peculiarities of their products [9]. In this regards, the quality of EVOO from Marche region is continuously improving, due to the work of producers and local associations that put the attention to all the steps of the production process [10]. However, the quality and the peculiarity of EVOO are influenced by various factors like the genetic factors, pedoclimatic condition, agricultural practices, extraction methods, processing techniques and storage conditions [11]. Marche region is placed at the center of the so-called "Italian boot", bathed by the Adriatic Sea in the east. Its territorial geography varies from hill (Pesaro-Urbino, Ancona) to mountains (Macerata, Fermo, Ascoli Piceno)**.** The climate, and also the precipitations, in Marche are variable depending on site, in fact the average annual precipitation varies from 769 mm in Macerata to 789 and 784 mm respectively in Ancona and Pesaro. The annual temperatures are between 11 and 14 °C. The olive growing has a long tradition in Marche with the presence of autochthonous varieties cultivated along the region. The value of olive oil from the Marche region was appreciated since the medieval period. It was sold to merchants from Florence and Venice at a much higher price compared to olive oils from other Italian regions, due to its sensory superiority [10]. Among the different olive varieties in the Mache region, this thesis work considers the monovarietal extra virgin olive oils (MEVOO) of Ascolana Tenera (Figure 1), Coroncina (Figure 2), Mignola (Figure 3), Piantone di Mogliano (Figure 4) and Raggia (Figure 5). These cultivars, produced in different areas of the region, are considered the most representative varieties of the Marche region because they covering all the territory.

Ascolana Tenera (ASC):

It is native to the Piceno area, in which it has maximum diffusion, but it is also occasionally cultivated in other Marche areas. It has light green big olives (8-10 grams) rich in pulp. The oil has a green/yellow color and sweet taste, with lightly fruity herbaceous. Its reported [12] fatty acid composition is: palmitic acid 13.9%, oleic acid 74.5% and linoleic acid 6.7%. The optimal collection period is around the end of September-beginning of October.

Figure 1. Cultivar Ascolana Tenera

Coroncina (COR):

It is native to Macerata province, with greater concentration in the municipalities of Caldarola and Serrapetrona, up to the internal areas, at altitudes above 600 m a.s.l. Their size and consequently the pit-pulp ratio results in a low oil yield. The oil has a yellow-gold color and very fruity, pungent and bitter taste. Its reported [12] fatty acid composition is: palmitic acid 13.3%, oleic acid 73.8% and linoleic acid 8.4%. The optimal collection period is around the end of November, first half of December.

Figure 2. Cultivar Coroncina

Mignola (MIG):

It is mainly diffused in Macerata, Ascoli Piceno and Ancona provinces, with greater concentration in the municipality of Cingoli. It has ovoid shape and small size olives (1-2 grams). This variety has a characteristic berry flavour and is very rich in polyphenols, responsible for its bitter taste. The oil has a yellow-gold colour. Its reported [12] fatty acid composition is: palmitic acid 14.8%, oleic acid 71.3% and linoleic acid 9.0%. The optimal collection period is around the middle of November.

Figure 3. Cultivar Mignola

Piantone di Mogliano (MOG):

It is a Marche cultivar, diffused mainly in the Macerata province, with greater concentration in the municipalities of Mogliano, Macerata and neighboring, up to the internal areas of the province, at altitudes above 600 m a.s.l. It has oval shape and quite big olives (2-4 grams). The ripe fruit has a characteristic red-violet color. The oil has a yellow-green colour and a pleasant taste. Its reported [12] fatty acid composition is: palmitic acid 12.4%, oleic acid 76.2% and linoleic acid 6.8%. The optimal collection period is around the middle of November.

Figure 4. Cultivar Piantone di Mogliano

Raggia (RAG):

It is a Marche cultivar, diffused mainly in the Ancona province, with greater concentration in the municipalities of Ostra, Monte San Vito and Morro d'Alba. It has ovoid shape and medium size olives (2 grams). The fruit is rich in pulp and for this reason is good even like table olive. The oil has a green/yellow-gold color and mild fruity taste. Its reported [12] fatty acid composition is: palmitic acid 13.3%, oleic acid 74.9% and linoleic acid 7.3%. The optimal collection period is around the middle of November.

Figure 5. Cultivar Raggia

2.1.3 Chemical characteristics of EVOO

For IOC an olive oil can be classified as “Extra Virgin” if it comes from virgin oil production only and has a superior taste. European Commission Regulation No. 61/2011, amending Regulation (EEC) No 2568/91, established the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. In the figure below (Figure 6) [13], the olive oils characteristics are shown.

Category	Fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs)	Acidity (%) (*)	Peroxide index mEq O_2/kg (*)	Waxes mg/kg (**)	2-glyceril monopalmitate (%)	Stigmastadiene mg/kg (1)	Difference: ECN42 (HPLC) and ECN42 (theoretical calculation)	K_{232} (*)	K_{270} (*)	Delta-K (*)	Organoleptic evaluation Median defect (Md) (*)	Organoleptic evaluation Fruity median (Mf) (*)
1. Extra virgin olive oil	Σ FAME + FAEE ≤ 75 mg/kg or 75 mg/kg <Σ FAME + FAEE ≤ 150 mg/kg and (FAEE/FAME) ≤ 1,5	≤ 0,8	≤ 20	≤ 250	≤ 0,9 if total palmitic acid % ≤ 14 % ≤ 1,0 if total palmitic acid % > 14 %	≤ 0,10	≤ 0,2	≤ 2,50	≤ 0,22	≤ 0,01	Md = 0	Mf > 0
2. Virgin olive oil	—	≤ 2,0	≤ 20	≤ 250	≤ 0,9 if total palmitic acid % ≤ 14 % ≤ 1,0 if total palmitic acid % > 14 %	≤ 0,10	≤ 0,2	≤ 2,60	≤ 0,25	≤ 0,01	Md ≤ 3,5	Mf > 0
3. Lampante olive oil	—	> 2,0	—	≤ 300 (3)	≤ 0,9 if total palmitic acid % ≤ 14 % ≤ 1,1 if total palmitic acid % > 14 %	≤ 0,50	≤ 0,3	—	—	—	Md > 3,5 (5)	—
4. Refined olive oil	—	≤ 0,3	≤ 5	≤ 350	≤ 0,9 if total palmitic acid % ≤ 14 % ≤ 1,1 if total palmitic acid % > 14 %	—	≤ 0,3	—	≤ 1,10	≤ 0,16	—	—
5. Olive oil composed of refined and virgin olive oils	—	≤ 1,0	≤ 15	≤ 350	≤ 0,9 if total palmitic acid % ≤ 14 % ≤ 1,0 if total palmitic acid % > 14 %	—	≤ 0,3	—	≤ 0,90	≤ 0,15	—	—
6. Crude olive-residue oil	—	—	—	> 350 (4)	≤ 1,4	—	≤ 0,6	—	—	—	—	—
7. Refined olive-residue oil	—	≤ 0,3	≤ 5	> 350	≤ 1,4	—	≤ 0,5	—	≤ 2,00	≤ 0,20	—	—
8. Olive-residue oil	—	≤ 1,0	≤ 15	> 350	≤ 1,2	—	≤ 0,5	—	≤ 1,70	≤ 0,18	—	—

(1) Total isomers which could (or could not) be separated by capillary column.
(2) Or where the median defect is less than or equal to 3,5 and the fruity median is equal to 0.
(3) Oils with a wax content of between 300 mg/kg and 350 mg/kg are considered to be lampante olive oil if the total aliphatic alcohol content is less than or equal to 350 mg/kg or if the erythrodiol and uvaol content is less than or equal to 3,5 %.
(4) Oils with a wax content of between 300 mg/kg and 350 mg/kg are considered to be crude olive-residue oil if the total aliphatic alcohol content is above 350 mg/kg and if the erythrodiol and uvaol content is greater than 3,5 %.

Category	Acid content (1)						Total transoleic isomers (%)	Total transli-noleic + transli-nolenic isomers (%)	Sterols composition						Total sterols (mg/kg)	Erythrodiol and uvaol (%) (**)
	Myristic (%)	Linolenic (%)	Arachidic (%)	Eicosenoic (%)	Behenic (%)	Lignoceric (%)			Cholesterol (%)	Brassic-asterol (%)	Campesterol (%)	Stig-masterol (%)	Betasi-tosterol (%) (2)	Delta-7-stig-mastenol (%)		
1. Extra virgin olive oil	≤ 0,05	≤ 1,0	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,05	≤ 0,05	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
2. Virgin olive oil	≤ 0,05	≤ 1,0	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,05	≤ 0,05	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
3. Lampante olive oil	≤ 0,05	≤ 1,0	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,10	≤ 0,10	≤ 0,5	≤ 0,1	≤ 4,0	—	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5 (3)
4. Refined olive oil	≤ 0,05	≤ 1,0	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,20	≤ 0,30	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
5. Olive oil composed of refined and virgin olive oils	≤ 0,05	≤ 1,0	≤ 0,6	≤ 0,4	≤ 0,2	≤ 0,2	≤ 0,20	≤ 0,30	≤ 0,5	≤ 0,1	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 000	≤ 4,5
6. Crude olive-residue oil	≤ 0,05	≤ 1,0	≤ 0,6	≤ 0,4	≤ 0,3	≤ 0,2	≤ 0,20	≤ 0,10	≤ 0,5	≤ 0,2	≤ 4,0	—	≥ 93,0	≤ 0,5	≥ 2 500	> 4,5 (4)
7. Refined olive-residue oil	≤ 0,05	≤ 1,0	≤ 0,6	≤ 0,4	≤ 0,3	≤ 0,2	≤ 0,40	≤ 0,35	≤ 0,5	≤ 0,2	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 800	> 4,5
8. Olive-residue oil	≤ 0,05	≤ 1,0	≤ 0,6	≤ 0,4	≤ 0,3	≤ 0,2	≤ 0,40	≤ 0,35	≤ 0,5	≤ 0,2	≤ 4,0	< Camp.	≥ 93,0	≤ 0,5	≥ 1 600	> 4,5

(1) Other fatty acids content (%): palmitic: 7,5-20,0; palmitoleic: 0,3-3,5; heptadecanoic: ≤ 0,3; heptadecenoic: ≤ 0,3; stearic: 0,5-5,0; oleic: 55,0-83,0; linoleic: 3,5-21,0.
(2) Total: Delta-5,23-stigmastadienol+chlerosterol+beta-sitosterol+sitostanol+delta-5-avenasterol+delta-5,24-stigmastadienol.
(3) Oils with a wax content of between 300 mg/kg and 350 mg/kg are considered to be lampante olive oil if the total aliphatic alcohol content is less than or equal to 350 mg/kg or if the erythrodiol and uvaol content is less than or equal to 3,5 %.
(4) Oils with a wax content of between 300 mg/kg and 350 mg/kg are considered to be crude olive-residue oil if the total aliphatic alcohol content is above 350 mg/kg and if the erythrodiol and uvaol content is greater than 3,5 %.

Notes:

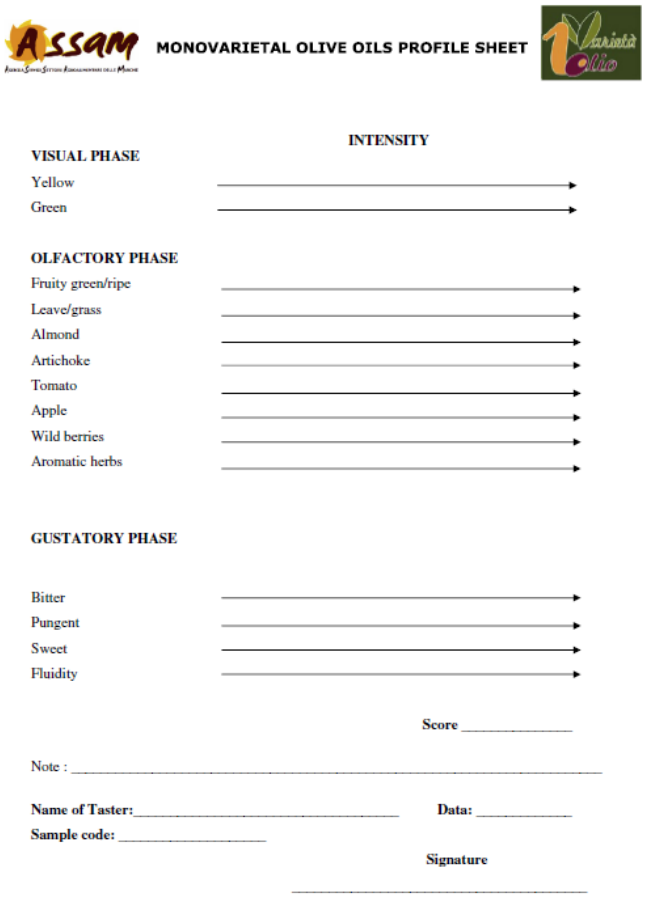
(a) The results of the analyses must be expressed to the same number of decimal places as used for each characteristic.
The last digit must be increased by one unit if the following digit is greater than 4.
(b) If just a single characteristic does not match the values stated, the category of an oil can be changed or the oil declared impure for the purposes of this Regulation.
(c) If a characteristic is marked with an asterisk (*), referring to the quality of the oil, this means the following:
— for lampante olive oil, it is possible for both the relevant limits to be different from the stated values at the same time,
— for virgin olive oils, if at least one of these limits is different from the stated values, the category of the oil will be changed, although they will still be classified in one of the categories of virgin olive oil.
(d) If a characteristic is marked with two asterisks (**), referring to the quality of the oil, this means that for all types of olive-residue oil, it is possible for both the relevant limits to be different from the stated values at the same time.

Figure 6. Olive oil characteristics [13]

EVOO must comply with strict chemical and organoleptic parameters of quality. Some parameters, like oil acidity (≤ 0.8% expressed in oleic acid, g per 100 g of oil) or oil peroxide values (≤ 20 mEq O_2/Kg), only help to do an initial screening permitting to discard oils that do not fulfil the legal limits for EVOO but they do not enable to conclude on high oil quality also because these parameters can have low values even if the oil has undergone a fraudulent refining process. Instead, other parameters like phenolic or volatiles substances are much more meaningful when dealing with the assessment of the quality, but they are not regulated by legislation. However (*cf.* paragraph 2.3.4), EFSA opens the possibility of using the claim relative to the olive oil polyphenols, when the product contains at least 5 mg of "hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol)" per 20 g of olive oil [6]. As regards volatile compounds, they are the main responsible for EVOO aroma. The presence of these compounds can determine the particular EVOO flavor, characterized by a series of positive sensory attributes that produce a particular balance of green, fruity, bitter and pungent sensory notes, but some of these can also produce negative sensory attributes (like moldy, winey or rancid). These compounds are enzymatically produced from the polyunsaturated fatty acids during oil extraction through the cascade of reactions collectively called "Lipoxygenase (LOX) pathway", activated by the mechanical break of olive fruit [14]-[15]. Moreover it has been observed how they can be formed starting from the amino acids valine and leucine that are converted into methyl-branched alkyl and acyl compounds of esters and into methyl-branched alcohols, which have the potential to change the sensory perception [16]. In addition to this, it is also important to consider the fatty acids composition, fatty acids alkyl esters (FAAEs) and sensorial evaluation of an oils. These parameters are regulated by the legislation (Figure 6). Anyway, it is important to highlight that EVOO characteristics have a wide range, as can be expected, because they are influenced by many factors. The three main factors are the agronomy/environment (state of olive grove and olive cultivars, growing area, fruit ripening, cultivation techniques, water resources, and soil management), the technology (for example the extraction system and malaxation conditions), and the storage conditions of oils (such as temperature and light exposure).

2.1.4 Sensorial analysis

There are several ways of defining the concept of "quality" of a food, and there is probably no single definition that adequately satisfies all situations. The quality of a food is defined by the UNI-ISO 8402 regulations as "the set of properties and characteristics of a product that give it the ability to satisfy the consumer needs". The quality of olive oil can be defined on the basis of a series of characteristics from a commercial, nutritional and organoleptic point of view. The latter aspect is based on the aroma, taste and color perception by the consumers. The importance of the organoleptic quality of EVOOs is linked to its uniqueness among vegetable oils, as it has the peculiarity of presenting, thanks to a purely physical-mechanical extraction process, characteristic sensorial attributes. The sensorial quality of an oil is defined by the organoleptic evaluation carried out according to the method defined by the IOC and adopted by the European Union. The Panel Test is the international method of assessing the sensory characteristics of VOO applicable for the classification of oils according to the intensity of the "fruity" and the possible presence of defects like fusty/sludge, mold, winey and rancid. The methodology establishes the tasting technique, the glass dimensions, the characteristics of the place where the panel (group of tasters) is found, the reference vocabulary for olive oil, the survey form and the calculation for the classification. The panel is made up of a group of tasters (from 8 to 10 judges) selected and trained. Each member individually tastes the oil in a special cabin. The oil is contained in a colored glass, to prevent its sight and to prevent that color influencing the taster. The analysis of the sample is carried out by evaluating its smell and taste. Positive (merits) and negative (defects) sensations are noted on a specific document. In the figure below (Figure 7), an example of profile sheet used by the Agency for Services in Agri-Food Sector of Marche (ASSAM) Regional Panel, is reported [9]. The panel leader is responsible for the consistency of the evaluation of his group and, by means of a mathematical calculation, of the assigning the merceological classification of the product. The score range for each attribute is from 0 to 9. An oil can to be classified as extra virgin if it has the defects median equal to zero, and the fruity median greater than zero.


MONOVARIETAL OLIVE OILS PROFILE SHEET

INTENSITY

VISUAL PHASE

Yellow

Green

OLFACTORY PHASE

Fruity green/ripe

Leave/grass

Almond

Artichoke

Tomato

Apple

Wild berries

Aromatic herbs

GUSTATORY PHASE

Bitter

Pungent

Sweet

Fluidity

Score _____

Note : _____

Name of Taster:_____ Data: _____

Sample code: _____

Signature

_____

Figure 7. Profile sheet used by trained panelists (ASSAM Regional Panel – Marche, acknowledged by International Olive Oil Council in 2000 and by the Italian Government in 2004), according to the methods described in EC Regulations 796/02 and 640/2008, for the merceological classification of the EVOO [9]

2.1.5 Aim of the work

There is nowadays a growing interest on the quality of food products due to hedonistic, nutritional and health aspects. EVOO is one of the products that have all of these properties. It is highly appreciated by consumers due to its intense colour, aromatic and taste characteristics but also for its health benefits. For these reasons EVOO is subject to numerous chemical analyses and continuous controls; in particular also to prevent and eliminate the possibility of eventual frauds or adulterations. In this research study, MEVOOs produced in the years 2015 and 2016, were analyzed. More in details, Ascolana Tenera (ASC), Coroncina (COR), Mignola (MIG), Piantone di Mogliano (MOG) and Raggia (RAG) cultivars were taken into account. The final objective of this work was focused on the assessment of chemical characteristics in order to contribute to the possible definition of new possible markers of quality, underlining in particular the peculiarities of the Marche varieties. In order to achieve this, a comparison with commercial oils was carried out. Therefore, high price and low price EVOOs, (HEVOO and LEVOO, respectively) and Olive Oil (OO), produced in the years 2014 and 2015 were bought and investigated.

2.2 Materials and Methods

2.2.1 Samples

A total of 80 samples of MEVOOs were provided by local producers in the years 2015 and 2016 in Marche Region (Italy) and stored away from light. In particular, 7 samples of Ascolana Tenera, 7 of Coroncina, 7 of Mignola, 9 of Piantone di Mogliano and 7 of Raggia for the 2015 year and 8 sample of Ascolana Tenera, 7 of Coroncina, 8 of Mignola, 8 of Piantone di Mogliano and 12 of Raggia for the 2016 year. In addition 30 bottles of commercial EVOOs and 10 of OOs were purchased from local shops and supermarkets during the year 2014 and 2015 in the area around Macerata (Marche Region, Italy) and stored away from the light. In turn, EVOO samples were divided in LEVOOs having a price range from 3.78 €/L to 5.80 €/L, and HEVOOs having a price range between 7.49 €/L and 25.80 €/L. OOs price ranged between 3.59 and 5.59 €/L. Regarding their origin, all the HEVOOs were produced in Italy and with Italian olives (as resulted from the label) and among LEVOOs, 13 were blends from European Union (EU) countries and 2 were blends from EU and non-EU countries.

2.2.2 Acidity and peroxide value

The determination of olive oil acidity (expressed as oleic acid g in 100 g olive oil) and peroxide value (expressed as milliequivalents of active oxygen per kg of oil) were carried out according to the EC Regulation No. 2568/1991 and subsequent modifications [17].

2.2.3 Fatty acids composition

Fatty acid methyl esters were obtained by reacting 5 mg of the oil dissolved in hexane (1 mL) with 2 N potassium hydroxide in methanol (0.1 mL) and then analysed by gas chromatography coupled with flame ionization detection (GC-FID) under reported conditions [18].

2.2.4 Fatty acids alkyl esters

The analyses were performed following the procedures reported in Biedermann et al. [19]. Briefly, 25 mg of oil were dissolved in 1.5 mL of heptane and injected into online coupled high performance liquid chromatography-gas chromatography-flame ionization detector (HPLC-GC-

FID) instrument. The separation was performed on a Spherisorb silica column (250 × 2.0 mm, 5 µm, from Grom, Rottenburg-Hailfingen, Germany). The mobile phase was hexane containing 5% methyl tert butyl ether (MTBE), at a flow rate of 300 µL min^{-1}. The injection volume was 80 µL.

2.2.5 Determination of α-tocopherol

The oil sample (100 mg) was dissolved in 5 mL of hexane and filtered through a 0.45 µm PTFE filter before high performance liquid chromatography coupled to a fluorescence detector (HPLC-FLD) analysis. The separation was performed on a Hypersil silica column (200 × 2.1 mm, 5 µm, from Thermo Fisher Scientific, Waltham, Massachusetts, United States). The mobile phase was hexane containing 0.25% *i*PrOH, at a flow rate of 0.5 mL min^{-1}. The injection volume was 10 µL. FLD was set with an excitation wavelength of 290 nm and an emission wavelength of 330 nm. For the quantification of commercial oils of 2014 and 2015 and MEVOOs of 2015, four standard stock solutions of α-tocopherol in hexane were prepared in the range 0.53 - 10.6 µg mL^{-1} and analysed to obtain the calibration curve (correlation coefficient R = 0.9992). For the quantification of MEVOOs of 2016, a standard solution of α-tocopherol in hexane was prepared at 2.5 µg mL^{-1} and analysed seven times, changing the injection volume, to obtain the calibration curve (correlation coefficient R = 0.9995).

2.2.6 Folin-Ciocalteu assay

The analyses were performed following the procedure reported in Ricciutelli et al. [20]. Briefly, 2.5 g of oil were dissolved in 2.5 mL of hexane and then extracted three times for 20 minutes under magnetic stirring with 2.5 mL of methanol/water 80:20 (v/v). The supernatants were collected, washed with 2 × 5 mL of hexane and stored in a 50 mL volumetric flask. An aliquot of 2.5 mL of FC reagent and 2.5 mL of saturated sodium carbonate solution were added and the solution was brought up to a volume of 50 mL with distilled water. After 120 minutes of reaction at ambient temperature in dark, the absorbance was measured at 765 nm in a UV-Vis spectrophotometer (Perkin-Elmer, Waltham, Massachusetts, United States). Total phenolics content was calculated and expressed as mg of gallic acid equivalent kg^{-1} of oil.

2.2.7 Quantification of polyphenols by HPLC-DAD-ESI/MS

The analyses were performed following the procedures reported in Ricciutelli et al. [20] and subsequent modifications. Briefly, 0.5 g of oil were dissolved in 0.5 mL of hexane and extracted with 4 × 0.5 mL of methanol/water (60:40, v/v). The methanolic extracted solutions were collected, evaporated to dryness and reconstituted with 0.25 mL of HPLC grade methanol before HPLC-DAD-ESI/MS analysis. HPLC-DAD-ESI/MS (ion trap) studies were performed using an Agilent 1100 (Santa Clara, CA, USA) with a diode-array detector (DAD) and a mass spectrometer detector (ion trap) equipped with an electrospray ionization (ESI) source. The separation was achieved on a Synergi Polar analytical column. The mobile phase was water (A) and methanol/*i*PrOH 90:10 v/v (B) both containing 0.1% formic acid, working in the gradient mode. HPLC-DAD analysis was performed monitoring different wavelengths: 260 nm for vanillic acid, 280 nm for hydroxytyrosol, tyrosol, and secoiridoids derivatives, pinoresinol, acetoxypinoresinol and syringic acid; 310 nm for *p*-coumaric acid, 325 nm for caffeic acid and ferulic acid, 338 nm for apigenin and 350 nm for luteolin. In HPLC-ESI/MS, ion source was operated in negative ionization (NI) mode and mass analyser in Full scan mode. Mass scan range was set in the range of m/z 70 - 1100 and extract ion chromatograms (EICs) from total ion chromatogram (TIC) were used for analytes quantification.

2.2.8 Volatile substances

An aliquot of 1.5 g of oil was weighted in a screw cap vial with pierceable septum, a small stirring magnet was added and the sample was conditioned at 40 °C for 10 minutes stirring at 300 rpm. A solid-phase microextraction (SPME) fibre coated with 50/30 µm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 1 cm long, was then exposed to the headspace of the sample for 30 minutes and then the fibre was retracted and exposed in the hot gas chromatograph injector kept at 260 °C. The instrument used for the analyses is a gas chromatograph coupled with a mass spectrometer detector (Agilent 6890 GC-MSD 5973 N, Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary column coated with polyethylene glycol (DB-WAX, length 60 m, internal diameter 0.25 mm, film thickness 0.25 µm). The instrumental conditions applied were: splitless injection with a splitless time of 4 minutes, carrier gas (helium) flow was 1.2 mL min^{-1}, the initial oven temperature was 40 °C held for 4 minutes, then the temperature was raised to 120 °C at 2.5 °C min^{-1} and then raised to 250 °C at 15 °C min^{-1} and held for 3.33 minutes. The temperature of the transfer line was held at 250 °C, ion source (electron impact was at 70 eV) at 230 °C and quadrupole was at

150 °C; mass scan range was 29 - 400 amu. Identification of eluted molecules was performed by comparison of the experimental retention indices, calculated with reference to linear alkanes, with those reported in literature, and with comparison of the experimental mass spectra with those of the NIST 08 library.

2.2.9 Sensory analysis

Sensory analysis of olive oil samples was carried out by ASSAM Marche Regional Panel, acknowledged by the International Olive Council (IOC) until 2004, and then, until today, by the Ministry of Agricultural, Food and Forestry Policies (MIPAAF) and according to the procedure reported in the EC Regulation No. 2568/1991 [17] and in its subsequent modifications. The trained tasters evaluated positive sensory, olfactory-gustatory and tactile attributes, as well as negative attributes.

2.2.10 Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's pairwise test using the software PAST [21] is applied to the data to determine the presence of significant differences among olive oils samples (significant level for $P < 0.05$).

2.3 Results and Discussion

2.3.1 Fatty acids composition

Fatty acid composition is a nutritional feature of undiscussed importance when dealing with the assessment of the quality of an oil. Fatty acids represent the major fraction of an oil and their typical composition in olive oil is represented mainly by oleic acid. The presence of oleic acid in such a high percentage in the fatty acid composition has made olive oil one of the key ingredients explaining the health benefits given by the MD. In this regard, the beneficial effects of EVOO associated with the oleic acid content, including protection against oxidative stress, blood pressure lowering, reduction of total and LDL-cholesterol levels, and even the reduction of the risk of breast, prostate, and colorectal cancer, are known [22]. It is well known [23] that oleic acid is the least oxidizable among unsaturated fatty acids and, together with the presence of numerous and effective natural antioxidants (biophenols) and a good conservation, makes the EVOO particularly stable in contrast to other oils. However oleic acid % can have a broad range even within olive oils. The IOC indicates that the content of oleic acid in olive oils can vary from 55% to 83% of total fatty acids. In the figure below (Figure 8), the comparisons between the different classes examined, for each fatty acid are shown.

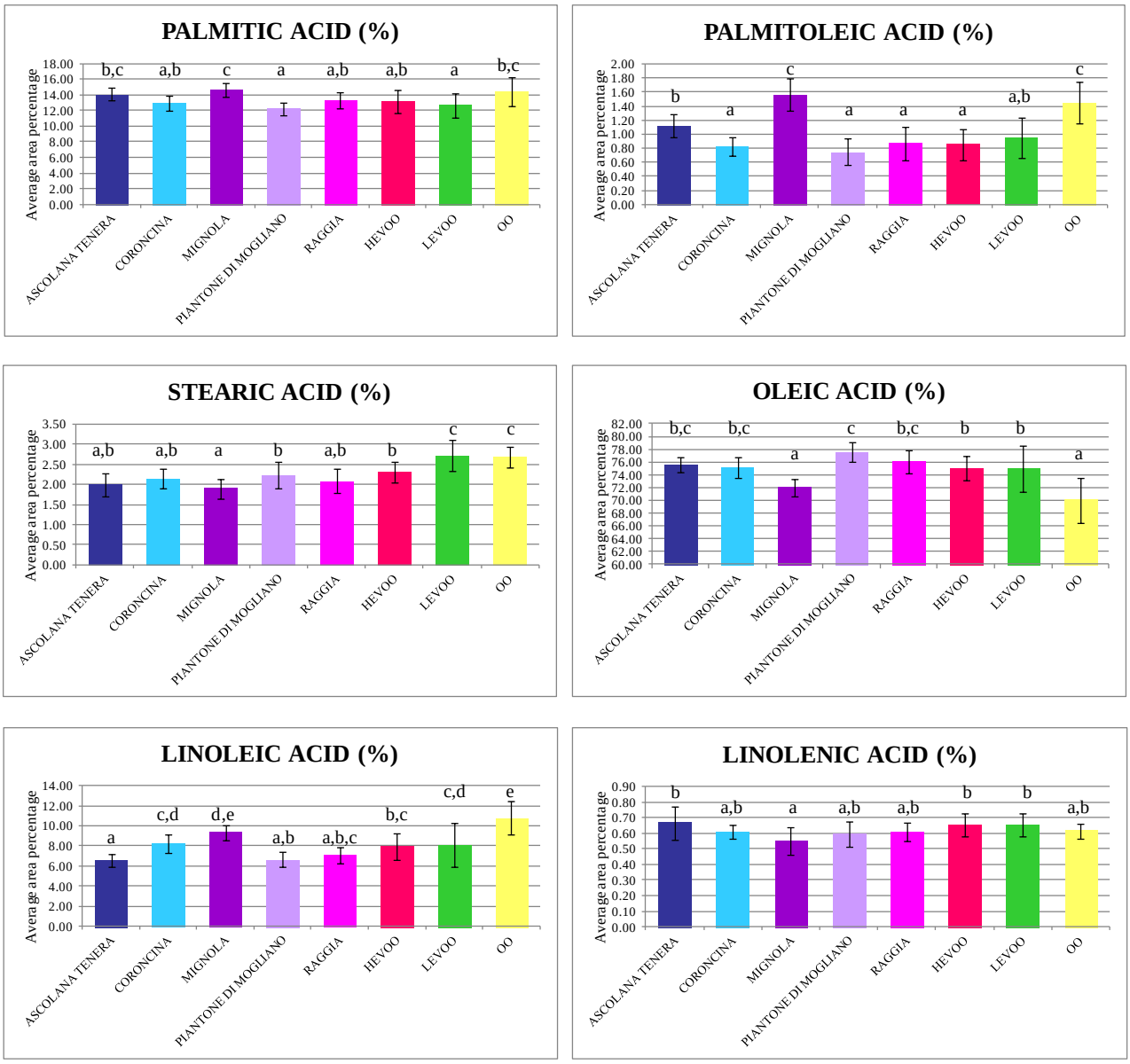


Figure 8. Average percentage of every fatty acid in each class of oils. Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

Considering palmitic acid and linolenic acid, the average area percentage for each class of oils are comparable and the respective ranges are 12.21% - 14.66% and 0.55% - 0.67%. In the case of palmitoleic acid and linoleic acid, Mignola cultivar shows the highest average percentage values (1.56% and 9.32%, respectively) as compared to the other cultivars, comparable only with the OO category. On the contrary, taking into account the oleic acid results, Mignola and OO show the lowest average percentage values (72.02% and 70.00% respectively), while Piantone di Mogliano cultivar has the highest content of oleic acid (77.55%). There aren't important differences for stearic acid (range from 1.90% - 2.71%), whose content seems to be comparable in each class of oils analyzed, showing the highest values for the commercial categories.

Nevertheless, the fatty acids composition of EVOO may differ, depending on the zone of production, the latitude, the overall pedoclimate growing conditions, the genetic characteristics of the olive cultivar, and fruit ripening stage. For these reasons, it is interesting to consider also the results obtained in the two different years of production, to underline possible differences within the same cultivar. Average fatty acids composition of commercial oils and monovarietal oils, for the two different years, are reported respectively in Table 7 and Table 8. It is possible to notice that for all of the fatty acids considered, with the only exception of stearic acid, the classes of analyzed oils show a comparable percentage abundance during the years taken into account. In the case of stearic acid, the situation is completely different. All the varieties of year 2015 have a higher % content of this acid than the same variety of the year 2016. This could be indicative that stearic acid, compared to the other fatty acids, is more affected by the external factors. About that, regarding fatty acid compositional changes occurring during ripening, even if not for all olive cultivars, it is known [24] that the stearic acid content increases during ripening and the use of too ripen olives leads to a low quality oil. More in detail, from Table 1 to Table 6, significant differences ($P < 0.05$) between all classes of oils for every fatty acid, are indicated in pink. Following (Figure 9) an example of chromatogram with the peaks identification is reported.

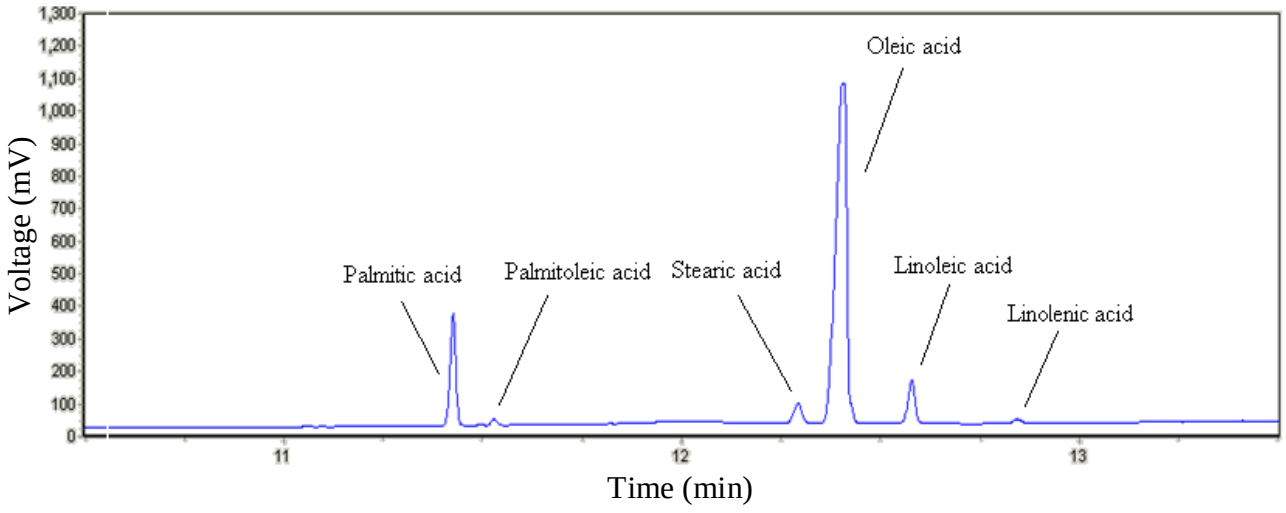


Figure 9. EVOO sample chromatogram

PALMITIC ACID																
	MOG 2015	LEVOO 2015	COR 2015	MOG 2016	HEVOO 2015	RAG 2015	OO 2015	RAG 2016	COR 2016	LEVOO 2014	MIG 2015	ASC 2016	ASC 2015	HEVOO 2014	MIG 2016	OO 2014
MOG 2015		1	1	0,9998	0,9889	0,9077	0,7215	0,07192	0,1118	0,08216	0,02395	0,008848	0,006938	0,01597	2.83E-04	7,86E-07
LEVOO 2015	0,09145		1	0,9999	0,9908	0,9147	0,7311	0,06591	0,1083	0,08064	0,02215	0,007809	0,006181	0,01511	1.79E-04	5.90E-04
COR 2015	0,8531	0,7871		1	1	0,9989	0,975	0,482	0,5064	0,373	0,1996	0,1097	0,08423	0,1227	2,51E-05	3.13E-02
MOG 2016	1,383	1,328	0,4675		1	1	0,9958	0,6764	0,686	0,5316	0,3214	0,19	0,1477	0,2028	4.75E-02	5.98E-02
HEVOO 2015	1,995	1,956	0,9879	0,5162		1	0,9997	0,8359	0,8342	0,6873	0,4621	0,2909	0,2297	0,3032	6.48E-02	9.04E-02
RAG 2015	2,558	2,53	1,607	1,192	0,7552		1	0,9975	0,994	0,9613	0,8974	0,7915	0,7033	0,7343	0,002877	0,002094
OO 2015	3,056	3,035	2,177	1,811	1,438	0,7096		1	1	0,9996	0,9984	0,9931	0,9807	0,9779	0,05852	0,03164
RAG 2016	4,784	4,828	3,531	3,149	2,785	1,725	0,7607		1	1	1	0,9995	0,9971	0,9963	0,02936	0,01968
COR 2016	4,547	4,565	3,483	3,13	2,79	1,876	1,003	0,3834		1	1	1	1	0,9999	0,1854	0,1024
LEVOO 2014	4,714	4,724	3,756	3,434	3,127	2,289	1,463	0,9771	0,5768		1	1	1	1	0,618	0,3893
MIG 2015	5,311	5,346	4,203	3,873	3,571	2,596	1,66	1,192	0,7199	0,08032		1	1	1	0,4946	0,2942
ASC 2016	5,74	5,791	4,558	4,234	3,947	2,898	1,902	1,489	0,9608	0,2798	0,2173		1	1	0,5467	0,332
ASC 2015	5,839	5,886	4,701	4,388	4,111	3,094	2,115	1,752	1,218	0,535	0,4981	0,2971		1	0,7509	0,5058
HEVOO 2014	5,49	5,514	4,495	4,193	3,917	3,028	2,147	1,79	1,316	0,684	0,6585	0,479	0,2038		0,9204	0,7302
MIG 2016	9,243	9,38	7,847	7,638	7,535	6,187	4,888	5,218	4,25	3,266	3,506	3,404	2,992	2,507		1
OO 2014	8,934	9,021	7,776	7,562	7,424	6,309	5,184	5,398	4,596	3,721	3,939	3,848	3,484	3,037	0,8626	

Table 1. Significant differences (P < 0.05) between the classes of oils are indicated in pink

PALMITOLEIC ACID																
	MOG 2015	MOG 2016	COR 2015	RAG 2015	HEVOO 2014	HEVOO 2015	LEVOO 2015	RAG 2016	COR 2016	LEVOO 2014	ASC 2015	ASC 2016	OO 2015	MIG 2015	OO 2014	MIG 2016
MOG 2015		1	1	1	1	0,9989	0,9931	0,958	0,981	0,2489	0,1124	0,03771	7.68E-02	5.75E-04	1.59E-03	1.77E-08
MOG 2016	0,07688		1	1	1	0,9996	0,9969	0,9775	0,9895	0,3086	0,1566	0,05911	0,0001505	1.58E-03	3.60E-03	7.73E-08
COR 2015	0,09682	0,0221		1	1	0,9998	0,9982	0,9862	0,9932	0,3617	0,2026	0,08511	0,0002776	4.14E-03	7.80E-03	3.39E-07
RAG 2015	0,8775	0,7822	0,736		1	1	1	1	1	0,6959	0,5195	0,2956	0,001786	4,12E-05	6.20E-02	4.89E-06
HEVOO 2014	1,072	0,9829	0,9375	0,2656		1	1	1	1	0,8868	0,7947	0,6005	0,01096	0,0006321	0,0006427	3.28E-04
HEVOO 2015	1,608	1,478	1,4	0,6015	0,2572		1	1	1	0,8368	0,6767	0,414	0,002336	3.55E-02	6.59E-02	1,57E-09
LEVOO 2015	1,9	1,762	1,673	0,8744	0,5028	0,3007		1	1	0,9129	0,8008	0,5565	0,004401	8.09E-02	0,0001366	4,23E-09
RAG 2016	2,312	2,151	2,041	1,214	0,7921	0,6556	0,3415		1	0,9542	0,8746	0,6523	0,005661	9.05E-02	0,0001646	3.09E-06
COR 2016	2,112	1,984	1,9	1,164	0,7969	0,6609	0,388	0,09458		0,9858	0,9607	0,8545	0,02421	0,001186	0,001299	2.95E-04
LEVOO 2014	4,057	3,904	3,781	3,109	2,633	2,783	2,537	2,336	2,047		1	1	0,7392	0,3076	0,2297	0,002277
ASC 2015	4,545	4,353	4,193	3,457	2,891	3,149	2,876	2,672	2,294	0,04687		1	0,6449	0,192	0,1462	0,0004628
ASC 2016	5,102	4,883	4,696	3,935	3,3	3,669	3,385	3,198	2,733	0,3791	0,3646		0,7613	0,2695	0,2063	0,0006784
OO 2015	7,478	7,251	7,04	6,369	5,65	6,267	6,022	5,921	5,306	3,018	3,212	2,969		1	1	0,7784
MIG 2015	9,029	8,719	8,421	7,685	6,75	7,734	7,461	7,424	6,521	3,906	4,228	4,002	0,6469		1	0,9232
OO 2014	8,718	8,465	8,222	7,55	6,744	7,53	7,284	7,221	6,487	4,111	4,394	4,182	1,094	0,5344		0,9977
MIG 2016	12,02	11,61	11,19	10,43	9,198	10,76	10,47	10,56	9,23	6,277	6,861	6,724	2,929	2,495	1,716	

Table 2. Significant differences (P < 0.05) between the classes of oils are indicated in pink

STEARIC ACID																
	MIG 2016	ASC 2016	RAG 2016	COR 2016	MOG 2016	MIG 2015	HEVOO 2014	ASC 2015	COR 2015	RAG 2015	HEVOO 2015	MOG 2015	OO 2015	LEVOO 2014	LEVOO 2015	OO 2014
MIG 2016		1	0,9148	0,7652	0,3037	0,06598	0,09611	0,0003641	2.64E-02	1.70E-03	1.28E-03	1.38E-04	6.88E-04	4.64E-07	2.04E-09	2.33E-10
ASC 2016	0,4443		0,9855	0,9145	0,512	0,1448	0,1878	0,001195	9.58E-02	6.67E-03	5.52E-03	6.24E-04	2.43E-03	1.81E-06	1.00E-08	6.92E-10
RAG 2016	2,529	2,051		1	0,9981	0,8517	0,8594	0,04544	0,00511	0,000424	0,0004191	4,95E-05	0,0001235	9.30E-05	5.34E-07	2.34E-08
COR 2016	2,959	2,53	0,7373		1	0,9958	0,9933	0,323	0,07979	0,01321	0,01759	0,003617	0,003277	7.91E-03	3,57E-07	5.40E-06
MOG 2016	3,917	3,472	1,686	0,8245		1	1	0,6647	0,2471	0,0536	0,07324	0,01752	0,01366	4.00E-02	2.05E-03	2.65E-05
MIG 2015	4,831	4,402	2,741	1,812	1,047		1	0,985	0,782	0,3695	0,4823	0,2061	0,1242	0,001032	0,0001456	1.37E-03
HEVOO 2014	4,633	4,243	2,718	1,895	1,198	0,2409		0,9986	0,938	0,6532	0,7748	0,4841	0,2965	0,006534	0,002075	2.17E-02
ASC 2015	6,957	6,528	5,016	3,87	3,173	2,058	1,638		1	0,9978	0,9999	0,9885	0,8928	0,08447	0,0388	0,0004205
COR 2015	7,847	7,418	5,969	4,732	4,063	2,92	2,425	0,8621		1	1	1	0,9955	0,3042	0,2081	0,0035
RAG 2015	8,719	8,29	6,903	5,577	4,935	3,765	3,196	1,707	0,8445		1	1	1	0,6787	0,6096	0,02228
HEVOO 2015	8,807	8,35	6,907	5,453	4,777	3,531	2,937	1,348	0,4333	0,4624		1	0,9994	0,3907	0,2695	0,004365
MOG 2015	9,485	9,028	7,639	6,106	5,455	4,184	3,527	2,001	1,086	0,1907	0,6982		1	0,6864	0,598	0,01803
OO 2015	8,998	8,608	7,332	6,145	5,563	4,49	3,934	2,611	1,824	1,054	1,524	0,9336		0,9917	0,9945	0,28
LEVOO 2014	11,15	10,76	9,602	8,236	7,71	6,581	5,87	4,702	3,915	3,145	3,719	3,129	1,936		1	0,9862
LEVOO 2015	12,69	12,23	11,11	9,197	8,661	7,275	6,32	5,092	4,178	3,282	4,003	3,305	1,859	0,3357		0,8817
OO 2014	13,41	13,02	11,99	10,44	9,974	8,785	7,91	6,906	6,119	5,348	6,032	5,442	3,976	2,04	2,649	

Table 3. Significant differences (P < 0.05) between the classes of oils are indicated in pink

OLEIC ACID																
	OO 2014	MIG 2016	OO 2015	MIG 2015	LEVOO 2014	COR 2016	HEVOO 2015	HEVOO 2014	LEVOO 2015	ASC 2015	ASC 2016	RAG 2016	COR 2015	RAG 2015	MOG 2015	MOG 2016
OO 2014		0,5837	0,7091	0,07335	0,01623	0,001287	2.23E-02	0,0004277	3,79E-06	1.69E-02	8.29E-03	2.12E-04	2.06E-03	2.02E-03	2.08E-06	2.47E-06
MIG 2016	3,333		1	0,9974	0,8486	0,4123	0,03785	0,1681	0,008616	0,02182	0,0138	0,0006927	0,00359	0,003523	5,27E-06	5.76E-03
OO 2015	3,082	0,08653		0,9997	0,9428	0,6701	0,1636	0,3501	0,0583	0,09324	0,07191	0,01053	0,02406	0,02372	0,0001844	0,0001706
MIG 2015	4,773	1,73	1,445		1	0,9938	0,7041	0,8761	0,3905	0,4937	0,4305	0,1085	0,1904	0,1882	0,002315	0,002109
LEVOO 2014	5,483	2,75	2,401	1,149		1	0,9992	0,9998	0,9805	0,9854	0,9803	0,8251	0,8606	0,8584	0,1306	0,1123
COR 2016	6,491	3,673	3,162	1,881	0,5686		1	1	0,9982	0,9987	0,998	0,9331	0,9516	0,9504	0,178	0,154
HEVOO 2015	7,885	5,1	4,327	3,092	1,554	1,052		1	1	1	1	0,9994	0,9995	0,9995	0,4671	0,4147
HEVOO 2014	6,889	4,31	3,807	2,668	1,406	0,9501	0,06937		1	1	1	1	1	1	0,8051	0,754
LEVOO 2015	8,449	5,751	4,89	3,719	2,118	1,678	0,6901	0,4941		1	1	1	1	1	0,7953	0,7395
ASC 2015	7,976	5,353	4,647	3,508	2,053	1,627	0,7125	0,5347	0,08618		1	1	1	1	0,9098	0,8712
ASC 2016	8,203	5,553	4,784	3,635	2,12	1,692	0,7533	0,5601	0,1026	0,01196		1	1	1	0,8871	0,8434
RAG 2016	9,329	6,717	5,667	4,564	2,814	2,45	1,51	1,144	0,7896	0,6216	0,6341		1	1	0,9674	0,9441
COR 2015	8,638	6,101	5,309	4,233	2,715	2,352	1,499	1,197	0,8727	0,7251	0,7369	0,1934		1	0,9965	0,9918
RAG 2015	8,644	6,109	5,315	4,24	2,722	2,359	1,507	1,203	0,8804	0,7322	0,7443	0,2014	0,007126		0,9967	0,9921
MOG 2015	10,68	8,346	7,182	6,27	4,459	4,275	3,561	2,865	2,889	2,55	2,632	2,244	1,781	1,773		1
MOG 2016	10,63	8,318	7,208	6,306	4,545	4,363	3,668	2,985	3,017	2,683	2,765	2,395	1,934	1,927	0,2132	

Table 4. Significant differences (P < 0.05) between the classes of oils are indicated in pink

LINOLEIC ACID																
	ASC 2015	MOG 2016	MOG 2015	ASC 2016	RAG 2016	HEVOO 2014	RAG 2015	COR 2015	LEVOO 2014	LEVOO 2015	HEVOO 2015	COR 2016	MIG 2015	MIG 2016	OO 2015	OO 2014
ASC 2015		1	1	0,9999	0,9919	0,9989	0,9965	0,5071	0,5451	0,1117	0,04422	0,03515	0,001975	0,0002028	7.56E-04	4.01E-04
MOG 2016	0,6019		1	1	0,9999	1	1	0,75	0,768	0,2448	0,1084	0,08419	0,005452	0,0005858	2.04E-03	1.07E-03
MOG 2015	1,135	0,5361		1	1	1	1	0,9077	0,9093	0,4314	0,2181	0,1678	0,01295	0,001486	4.95E-03	2.59E-03
ASC 2016	1,284	0,7058	0,1902		1	1	1	0,9579	0,9545	0,588	0,3441	0,2628	0,02712	0,003879	1.46E-02	7.85E-03
RAG 2016	1,933	1,331	0,7874	0,5582		1	1	0,9897	0,9874	0,7064	0,4292	0,3364	0,03174	0,003674	1.10E-02	5.63E-03
HEVOO 2014	1,603	1,1	0,6572	0,4809	0,03631		1	0,9991	0,9982	0,947	0,8273	0,7079	0,208	0,0639	0,0005527	0,0003342
RAG 2015	1,781	1,238	0,7546	0,5562	0,0695	0,02344		0,9979	0,9965	0,8918	0,7036	0,573	0,1114	0,02404	0,0001233	6.97E-02
COR 2015	3,482	2,994	2,558	2,312	1,98	1,575	1,7		1	1	0,9999	0,9984	0,8115	0,4708	0,008277	0,005151
LEVOO 2014	3,407	2,953	2,552	2,334	2,021	1,671	1,781	0,2292		1	1	0,9999	0,9482	0,7515	0,03573	0,02427
LEVOO 2015	4,548	4,068	3,633	3,324	3,088	2,378	2,616	0,7716	0,4492		1	1	0,9569	0,7122	0,01667	0,0103
HEVOO 2015	5,026	4,565	4,145	3,821	3,638	2,808	3,093	1,249	0,8791	0,5265		1	0,995	0,902	0,04282	0,0276
COR 2016	5,135	4,701	4,311	4,019	3,838	3,084	3,353	1,653	1,28	1,022	0,5437		1	0,9949	0,1829	0,1327
MIG 2015	6,331	5,936	5,58	5,255	5,182	4,176	4,549	2,849	2,372	2,319	1,841	1,196		1	0,668	0,5704
MIG 2016	7,149	6,777	6,437	6,071	6,092	4,844	5,309	3,553	2,99	3,075	2,579	1,846	0,6107		0,8665	0,7929
OO 2015	8,945	8,641	8,365	8,022	8,113	6,798	7,319	5,767	5,127	5,471	5,041	4,258	3,166	2,698		1
OO 2014	9,137	8,839	8,567	8,22	8,325	6,976	7,511	5,959	5,305	5,676	5,247	4,45	3,358	2,895	0,1778	

Table 5. Significant differences (P < 0.05) between the classes of oils are indicated in pink

LINOLENIC ACID																
	MIG 2016	MOG 2016	LEVOO 2014	OO 2014	MIG 2015	RAG 2016	COR 2016	MOG 2015	COR 2015	RAG 2015	HEVOO 2014	OO 2015	ASC 2015	HEVOO 2015	ASC 2016	LEVOO 2015
MIG 2016		0,9082	0,9631	0,9258	0,6772	0,2761	0,4838	0,2528	0,2641	0,1457	0,06589	0,07535	0,01265	0,003298	0,001134	4.92E-02
MOG 2016	2,556		1	1	1	1	1	0,9997	0,9993	0,9921	0,9	0,9178	0,6905	0,5282	0,2773	0,05353
LEVOO 2014	2,276	0,03449		1	1	1	1	1	0,9999	0,9983	0,9597	0,968	0,8574	0,7723	0,5195	0,1895
OO 2014	2,483	0,2414	0,1865		1	1	1	1	1	0,9996	0,9808	0,9855	0,9164	0,8562	0,6257	0,2624
MIG 2015	3,148	0,6784	0,566	0,3646		1	1	1	1	1	0,9924	0,9947	0,9494	0,8965	0,6671	0,2569
RAG 2016	3,985	1,185	0,9788	0,7572	0,3986		1	1	1	1	0,997	0,9981	0,9654	0,9101	0,6627	0,2
COR 2016	3,528	1,058	0,9018	0,7003	0,3678	0,01476		1	1	1	0,999	0,9994	0,9888	0,9725	0,8349	0,4298
MOG 2015	4,046	1,416	1,198	0,987	0,6688	0,3344	0,2787		1	1	0,9998	0,9999	0,9951	0,9853	0,872	0,4503
COR 2015	4,016	1,547	1,334	1,132	0,8408	0,5463	0,4729	0,2229		1	1	1	0,9994	0,9981	0,9598	0,6891
RAG 2015	4,396	1,927	1,669	1,468	1,209	0,9597	0,8408	0,6131	0,3678		1	1	1	0,9999	0,9924	0,8575
HEVOO 2014	4,828	2,587	2,3	2,114	1,919	1,755	1,583	1,41	1,151	0,8155		1	1	1	1	0,9991
OO 2015	4,759	2,518	2,238	2,052	1,852	1,681	1,516	1,339	1,084	0,7483	0,06217		1	1	1	0,9985
ASC 2015	5,59	3,121	2,725	2,523	2,365	2,259	1,997	1,839	1,524	1,156	0,2398	0,307		1	1	0,9996
HEVOO 2015	6,135	3,44	2,944	2,728	2,599	2,549	2,2	2,054	1,687	1,288	0,2872	0,359	0,0342		1	0,999
ASC 2016	6,537	3,981	3,457	3,251	3,168	3,177	2,788	2,681	2,3	1,92	0,9053	0,9743	0,7259	0,7565		1
LEVOO 2015	7,627	4,933	4,236	4,02	4,035	4,202	3,636	3,594	3,123	2,724	1,579	1,651	1,47	1,583	0,7357	

Table 6. Significant differences (P < 0.05) between the classes of oils are indicated in pink

		HEVOO 2014		HEVOO 2015		LEVOO 2014		LEVOO 2015		OO 2014		OO 2015	
		Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%
Volatile substances (peak area units)	Ethanol	9.73E+06	81.3	1.89E+07	68.0	2.45E+07	75.5	3.49E+07	98.1	5.16E+06	44.4	5.66E+06	61.5
	3-Pentanone	6.98E+06	17.3	7.68E+06	38.8	6.00E+06	24.2	4.24E+06	41.0	2.30E+06	41.5	nd	
	1-Penten-3-one	6.88E+06	144.7	2.62E+06	64.8	1.46E+06	67.1	1.14E+06	83.9	nd		nd	
	1-Penten-3-ol	3.39E+06	29.5	3.02E+06	20.1	1.94E+06	29.4	1.44E+06	64.8	nd		nd	
	Hexanal	1.60E+07	97.7	1.16E+07	43.3	7.77E+06	155.9	6.55E+06	61.7	4.15E+06	45.0	2.32E+06	26.7
	(*E*)-2-Hexenal	1.42E+08	106.3	2.04E+08	63.8	5.25E+06	223.6	6.03E+07	55.1	3.89E+06	118.2	1.98E+06	40.7
	Acetic acid, hexyl ester	1.32E+07	60.4	1.50E+06	83.7	1.66E+07	101.3	3.14E+06	19.8	2.71E+06	141.2	2.27E+04	223.6
	(*Z*)-3-Hexen-1-ol, acetate	nd		7.17E+06	102.8	nd		1.49E+07	27.6	nd		4.69E+05	47.5
	(*Z*)-2-Penten-1-ol	1.98E+06	142.7	1.38E+06	114.6	nd		9.15E+05	109.6	nd		nd	
	1-Hexanol	8.32E+06	92.1	1.61E+07	114.5	5.38E+06	103.1	6.72E+06	42.0	7.02E+05	99.2	2.40E+05	137.4
	(*Z*)-3-Hexen-1-ol	6.17E+06	109.8	1.32E+07	59.5	1.00E+07	95.1	1.09E+07	29.9	1.01E+06	97.5	6.88E+05	25.3
	(*E*)-2-Hexen-1-ol	1.54E+07	112.3	2.37E+07	50.9	3.79E+06	5.2	8.66E+06	40.9	1.70E+06	45.5	2.00E+05	94.1
	Ratio Hexanal/(*E*)-2-Hexenal	0.11	92.0	0.06	67.9	1.48	69.7	0.11	112.1	1.07	38.1	1.17	65.5
α-Tocopherol (mg kg^{-1})		171.69	10.7	236.29	10.7	190.03	21.5	240.12	17.0	140.36	17.6	138.49	19.4
Fatty acids (%)	Palmitic acid	14.23	12.4	12.65	9.0	13.91	14.0	12.01	7.6	15.64	12.6	13.23	4.6
	Palmitoleic acid	0.84	38.5	0.86	19.9	1.10	36.9	0.88	22.1	1.50	27.1	1.40	12.2
	Stearic acid	2.12	5.9	2.41	11.2	2.68	13.5	2.73	15.2	2.88	7.0	2.50	5.8
	Oleic acid	75.08	2.9	75.02	2.5	73.74	6.7	75.48	3.8	68.54	6.6	71.46	2.1
	Linoleic acid	7.08	9.9	8.40	16.7	7.99	34.3	8.20	25.2	10.85	21.2	10.75	7.6
	Linolenic acid	0.65	3.6	0.66	14.3	0.58	7.8	0.69	6.8	0.58	6.0	0.65	4.2
	Ratio oleic acid/linoleic acid	10.68	10.6	9.17	17.9	10.11	32.9	9.76	26.0	6.60	26.2	6.68	8.9
Polar polyphenols (mg kg^{-1})	Hydroxytyrosol	9.32	49.8	13.23	73.1	10.07	27.2	13.34	45.5	1.02	47.9	2.15	45.6
	Tyrosol	14.22	45.6	13.63	56.7	10.66	22.5	14.01	58.5	1.31	37.0	2.13	39.1
	Vanillic acid	0.25	35.3	0.40	38.8	0.21	24.0	0.26	40.6	nd		0.02	31.9
	p -Coumaric acid	0.12	28.5	0.15	42.8	0.19	23.7	0.15	19.4	0.03	61.5	0.02	56.5
	Caffeic acid	nd		0.01	198.4	nd		0.00	316.2	nd		nd	
	Ferulic acid	0.04	33.0	0.06	36.6	0.04	57.3	0.04	24.2	nd		nd	
	Luteolin	1.98	35.0	2.64	31.3	2.04	22.5	2.26	24.8	0.16	47.7	0.10	68.2
	Apigenin	0.93	18.5	1.15	29.8	0.86	12.1	1.12	29.8	0.12	41.6	0.06	54.4
	Secoiridoid derivatives	300.65	22.8	262.05	27.3	250.61	17.0	241.06	35.8	27.45	28.3	16.25	46.3
	Pinoresinol	8.91	5.5	5.88	49.3	5.65	59.0	5.22	42.0	0.97	37.5	0.64	40.6
	Acetoxypinoresinol	33.03	13.8	18.18	34.5	18.13	38.1	12.31	42.2	1.48	44.9	0.78	29.8
	Total	369.46	21.9	317.38	23.0	298.45	17.9	289.78	29.5	32.53	29.0	22.14	40.4
	Ratio "R"	0.07	21.0	0.10	65.2	0.08	16.0	0.12	67.0	0.08	22.1	0.23	45.5
Total polyphenols (Folin Ciocalteu, gallic acid mg kg^{-1})		80.73	23.0	149.69	16.9	82.38	26.1	148.81	27.9	12.15	43.5	37.17	30.3
Acidity (oleic acid %)		0.40	25.1	0.30	42.9	0.37	24.3	0.41	35.8	0.12	32.0	0.10	19.2
Peroxide value (meq O_2 kg^{-1})		11.7	25.2	14.67	46.9	10.42	17.8	14.21	35.0	6.24	18.5	4.90	42.3

Table 7. Average results obtained for the classes of commercial oil investigated

		ASC 2015		ASC 2016		COR 2015		COR 2016		MIG 2015		MIG 2016		MOG 2015		MOG 2016		RAG 2015		RAG 2016	
		Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%	Average	R.S.D.%
Volatile substances (peak area units)	Ethanol	2.30E+07	89.7	1.04E+06	73.8	3.04E+07	140.5	3.24E+06	108.5	4.39E+07	115.1	4.19E+06	76.1	1.21E+07	77.8	2.36E+06	104.8	1.15E+07	44.3	2.23E+06	69.9
	3-Pentanone	4.07E+06	60.5	3.25E+06	65.8	3.45E+06	65.9	1.66E+06	55.1	9.53E+06	33.1	1.67E+06	25.1	8.03E+06	143.5	1.34E+06	34.1	3.89E+06	96.8	1.37E+06	74.4
	1-Penten-3-one	1.64E+07	96.7	4.69E+06	68.3	1.20E+07	50.3	3.85E+06	49.7	7.49E+06	77.2	2.85E+06	46.1	8.30E+06	61.2	2.90E+06	66.9	1.21E+07	57.0	3.29E+06	44.2
	1-Penten-3-ol	7.15E+06	71.8	1.68E+06	51.0	3.80E+06	42.9	1.25E+06	48.6	3.53E+06	51.6	1.04E+06	46.4	4.31E+06	44.4	9.96E+05	52.9	5.70E+06	50.3	9.82E+05	45.9
	Hexanal	1.73E+07	87.9	8.14E+06	48.2	1.02E+07	54.1	4.50E+06	53.1	7.57E+06	74.6	4.96E+06	48.4	1.99E+07	37.5	5.48E+06	52.3	1.42E+07	46.9	5.28E+06	61.5
	(*E*)-2-Hexenal	1.69E+08	100.5	1.11E+08	45.6	2.91E+08	87.7	9.91E+07	22.2	1.78E+08	81.2	8.56E+07	52.9	2.56E+08	74.4	9.48E+07	43.6	6.36E+08	41.0	1.48E+08	35.3
	Acetic acid, hexyl ester	5.27E+06	121.6	2.24E+06	81.5	1.79E+06	57.3	1.86E+06	71.2	5.55E+06	36.8	3.05E+06	70.5	9.50E+05	-	7.54E+05	70.5	1.26E+06	47.9	8.66E+05	29.5
	(*Z*)-3-Hexen-1-ol, acetate	1.86E+07	58.2	1.07E+07	56.0	6.99E+06	44.9	9.09E+06	61.4	2.51E+07	52.1	1.44E+07	106.4	2.68E+06	76.7	1.82E+06	65.5	3.18E+06	69.3	2.00E+06	52.1
	(*Z*)-2-Penten-1-ol	1.01E+06	52.4	1.93E+06	43.8	9.89E+05	28.6	2.03E+06	41.7	7.77E+05	12.3	1.54E+06	35.6	8.36E+05	13.1	1.64E+06	83.5	9.75E+05	31.1	1.39E+06	60.7
	1-Hexanol	1.19E+07	96.8	3.97E+06	99.4	9.80E+06	109.9	2.08E+06	66.0	1.83E+07	66.5	3.10E+06	36.9	7.61E+06	67.5	3.13E+06	116.1	1.09E+07	82.4	3.13E+06	83.9
	(*Z*)-3-Hexen-1-ol	3.11E+07	114.1	8.80E+06	31.9	1.69E+07	82.7	4.45E+06	51.1	1.75E+07	66.8	4.33E+06	50.3	2.43E+07	43.7	4.87E+06	46.2	6.03E+06	26.9	2.37E+06	38.9
	(*E*)-2-Hexen-1-ol	2.95E+06	56.5	1.23E+07	106.8	8.32E+06	59.1	3.58E+06	64.2	2.61E+07	103.1	5.02E+06	44.1	1.13E+07	95.1	7.74E+06	103.3	1.80E+07	92.7	6.34E+06	61.6
	Ratio Hexanal/(*E*)-2-Hexenal	0.10	87.5	0.07	105.6	0.04	61.7	0.05	239.2	0.04	91.9	0.06	91.6	0.08	50.5	0.06	120.0	0.02	114.5	0.04	174.1
α-Tocopherol (mg kg^{-1})		299.50	28.1	375.24	17.0	232.43	6.6	363.77	10.7	292.35	7.9	397.26	9.3	196.81	20.0	253.14	16.4	218.97	20.0	286.66	23.5
Fatty acids (%)	Palmitic acid	14.14	3.8	14.03	7.6	12.30	3.0	13.67	6.3	13.95	5.5	15.28	2.6	11.98	6.6	12.48	7.5	12.93	9.5	13.53	6.7
	Palmitoleic acid	1.11	16.3	1.13	13.8	0.75	10.0	0.91	14.2	1.46	16.3	1.66	10.9	0.75	19.5	0.75	31.1	0.81	34.4	0.91	23.8
	Stearic acid	2.27	6.4	1.75	6.7	2.34	5.1	1.95	8.5	2.10	7.0	1.72	7.4	2.42	6.6	2.02	17.5	2.40	7.0	1.90	9.1
	Oleic acid	75.55	1.0	75.55	2.0	76.13	1.3	74.24	2.2	72.73	1.8	71.39	1.6	77.47	1.8	77.63	2.3	76.13	2.9	75.99	2.1
	Linoleic acid	6.28	9.1	6.85	8.2	7.87	12.9	8.63	8.2	9.17	7.8	9.44	8.2	6.77	12.0	6.55	11.2	7.10	12.5	7.07	10.0
	Linolenic acid	0.66	13.3	0.68	18.6	0.61	5.7	0.60	8.7	0.59	13.8	0.51	15.1	0.61	13.9	0.58	12.9	0.63	8.0	0.60	11.2
	Ratio oleic acid/linoleic acid	12.11	9.5	11.10	9.5	9.81	13.0	8.67	10.7	7.99	10.7	7.61	9.5	11.59	12.2	12.00	13.0	10.92	16.7	10.87	11.8
Alkyl esters (mg kg^{-1})	Methyl ester	4.83	72.2	6.75	73.8	4.43	38.8	7.29	56.4	3.43	58.0	5.13	48.3	3.22	37.3	7.75	64.6	3.86	64.3	7.25	82.6
	Ethyl ester	2.67	81.0	5.25	61.7	4.43	25.1	4.29	70.9	4.29	79.4	6.88	49.4	2.22	43.7	8.38	84.7	2.43	40.2	7.00	57.5
	Total	7.50	80.8	11.88	64.4	7.43	35.5	11.57	58.2	7.86	62.5	11.75	50.0	5.22	36.8	16.00	72.5	6.29	49.2	14.25	65.0
Polar polyphenols (mg kg^{-1})	Hydroxytyrosol	5.09	43.0	5.01	57.2	4.06	39.9	5.32	40.6	8.75	59.0	6.37	31.1	2.38	64.5	4.94	110.5	6.79	41.1	3.83	54.3
	Tyrosol	6.08	44.7	6.24	60.3	4.67	29.8	5.78	48.1	9.24	66.7	5.53	43.6	5.11	24.0	7.47	89.0	6.20	59.1	6.86	37.7
	Vanillic acid	0.36	44.8	0.31	61.0	0.32	54.0	0.35	71.5	0.49	47.2	0.52	89.3	0.46	45.6	0.35	45.3	0.34	40.8	0.44	58.6
	p -Coumaric acid	0.11	45.7	0.12	34.5	0.11	39.6	0.14	44.8	0.09	39.8	0.09	52.5	0.12	29.6	0.18	65.8	0.05	35.3	0.13	67.5
	Caffeic acid	0.01	264.6	0.05	89.7	0.01	264.6	0.04	106.8	nd		0.08	100.9	nd		0.11	80.0	nd		0.07	62.4
	Ferulic acid	0.03	59.7	0.06	37.7	0.03	87.5	0.05	55.1	0.03	87.8	0.05	40.2	0.08	31.3	0.07	53.4	0.05	34.3	0.07	49.5
	Luteolin	2.20	27.3	3.04	32.6	3.24	35.9	3.61	21.0	2.10	22.5	2.51	25.0	1.49	63.4	2.06	36.5	3.08	47.7	4.05	52.8
	Apigenin	0.85	39.8	0.92	26.5	1.25	25.1	1.20	15.9	0.49	29.8	0.59	46.9	0.94	32.5	0.92	31.7	1.25	14.3	1.10	36.8
	Secoiridoid derivatives	342.05	30.3	334.77	31.1	340.19	32.0	384.59	33.2	282.14	40.3	339.73	31.1	248.22	45.3	335.27	33.6	429.75	40.1	349.47	37.7
	Pinoresinol	11.21	54.5	18.45	76.4	4.72	33.1	6.27	19.9	6.48	24.3	9.70	17.9	7.63	30.5	10.36	60.1	3.83	14.1	5.98	38.7
	Acetoxypinoresinol	7.92	108.4	8.94	103.2	8.86	94.1	5.52	84.2	8.13	20.8	10.30	48.8	5.31	81.5	9.03	71.0	35.47	29.1	31.77	39.0
	Total	375.91	29.9	377.90	27.3	367.45	30.9	412.86	32.3	317.94	38.1	375.47	28.0	271.73	41.5	370.68	32.2	487.48	36.1	403.13	33.0
	Ratio "R"	0.03	18.6	0.03	55.9	0.03	40.3	0.03	33.4	0.06	34.9	0.04	35.6	0.03	46.3	0.03	78.0	0.04	92.4	0.03	54.2
Total polyphenols (Folin Ciocalteu, gallic acid mg kg^{-1})		207.38	32.1	367.82	24.5	206.66	23.5	287.14	48.7	213.45	42.5	345.97	46.4	163.80	36.7	346.77	20.2	192.32	32.2	384.46	32.5
Acidity (oleic acid %)		0.26	54.8	0.34	39.3	0.17	14.3	0.22	24.2	0.18	22.2	0.25	35.0	0.15	17.2	0.25	31.0	0.21	11.4	0.35	59.7
Peroxide value (meq O_2 kg^{-1})		7.89	32.9	18.63	40.8	12.39	21.5	14.50	26.9	10.71	37.1	18.34	46.7	10.45	41.6	12.25	32.5	9.57	34.4	19.19	35.2

Table 8. Average results obtained for the classes of monovarietal oil investigated

2.3.2 Fatty acids alkyl esters

The European Commission Regulation No. 61/2011 [13] (as discussed in the paragraph 2.1.3), established the following limits:

Σ (FAME + FAEE) ≤ 75 mg/Kg

or

75 mg/Kg < Σ (FAME + FAEE) ≤ 150 mg/Kg

as long as it appears that **(FAME/FAEE) ≤ 1.5**

It is essential to remark that the European law authorizes a higher content of alkyl esters in virgin olive oil only if the ratio between ethyl esters and methyl is less or equal to the above value, since, the methyl esters usually are formed with the technological transformations of overripe olives.

Following, an example of a sample chromatogram (Figure 10) with the relative peaks identification is reported.

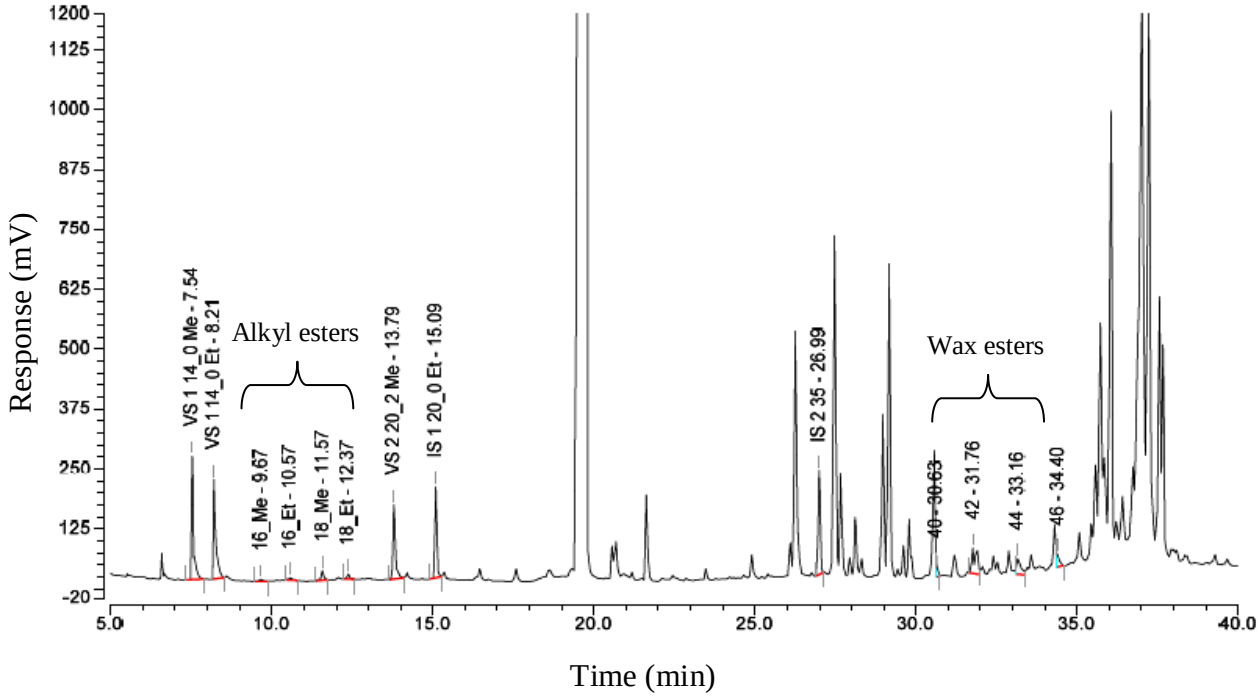


Figure 10. EVOO sample HPLC-GC-FID chromatogram

VS: verification standard, IS: internal standard

In the figure below (Figure 11), the total average alkyl esters (in mg kg^{-1}) in each class of monovarietal oil investigated, are shown. All the samples are largely within the legal limits. Among the cultivars examined, very similar values of alkyl esters are obtained. In particular, Coroncina is the variety with the lowest average content of alkyl esters (9.5 mg kg^{-1}), while Raggia is the cultivar with the highest average content of alkyl esters (11.3 mg kg^{-1}).

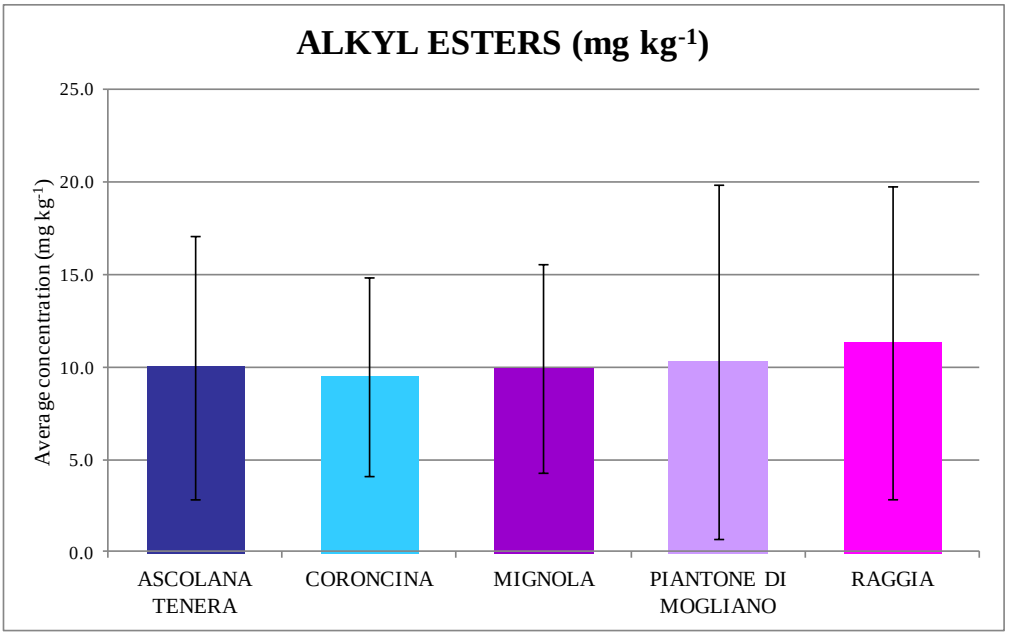


Figure 11. Total average alkyl esters in each class of monovarietal oil.

Also in this case, it is interesting to note that each of the varieties undergoes an increase not statistically significant in the total content of alkyl esters, passing from the year 2015 to the year 2016 (Table 8), showing a generally lower quality in 2016. This trend is in agreement with the results of sensorial analysis (see paragraph 2.3.6). Nevertheless, considering all cultivars, there are not significant differences ($P > 0.05$) between them (Table 9). However, analyzing the two years in consideration, it is not possible to find the same trend among the varieties analyzed; in fact, if in year 2015 Piantone di Mogliano variety showed the lowest average alkyl content compared to all the other varieties of that year, in 2016 it is the cultivar with the highest quantity of alkyl esters. On the contrary, in 2015 year Mignola is the variety with more alkyl esters, in 2016, together with the Coroncina variety, it is the one with the minor alkyl esters average content. It can be observed that the increase in the average alkyl esters content, passing from the year 2015 to the year 2016, corresponds to an increase of the average free acidity values and the average peroxides value. As shown in Table 8, this trend is observable for both parameters in all the cultivars examined.

ALKYL ESTERS										
	MOG 2015	RAG 2015	COR 2015	ASC 2015	MIG 2015	COR 2016	MIG 2016	ASC 2016	RAG 2016	MOG 2016
MOG 2015		1	0,9997	0,9998	0,9989	0,7133	0,6338	0,6086	0,105	0,05635
RAG 2015	0,4338		1	1	1	0,9108	0,8733	0,8577	0,3225	0,1831
COR 2015	0,9	0,4395		1	1	0,9802	0,9677	0,9613	0,5441	0,3377
ASC 2015	0,8885	0,4487	0,02639		1	0,9866	0,978	0,9734	0,6274	0,4099
MIG 2015	1,075	0,6044	0,1648	0,132		0,9907	0,9838	0,9799	0,6331	0,4104
COR 2016	2,59	2,033	1,593	1,504	1,429		1	1	0,9981	0,9622
MIG 2016	2,762	2,17	1,717	1,618	1,546	0,07093		1	0,9984	0,9638
ASC 2016	2,815	2,22	1,766	1,665	1,596	0,1206	0,05139		0,9989	0,9701
RAG 2016	4,209	3,443	2,949	2,775	2,763	1,158	1,126	1,07		0,9999
MOG 2016	4,56	3,859	3,405	3,236	3,234	1,759	1,747	1,696	0,7882	

Table 9. Significant differences ($P < 0.05$) between the classes of oils are indicated in pink

Inappropriate practices during the olive oil extraction process and above all bad quality of the olive fruits promote alkyl esters formation. In the case of olive fruits stored for days before milling, the increase in free fatty acids (FFAs) formed by lipolysis of triglycerides and the alcohols formed by the action of microorganisms, react producing FAAEs. Thus, molecules of triglycerides are transformed into methyl or ethyl esters producing di- and monoacylglycerols as byproducts. Low amounts of MeOH and EtOH are accepted since small quantities of these alcohols may be formed during the maturation of olives. Anyway, high volumes of EtOH appear during the fermentation processes occurred mainly throughout olive oil storage [25]. It is known in fact that the alkyl esters are formed effectively as a result of degradation processes or fermentation in the olives of low quality that can be over-ripe, deteriorated or only badly stored before processing. For example, in a recent study [26], alkyl esters were checked in a variety of Italian extra virgin olive oils from Sicily, produced in the years 2014 - 2015 and in a set of very aged oils produced in the years 1996 - 2000. The concentration range found for the first one was 11.6-58.9 mg kg^{-1} and for the latter was 110.6-144.5 mg kg^{-1}, and this indicates an increment of FAAEs in aged EVOOs, where also deterioration processes like oxidation and lipolysis took place. As compared to Italian extra virgin olive oils from Marche region investigated in the present study, some of the Sicilian varieties such us Nocellara etnea and Coratina have a minor content, others such as Nocellara del Belice and Sanbenedettese have a greater content and others, like Nerba, have about the same content of FAAEs. It was also demonstrated that the methyl esters usually are formed with technological transformations of over-ripe olives, while the ethyl appear to be more related to poor raw materials or at mixing, since their formation is related to the damage of the cell structure of the olives that causes escape of aqueous fraction with consequent processes of alcoholic fermentation of the sugars with the production of ethanol. What is worthy to note is that oils obtained from fermented fruits are low-quality virgin olive

oils, having unpleasant sensorial features that prevent them from being classified as extra-virgin olive oils, thus leading to the decrease in their commercial value. In fact, a connection between the presence of large quantities of FAAEs and fermentative organoleptic defects, has been proven [27]-[28]. In the study conducted by Coca et al. [28], it has been demonstrated that FAAEs and organoleptic data are complementary criteria when classifying olive oils. Fermentative defects correspond to very high concentrations of FAAEs, while other kinds of defects, such as oxidative ones and frozen olives, do not correspond to high levels of FAAEs. Actually, to these molecules, two meanings are attributed: they are in connection with the quality of the olives and then oil, but they also can be regarded as indicators of a possible mild deodorization, given that they are not removed by this illegal treatment. In fact, the presence of a high content of alkyl esters, without the existence of a defect sensory distinctly recognizable can be reasonably explained by the use of "mild deodorization" [29]-[30].

2.3.3 α-Tocopherol

The stability of olive oil is related to the low levels of polyunsaturated fatty acids in triacylglycerols and the presence of natural antioxidants. Tocopherols and in particular α-tocopherol, representing about 95% of the total of tocopherols in olive oils, are very important molecules effectively inhibiting lipid oxidation in foods and biological systems. α-Tocopherol is traditionally considered as the major antioxidant of olive oil and its concentration depends particularly on the variety of the plant and usually, values found in EVOO range from 100 mg to 300 mg kg^{-1} [31]. This compound is an effective antioxidant against large number of diseases such as Alzheimer, Parkinson, cardiovascular inflammation and other age-related diseases. It has also been reported that the progressive neuromuscular disease in children is due to deficiency of vitamin E. For these reasons, vitamin E is an essential and primary component of the balanced diet of humans and other animals [32]. It is reported [33] that α-tocopherol, at physiological concentrations, inhibits vascular smooth muscle proliferation, an important process in the formation of the so-called intermediate atherosclerotic lesion. It also seems that it exerts direct effect on the expression genes such those of adhesive molecule or on the activity of enzymes such us 5-lipoxygenase or protein kinase C. Moreover, Vitamin E has regulatory cellular and molecular roles. As antioxidant, it inhibits lipid oxidation in food and organisms by stopping the radical oxygen species (ROS) chain reaction, preventing the peroxidation of polyunsaturated fatty acids from cellular and subcellular membranes [34]. Nevertheless high doses of α-tocopherol supplements may cause hemorrhage due to inhibition of platelet aggregation and hence interruption of blood coagulation [35]. It is known that the intake of high amounts of vitamin E from food or supplements has been linked with various adverse effects. For example it has been demonstrated that taking vitamin E supplements increased the risk of prostate cancer, or it is also reported that α-tocopherol attenuated the effects of crizotinib, an inhibitor of anaplastic lymphoma kinase (ALK) [36]. In the figures below, an example of sample chromatogram (Figure 12) and the average content (in mg kg^{-1}) of α-tocopherol content found in each class of oils (Figure 13) investigated are shown.

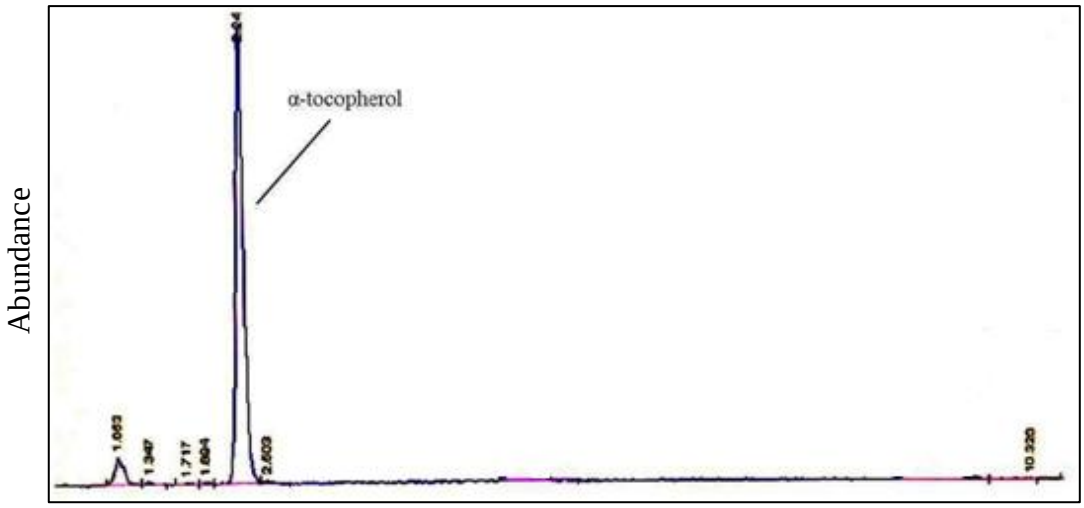


Figure 12. EVOO sample HPLC-FLD chromatogram

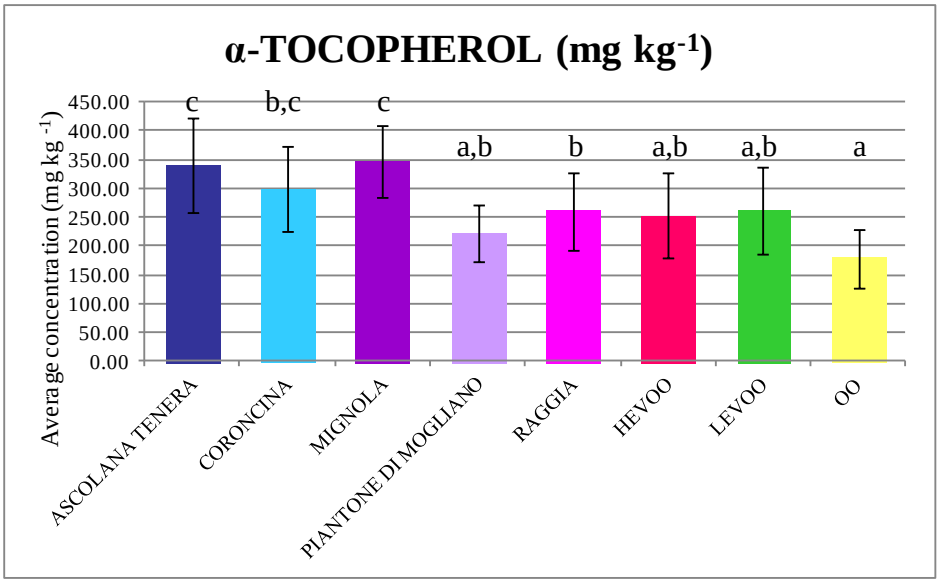


Figure 13. Average content of α-tocopherol in each class of oils.

Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

Among the classes of oils, there are significant differences ($P < 0.05$) (Figure 13). Among the different varieties, Piantone di Mogliano has the lowest avarage content f α-tocopherol (223.32 mg kg^{-1}) while Mignola has the highest content of α-tocopherol (348.30 mg kg^{-1}). The differences in the amounts of α-tocopherol among them may be due to the type of cultivar. In fact it is known how the cultivar is an important factor that influences the antioxidant activity and therefore the content of tocopherol in olive oil [37]**.** Instead, for the commercials oils, OO class has the lowest α-tocopherol content respect to HEVOO and LEVOO classes, as can be

expected due to the refining process to which most part of an OO is undergone. Taking into account Table 7 and Table 8, where the average results for the various classes of oils per year are reported, it is possible to observe that in the two years, the varieties have the same trend and all monovarietal oils undergo an increase in terms of α-tocopherol content, passing from year 2015 to year 2016. Moreover, for both years Piantone di Mogliano and Raggia are the varieties with the lowest average content of α-tocopherol (respectively 196.81 mg kg^{-1} and 218.97 mg kg^{-1} for year 2015 and 253.14 mg kg^{-1} and 286.66 mg kg^{-1} for year 2016); while Ascolana Tenera and Mignola have the highest α-tocopherol average value (respectively 299.50 mg kg^{-1} and 292.35 mg kg^{-1} for year 2015 and 375.24 mg kg^{-1} and 397.26 mg kg^{-1} for year 2016). This great concentration variability is mainly due to the olives variety, but also to the different way of oil production. For example, in the case of oils not classified "extra virgin", the treatments and the possible refining of the oil (in case in which an oil has been fraudulently mixed with a refined oil), besides altering its composition, taste, nutritional and biological values, can also cause considerable losses of vitamin E content. Another important aspect influencing the total content of α-tocopherol is the different maturation degree of the drupe at the time of collection. Scientific researches demonstrated [31] that drupe antioxidants decrease with the maturation, so there is a tendency towards early collection to increase the content of these compounds (tocopherols). Significant differences ($P < 0.05$) of α-tocopherol content between all classes of oils are indicated in the table below (Table 10).

α-TOCOPHEROL																
	OO 2015	OO 2014	HEVOO 2014	LEVOO 2014	MOG 2015	RAG 2015	COR 2015	HEVOO 2015	LEVOO2015	MOG 2016	RAG 2016	MIG 2015	ASC 2015	COR 2016	ASC 2016	MIG 2016
OO 2015		1	0,9986	0,9123	0,6217	0,1756	0,04663	0,01342	0,007967	0,002332	1.83E-03	8,65E-06	2.57E-03	1.97E-08	7.20E-10	9.78E-11
OO 2014	0,09205		0,9993	0,9336	0,6741	0,2056	0,05708	0,01717	0,0103	0,003043	2.60E-03	1.18E-02	3,54E-06	2.81E-08	1.01E-09	9.12E-11
HEVOO 2014	1,636	1,544		1	0,9998	0,9181	0,6345	0,4108	0,3126	0,1321	0,000665	0,001548	0,0005453	1.01E-05	4.25E-07	5.84E-09
LEVOO 2014	2,54	2,448	0,9039		1	0,9993	0,9657	0,887	0,8111	0,5232	0,01078	0,01767	0,007164	2.79E-04	1.42E-05	2.09E-07
MOG 2015	3,258	3,154	1,403	0,3784		0,9998	0,9721	0,8728	0,7746	0,441	0,001874	0,005845	0,001874	7.09E-06	1.38E-07	9.53E-10
RAG 2015	4,283	4,184	2,517	1,54	1,371		1	1	0,9999	0,985	0,1361	0,177	0,08516	3.87E-03	1.61E-04	1,69E-09
COR 2015	5	4,901	3,233	2,257	2,203	0,785		1	1	0,9999	0,4708	0,5011	0,3059	4.48E-02	2.34E-03	2,81E-08
HEVOO 2015	5,565	5,458	3,676	2,632	2,678	1,095	0,2437		1	1	0,4152	0,4748	0,2711	1.29E-02	4.12E-04	2.96E-06
LEVOO2015	5,783	5,677	3,894	2,85	2,938	1,338	0,4863	0,2673		1	0,5555	0,599	0,3737	2.73E-02	9.45E-04	7.11E-06
MOG 2016	6,268	6,166	4,453	3,45	3,613	2,058	1,247	1,108	0,8555		0,9614	0,9501	0,8337	0,000808	5.17E-02	6.69E-04
RAG 2016	8,675	8,566	6,732	5,658	6,351	4,436	3,553	3,667	3,387	2,288		1	1	0,04181	0,004114	6.17E-02
MIG 2015	8,189	8,09	6,422	5,446	5,909	4,278	3,493	3,545	3,303	2,361	0,3727		1	0,212	0,04746	0,002046
ASC 2015	8,57	8,47	6,803	5,826	6,351	4,695	3,91	3,997	3,755	2,791	0,8412	0,4168		0,3772	0,1091	0,006168
COR 2016	11,99	11,89	10,22	9,247	10,33	8,442	7,657	8,062	7,819	6,661	5,053	4,164	3,747		1	0,9877
ASC 2016	12,94	12,84	11,13	10,12	11,44	9,41	8,599	9,13	8,877	7,61	6,048	4,991	4,561	0,691		0,9998
MIG 2016	14,15	14,04	12,33	11,33	12,86	10,74	9,925	10,58	10,32	8,983	7,552	6,317	5,887	2,017	1,372	

Table 10. Significant differences ($P < 0.05$) between the classes of oils are indicated in pink

2.3.4 Polyphenols

The results of the total phenolics content found in the different olive oil classes as resulted from the Folin-Ciocalteu test and major hydrophilic olive oil polyphenols quantified by HPLC-DAD/MS, are reported in Table 7 and Table 8. In the figure below (Figure 14), the average total content (expressed in mg kg^{-1} of gallic acid) of polyphenols in each class of oils, obtained from the Folin-Ciocalteu analysis, is shown.

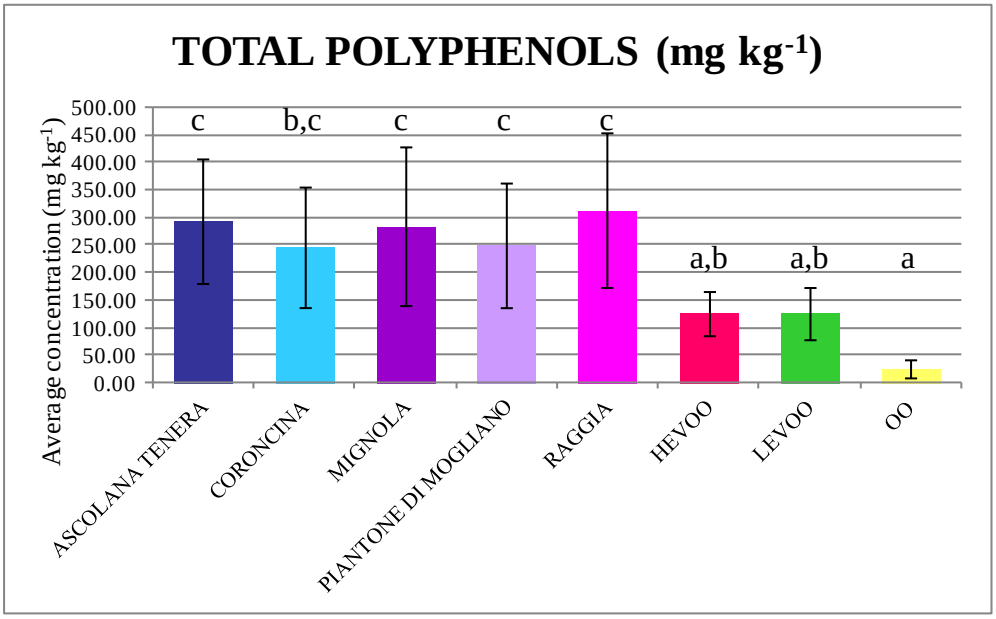


Figure 14. Average content of total polyphenols in each class of oils.
Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

There is a clear difference of total polyphenols content between the monovarietal and commercial oils, confirmed also by the statistical analysis, that underline significant differences ($P < 0.05$) between these category of oils. For monovarietal oils, polyphenols are more abundant in Raggia cultivar and less abundant in Coroncina cultivar; regarding commercial oils, OO has the lowest quantity of polyphenols and HEVOO shows the highest quantity. These differences indicate that the type of cultivar (in addition to the oil production methods, the maturation degree of the olives and other factors) affects strongly the total content of polyphenols. During the olives processing, a loss of phenols can occur; in particular, the polysaccharides that are found inside the drupe can bind to the hydrophilic phenols in the pastes, reducing their release in the oil during crushing and grinding phases. Several studies have indicated that certain phenolic fractions are strongly influenced by particular processes depending on both the size and the

spatial structure of the olive paste. For these reasons it is important to define the average kneading times, which allow to obtain well-flavored polyphenols and pleasing to the taste, obtaining the best possible yield. Extraction systems, such as pressure and centrifugation, play an important role in the phenolic composition of the oil. Since the presence of hydrophilic phenols in oils is closely related to the activities of various endogenous enzymes present in olives, their concentration in the oil is strongly influenced by the extraction conditions. Considering the two years of production, as regards monovarietal oils, it is interesting to observe an increase of total polyphenols content for all varieties passing from year 2015 to year 2016. Significant differences ($P < 0.05$) between all classes of oils are obtained and indicated in the table below (Table 11).

Moreover, in order to have a clear and complete results, it is good to correlate this data with that of the polyphenols performed using HPLC-DAD-ESI/MS. In the figure below (Figure 15), the average total content (in mg kg^{-1}) of polyphenols in each class of oils is shown.

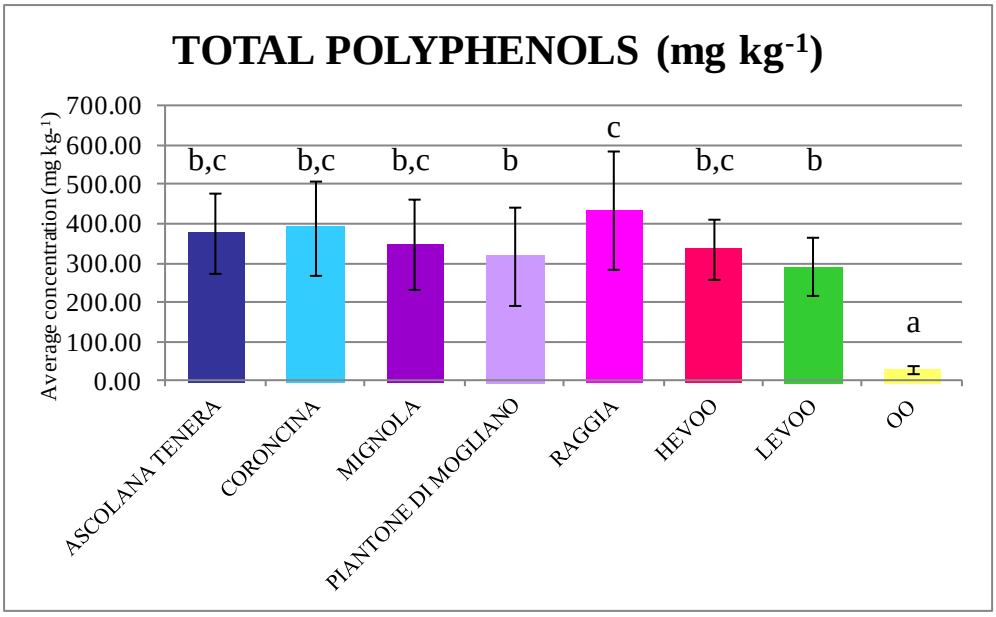


Figure 15. Average content of total polyphenols in each class of oils.
Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

Comparing the two analytical techniques used for the quantification of polyphenols, one would expect that the results for the different classes of oils would be the similar. In fact, observing the results (Figure 14 and Figure 15) it is possible to see how the trend for monovarietal and commercial oils is the same for both techniques; the only exception is for the Coroncina cultivar, which, with Folin-Ciocalteu method, results to be the variety with the smaller quantity of

polyphenols (246.90 mg kg $^{-1}$) while with the HPLC-DAD results to be the second cultivar richer in polyphenols content (390.16 mg kg $^{-1}$) after the Raggia cultivar. Considering the two years of production, also in this case the same trend is maintained: all cultivars have an higher content of polyphenols in the year 2016 than in the year 2015, except for Ascolana Tenera in which the total polyphenols content is very similar in both years and Raggia cultivar that shows an higher content in year 2015 respect in the year 2016. Significant differences ($P < 0.05$) between of the classes of oils investigated are obtained and indicated in the table below (Table 12).

The HPLC-DAD-ESI/MS analysis performed allows to determine and quantify the phenolic acids (caffeic acid, *p*-coumaric acid, vanillic acid and ferulic acid), the phenolic alcohols (tyrosol and hydroxytyrosol), secoiridoid derivatives, the lignans pinoresinol and acetoxypinoresinol and the flavonoids luteolin and apigenin present in the oils samples. In the figures below, the comparisons between the different classes of oils, for each detected compound are shown.

Polyphenols are an important group of natural compounds, which are produced in the secondary metabolism of many plants in nature. Their content in EVOO can be strongly influenced by many agrochemical and technological factors including cultivar, degree of maturation, and climate as well as type of crushing machine, conditions during malaxation, and others [38]. The importance of these compounds resides in their antioxidant activity (demonstrated in *in vivo* and *in vitro* experiments), their possible effects against degenerative diseases, and some pharmaceutical effects, such as anti-carcinogenic, anti-atherogenic and anti-microbial properties. Moreover these compounds can extend the shelf life of olive oil, prevent oxidation reactions and they can contribute to the satisfactory organoleptic characteristics of oil, including aroma and flavor [37]-[39]-[40].

Phenolic acids

Phenolic acids are secondary aromatic plant metabolites that are widely spread throughout the plant kingdom. Their chemical structure is simple and consists mostly in a phenolic ring and a carboxylic acid function. The two most important natural phenolic acids are hydroxybenzoic acid (and its derivatives) and hydroxycinnamic acid (and its derivatives). These acids have been associated with color and organoleptic properties (flavor, astringency, and hardness), as well as with the health-related and antioxidant properties of foods. In addition, they are involved in fruit maturation, enzymatic browning prevention and food conservation. For these reasons there are many ongoing studies, but the recent interest in phenolic acids stems also from their potential

protective role, through ingestion of fruit and vegetables, against diseases that may be related to oxidative damage such as coronary heart disease, stroke, and cancers [41]. The phenolic acids are present in low amounts in EVOO. Considering the results obtained (Figure 16), caffeic acid is present in a range from 0.01 mg kg^{-1} to 0.05 mg kg^{-1}, Coroncina cultivar has the lowest content (0.02 mg kg^{-1}) and Piantone di Mogliano the highest content (0.05 mg kg^{-1}) of this acid, while it is completely absent in OO and LEVOO categories. The range obtained for vanillic acid is 0.01 mg kg^{-1} to 0.51 mg kg^{-1} with the lowest content in Ascolana Tenera cultivar (0.33 mg kg^{-1}) and with the highest in Mignola cultivar (0.51 mg kg^{-1}). As concerns *p*-coumaric acid and ferulic acid the average concentration range is respectively 0.02 - 0.16 mg kg^{-1} and 0.04 - 0.07 mg kg^{-1}. In both cases, Mignola is the cultivar with the lowest content (0.09 mg kg^{-1} and 0.04 mg kg^{-1}) while Piantone di Mogliano is the cultivar with the highest content (0.15 mg kg^{-1} and 0.07 mg kg^{-1}).

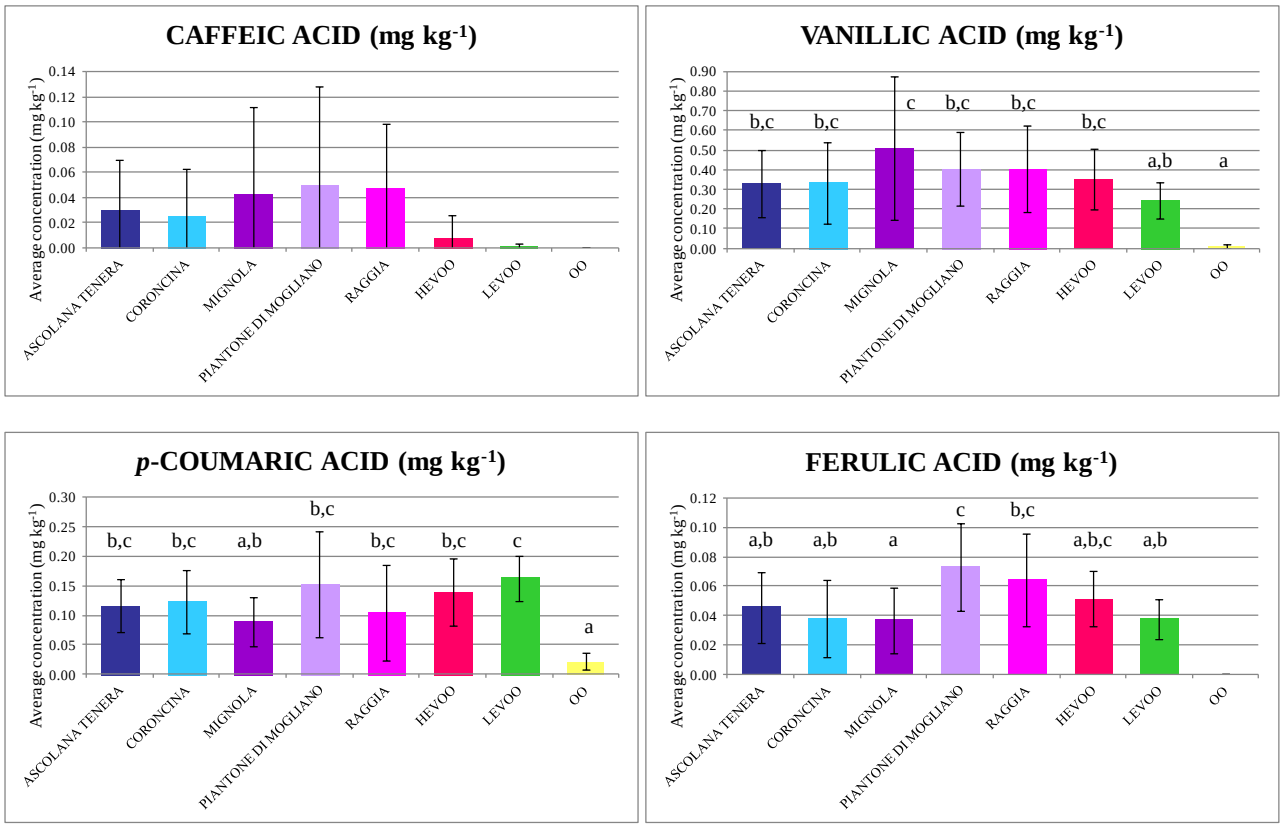


Figure 16. Average content of phenolic acids in each class of oils.
Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

Flavonoids

Flavonoid compounds are secondary plant metabolites. Research studies have proved health benefits of these species in fighting cancer, coronary heart diseases and neurodegenerative disorder [42]. Structurally they are large planar molecules that differ from one another by the presence of carbonyl carbon at C4, hydroxyl groups at C3, unsaturation between C2 and C3, and a combination of no carbonyl at C4 and hydroxyl group at C3. The main flavonoids found in EVOOs are luteolin (derived from luteolin-7-glucoside) and apigenin (derived from apigenin glucoside). Luteolin and apigenin are usually present in EVOO, in a range of 0.6 - 7.5 mg kg^{-1} and 0.3 - 32.0 mg kg^{-1} respectively. It has been demonstrated [42] how luteolin in oils is affected by irrigation of the trees while apigenin is not; so luteolin content is greater in the oils from olives coming from rainfed trees. Also, luteolin content increases with the maturity index of the fruit while apigenin does not show any definite trend. Anyway, considering the samples under investigation (Figure 17), the content of luteolin and apigenin varies in a range of 0.13 - 3.70 mg kg^{-1} and 0.09 - 1.23 mg kg^{-1} respectively. Piantone di Mogliano is the cultivar with the lowest content of luteolin (1.76 mg kg^{-1}) and Raggia is the variety with the highest content of this flavonoid (3.70 mg kg^{-1}); in the case of apigenin, Mignola cultivar exhibits the lowest content (0.54 mg kg^{-1}), while Coroncina the highest apigenin content (1.23 mg kg^{-1}).

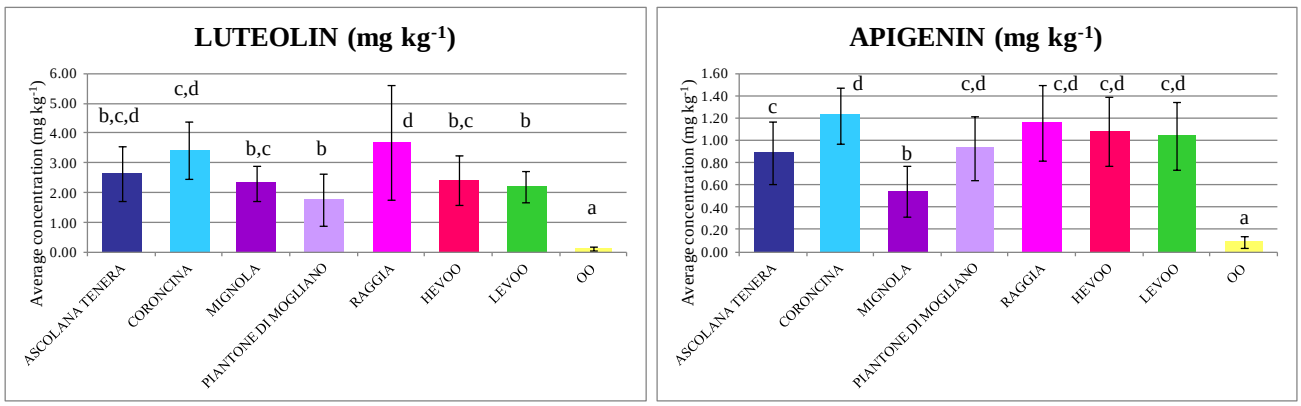


Figure 17. Average content of flavonoids in each class of oils.
Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

Lignans

Acetoxypinoresinol and pinoresinol, the most abundant lignans in olive oil, have been also investigated in the present study. It has been recently reported [43] that their concentration in oil slightly decreases and increases, respectively, with the maturation of olives and that these, together with tyrosol, seem to be among the most stable phenolics during oil storage. Beyond the antioxidant activity, some authors [44] reported that pinoresinol exerts anti-inflammatory activity and it is thought that both have potential pharmacological properties. Lignans composition can be used as varietal marker and for authentication analysis purposes because of their inter-oil variation. The most abundant compounds in this class are (+)-pinoresinol, (+)-1-acetoxypinoresinol and (+)-1-hydroxypinoresinol. (+)-Pinoresinol is a common component of the lignan fraction of several plants such as *Forsythia* species and *Sesamum indicum* seeds, whereas (+)-1-acetoxypinoresinol and (+)-1-hydroxypinoresinol and their respective glucosides have been detected in the bark of the olive tree (*Olea europaea L.*). (+)-Pinoresinol and (+)-1-acetoxypinoresinol are usually present in EVOO, in a range of 4.2 - 67 mg kg^{-1} and 6.7 - 41.0 mg kg^{-1}, respectively. Considering the results obtained (Figure 18), pinoresinol is present in the range 0.80 - 15.07 mg kg^{-1} while acetoxypinoresinol shows a range of 1.13 - 33.14 mg kg^{-1}. For commercial oils, OOs class and HEVOOs class have respectively the lowest and highest content of both compounds. Pinoresinol, for monovarietal oils, is lower in Raggia cultivar (5.19 mg kg^{-1}) and higher in Ascolana Tenera cultivar (15.07 mg kg^{-1}) while acetoxypinoresinol has the lowest content in Piantone di Mogliano cultivar (7.06 mg kg^{-1}) and the highest one in Raggia cultivar (33.14 mg kg^{-1}).

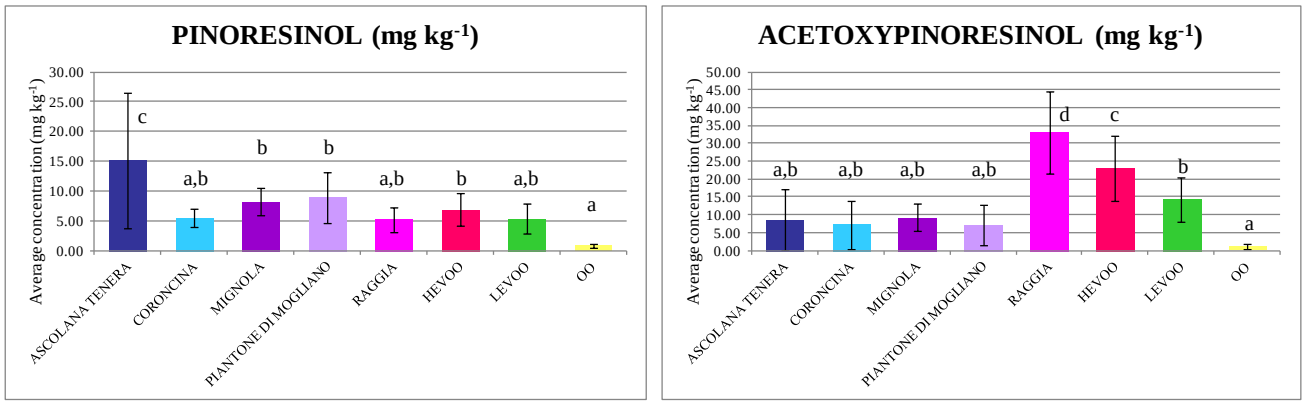


Figure 18. Average content of lignans in each class of oils.

Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

Secoiridoid derivatives

Secoiridoid derivatives are major polyphenols in EVOO. These species can be found only in plants belonging to the family of O*leaceae,* that includes *Olea europaea,* and are characterized by the presence, in their structures, of elenolic acid both in glycosidic form and in aglyconic form. Glycosidic forms are produced by the secondary metabolism of terpenes, while aglyconic forms are due to the hydrolysis of endogenous β-glucosidases during crushing and malaxation. Their structure is characterized by the presence of either elenolic acid or elenolic acid derivatives. The principal glycosylated species are oleuropein, demethyloleuropein and ligstroside. Oleuropein and ligstroside are the major phenolic constituents of olive fruits and, through their breakdown, they form the major phenolic constituent of virgin olive oil, the aglyconic forms. The amphiphilic characteristic of secoiridoids causes them to be partitioned between oily layer and vegetation water, and most of them end up in vegetation water because of their polar functional groups. During the storage of oil, simpler species may be formed by hydrolytic mechanisms: phenolic alcohols. It is possible to find in oil the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA) and tyrosol (*p*-HPEA-EDA), oleuropein and ligstroside aglycon and their aldehydic isomers. Usually 3,4-DHPEA-EDA, 3,4-DHPEA-EA, *p*-HPEA-EDA and *p*-HPEA-EA are present in EVOO, in a range of 25 - 453.3 mg kg^{-1}, 72 - 310 mg kg^{-1}, 5.4 - 152.1 mg kg^{-1} and 3.7 - 32.4 mg kg^{-1}, respectively. Considering the results obtained (Figure 19), they were found to be present in a range 21.85 - 379.05 mg kg^{-1}. For commercial oils, the LEVOOs class and HEVOOs class have, respectively, the lowest (244.25 mg kg^{-1}) and the highest (274.92 mg kg^{-1}) content of secoiridoid derivatives while for the monovarietal oils Piantone di Mogliano cultivar has the lowest content (289.19 mg kg^{-1}) and Raggia cultivar has the highest content (379.05 mg kg^{-1}). In general, the content of these compounds depends on agronomic factors, in fact, it is known [45] that they decrease during the ripening process due to the fact that along this process the precursor compounds of the phenolic compounds present in the fruits decrease. Moreover, it is also known [45], that the olive oils from olives cultivated without irrigation have more secoiridoid derivatives than olive oils from olives cultivated with irrigation. They also depend on technological factors linked to the production process, in particular the olive paste malaxation step, and the conditions in which it is performed (such us temperature and time).

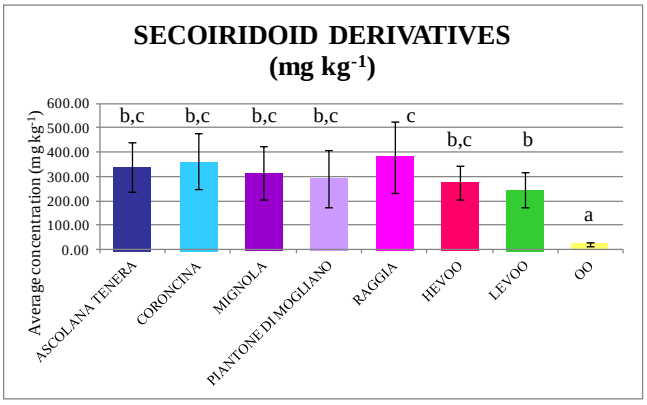


Figure 19. Average content of secoiridoid derivatives in each class of oils.
Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

Phenyl alcohols

Phenyl alcohols are formed during the oil storage by hydrolytic decomposition of oleuropein. The main molecules found in EVOOs are tyrosol and hydroxytyrosol. Tyrosol and hydroxytyrosol are usually present in EVOO, in a range of 0.7 - 35.0 mg kg^{-1} and 0.01 - 34.7 mg kg^{-1}, respectively. Considering the results obtained (Figure 20), hydroxytyrosol is present in a range of 1.58 - 12.25 mg kg^{-1} while tyrosol shows a range 1.72 - 13.83 mg kg^{-1}. Important differences, for both compounds, are observed between commercial and monovarietal oils, LEVOO and HEVOO categories have an higher content with respect to the monovarietal oils with differences statistically significant ($P < 0.05$). In particular, for both analytes the class with the lowest content is OO, as expected considering that the refined processes remove the most of these substances, while HEVOO and LEVOO have the greatest content. As regards the monovarietal oils, for hydroxytyrosol Piantone di Mogliano cultivar has the lowest content (3.58 mg kg^{-1}) and Mignola cultivar has the highest content (7.48 mg kg^{-1}) whereas, for tyrosol Coroncina cultivar has the lowest content (5.22 mg kg^{-1}) and Mignola cultivar has the highest content (7.26 mg kg^{-1}).

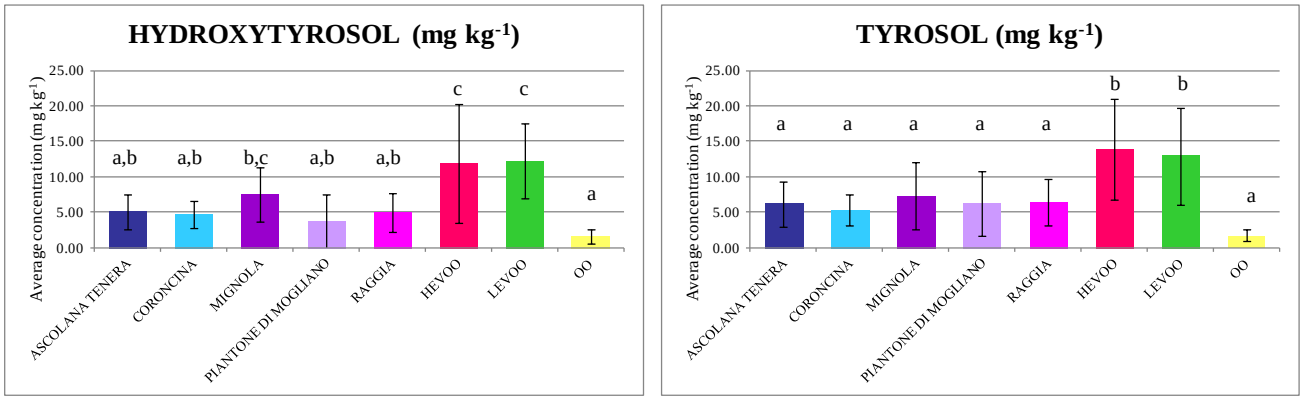


Figure 20. Average content of phenyl alcohols compound in each class of oils. Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

In particular, hydroxytyrosol is a product of the degradation of oleuropein and besides having antioxidant properties, recent studies [46] show how it gives good results against diabetes, inflammation, nervous disorders, angiogenesis, cancer, oxidative stress, heavy metal toxicity, hemolysis, LDL oxidation, muscle damage, and nephrotoxicity. For example, as regards anti-inflammatory action, it was demonstrated [33] that inhibits, in a dose dependent manner, the formation of a pro-inflammatory eicosanoid, leukotriene B4. Given its importance, this bioactive compound is related, together with its derivatives, to olive oil's health claim. In this regards, EU Regulation 432/2012 EFSA allowed an health claim related to olive oil polyphenols (Figure 21) [47].

Nutrient, substance, food or food category	Claim	Conditions of use of the claim	Conditions and/or restrictions of use of the food and/or additional statement or warning	EFSA Journal number	Relevant entry number in the Consolidated List submitted to EFSA for its assessment
Olive oil polyphenols	Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress	The claim may be used only for olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil. In order to bear the claim information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil.		2011;9(4):2033	1333, 1638, 1639, 1696, 2865

Figure 21. Olive oil polyphenols health claims

More in details, from Table 13 to Table 23, significant differences between all classes of oils for every detected compound ($P < 0.05$, one-way ANOVA and Tukey's test for pairwise comparison) are indicated in pink.

Among all these compounds, an important meaning is that of tyrosol and hydroxytyrosol. As previously mentioned, they mainly derive from the hydrolysis of secoiridoid derivatives, thus the content of their free forms can be inversely related with the freshness of the oil or of the olives and thus a higher value in lower quality oils could be expected. For this reason, it is important to evaluate another parameter which could give more information than free tyrosol and free hydroxytyrosol alone, the *"R" ratio*:

$$"R"\ ratio = \frac{hydroxytyrosol + tyrosol}{secoiridoid\ derivatives + hydroxytyrosol + tyrosol}$$

The concentration of free hydroxytyrosol and tyrosol increases during the storage, while the concentration of secoiridoid derivatives decreases. Thus, this parameter is a measure of the extent of secoiridoids hydrolysis, factor that depends on the EVOO age. Higher is the "R" ratio, lower is the quality of the oil. In the figure below (Figure 22), the average "R" ratio values calculated for each category of oil are shown.

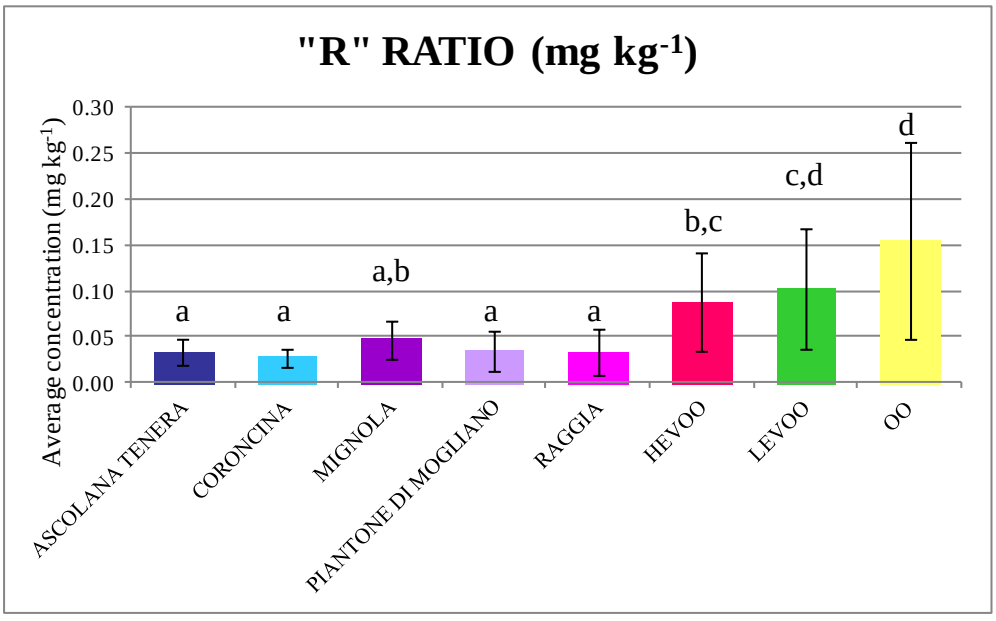


Figure 22. "R" ratio in each class of oils.

Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

It is possible to see a clear distinction between the monovarietal and commercial oils: the average value of "R" ratio indeed is very low and changes slightly among the different cultivars, while it is rather high for commercial categories. These important differences are also confirmed

by the statistical analysis, that underlines significant differences ($P < 0.05$). Table 24 shows the significant differences ($P < 0.05$) between all classes of oils.

Following (Figure 23), a typical chromatogram obtained from the analysis of EVOO and OO extracts is shown.

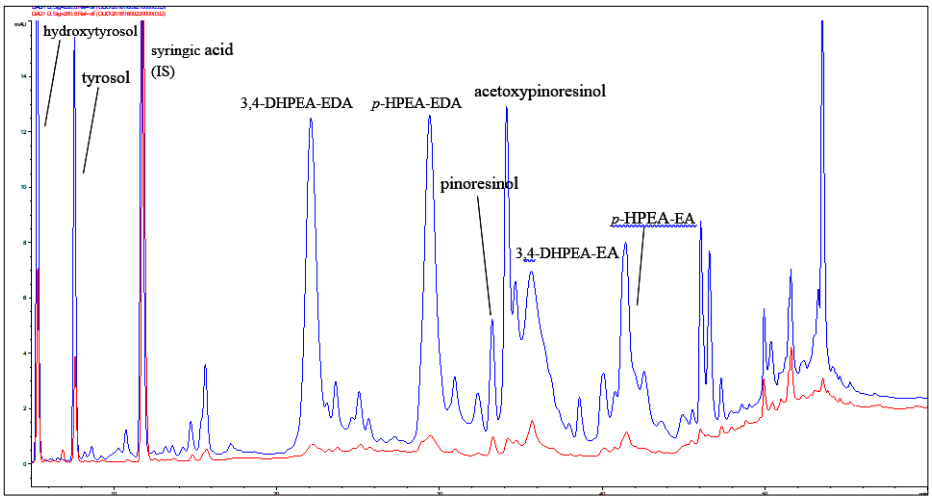


Figure 23. Overlaid HPLC-DAD chromatograms ($\lambda = 280$ nm) obtained from an EVOO polyphenol extract (blue) and from an OO polyphenol extract (red). 3,4-DHPEA-EDA: Decarboxymethyloleuropein aglycon. ρ-HPEA-EDA: Decarboxymethyl ligstroside aglycon. 3,4-DHPEA-EA: Oleuropein aglycon. ρ-HPEA-EA: Ligstroside aglycon

TOTAL POLYPHENOLS by UV																
	OO 2014	OO 2015	HEVOO 2014	LEVOO 2014	LEVOO 2015	HEVOO 2015	MOG 2015	RAG 2015	COR 2015	ASC 2015	MIG 2015	COR 2016	MIG 2016	MOG 2016	ASC 2016	RAG 2016
OO 2014		1	0,9953	0,9939	0,195	0,1868	0,1023	0,03045	0,0119	0,01134	0,007428	1.89E-02	3,08E-08	2.84E-05	3.28E-06	3.57E-08
OO 2015	0,6688		1	1	0,5255	0,5117	0,3366	0,1262	0,05793	0,05559	0,03872	0,0001666	3.76E-04	3.47E-04	4.24E-05	6.02E-07
HEVOO 2014	1,833	1,164		1	0,9818	0,9796	0,9177	0,6403	0,4301	0,4201	0,3393	0,00515	2.37E-02	2.21E-02	3.08E-03	7.28E-05
LEVOO 2014	1,877	1,208	0,04416		0,9856	0,9837	0,9293	0,6642	0,4535	0,4433	0,3605	0,005802	2.76E-02	2.56E-02	3.61E-03	8.69E-05
LEVOO 2015	4,218	3,446	2,101	2,05		1	1	0,9995	0,9894	0,9881	0,9706	0,07745	0,0002892	0,0002662	2.86E-02	2.31E-04
HEVOO 2015	4,245	3,473	2,128	2,077	0,03323		1	0,9996	0,9909	0,9897	0,9739	0,08203	0,0003164	0,0002913	3.14E-02	2.59E-04
MOG 2015	4,596	3,838	2,518	2,468	0,5515	0,5192		1	0,9997	0,9996	0,9983	0,2208	0,001941	0,001801	0,0002354	3.67E-03
RAG 2015	5,201	4,479	3,222	3,174	1,492	1,462	0,9565		1	1	1	0,7477	0,04487	0,04248	0,009002	0,0005056
COR 2015	5,616	4,893	3,636	3,588	1,984	1,954	1,438	0,4537		1	1	0,9109	0,1113	0,1061	0,02657	0,002034
ASC 2015	5,636	4,914	3,656	3,609	2,009	1,979	1,462	0,4762	0,02254		1	0,9166	0,116	0,1106	0,02796	0,002175
MIG 2015	5,811	5,089	3,832	3,784	2,217	2,187	1,665	0,6682	0,2146	0,192		0,955	0,1631	0,1561	0,04272	0,003807
COR 2016	7,939	7,217	5,959	5,912	4,745	4,715	4,137	2,999	2,545	2,523	2,331		0,9923	0,9912	0,886	0,5186
MIG 2016	9,899	9,157	7,865	7,816	7,026	6,995	6,337	5,019	4,55	4,527	4,328	1,921		1	1	0,9997
MOG 2016	9,923	9,181	7,889	7,84	7,055	7,024	6,366	5,045	4,576	4,553	4,355	1,948	0,02734		1	0,9998
ASC 2016	10,55	9,805	8,513	8,464	7,805	7,774	7,098	5,732	5,264	5,241	5,042	2,635	0,7389	0,7116		1
RAG 2016	11,82	11,03	9,646	9,594	9,304	9,269	8,459	6,83	6,32	6,294	6,079	3,459	1,426	1,396	0,6163	

Table 11. Significant differences ($P < 0.05$) between the classes of oils are indicated in pink

TOTAL POLYPHENOLS by HPLC-DAD-ESI-MS																
	OO 2015	OO 2014	MOG 2015	LEVOO 2015	LEVOO 2014	HEVOO 2015	MIG 2015	COR 2015	HEVOO 2014	MOG 2016	MIG 2016	ASC 2015	ASC 2016	RAG 2016	COR 2016	RAG 2015
OO 2015		1	0,007438	0,0019	0,01038	0,000312	0,001066	4.47E-02	0,0002136	1.89E-02	1.34E-02	2,52E-05	1.13E-02	2.37E-04	1.89E-03	7.21E-06
OO 2014	0,2133		0,01328	0,003612	0,0171	0,0006257	0,00198	8.94E-02	0,0003939	3.90E-02	2.80E-02	5.09E-02	2.36E-02	5.51E-04	3.96E-03	1.60E-05
MOG 2015	5,811	5,569		1	1	0,9999	1	0,9296	0,9632	0,8833	0,8406	0,8708	0,8162	0,3229	0,4296	0,01358
LEVOO 2015	6,345	6,099	0,51		1	1	1	0,9858	0,9935	0,9708	0,9525	0,964	0,9407	0,5295	0,6293	0,02992
LEVOO 2014	5,673	5,46	0,622	0,2056		1	1	0,9994	0,9996	0,9985	0,997	0,9976	0,9959	0,9087	0,9129	0,2029
HEVOO 2015	7	6,753	1,29	0,8014	0,4487		1	0,9999	1	0,9996	0,999	0,9993	0,9984	0,896	0,9181	0,1255
MIG 2015	6,56	6,33	1,191	0,7421	0,4322	0,01484		1	1	0,9999	0,9997	0,9998	0,9995	0,9558	0,9588	0,2271
COR 2015	7,658	7,428	2,466	2,047	1,53	1,319	1,203		1	1	1	1	1	1	1	0,784
HEVOO 2014	7,131	6,918	2,275	1,889	1,458	1,235	1,142	0,04451		1	1	1	1	1	1	0,8912
MOG 2016	7,939	7,703	2,644	2,215	1,645	1,459	1,323	0,08118	0,02798		1	1	1	1	1	0,7778
MIG 2016	8,049	7,812	2,773	2,346	1,755	1,591	1,444	0,2014	0,1372	0,1245		1	1	1	1	0,8265
ASC 2015	7,846	7,615	2,684	2,27	1,718	1,542	1,408	0,2055	0,1431	0,1311	0,01084		1	1	1	0,8622
ASC 2016	8,104	7,867	2,837	2,412	1,81	1,657	1,504	0,2622	0,1924	0,1874	0,06295	0,04997		1	1	0,8488
RAG 2016	9,295	9,041	3,87	3,438	2,554	2,601	2,326	0,9744	0,8217	0,9233	0,7869	0,7434	0,718		1	0,9593
COR 2016	8,665	8,435	3,637	3,243	2,537	2,516	2,306	1,103	0,9627	1,058	0,9381	0,8978	0,8773	0,2656		0,9958
RAG 2015	10,32	10,09	5,559	5,21	4,192	4,482	4,119	2,916	2,618	2,931	2,81	2,711	2,75	2,303	1,813	

Table 12. Significant differences ($P < 0.05$) between the classes of oils are indicated in pink

HYDROXYTYROSOL																
	OO 2014	OO 2015	MOG 2015	RAG 2016	COR 2015	MOG 2016	ASC 2016	ASC 2015	COR 2016	MIG 2016	RAG 2015	MIG 2015	HEVOO 2014	LEVOO 2014	HEVOO 2015	LEVOO 2015
OO 2014		1	1	0,9974	0,9979	0,9661	0,9603	0,9626	0,9411	0,7097	0,636	0,1627	0,1694	0,08547	0,000126	0,0001034
OO 2015	0,5859		1	1	1	0,9989	0,9985	0,9985	0,9966	0,9371	0,897	0,4006	0,3901	0,231	0,0008681	0,0007213
MOG 2015	0,8001	0,1357		1	1	0,9975	0,9966	0,9969	0,9926	0,867	0,8042	0,2181	0,24	0,1171	3.43E-02	2.69E-02
RAG 2016	1,729	1,033	1,075		1	1	1	1	1	0,9953	0,9861	0,5512	0,556	0,3325	0,0001731	0,0001346
COR 2015	1,7	1,068	1,09	0,1581		1	1	1	1	0,9996	0,9982	0,7988	0,7694	0,565	0,003547	0,002927
MOG 2016	2,254	1,604	1,726	0,7983	0,5588		1	1	1	1	1	0,9399	0,9166	0,7682	0,008965	0,007426
ASC 2016	2,296	1,646	1,776	0,8513	0,6055	0,04837		1	1	1	1	0,9485	0,9265	0,7864	0,01013	0,008409
ASC 2015	2,28	1,647	1,763	0,8712	0,6345	0,09651	0,04978		1	1	1	0,9671	0,9483	0,8343	0,01882	0,01587
COR 2016	2,41	1,777	1,915	1,032	0,7774	0,244	0,1973	0,1429		1	1	0,9817	0,9678	0,8787	0,02662	0,02257
MIG 2016	3,081	2,431	2,696	1,831	1,47	0,9428	0,8944	0,8143	0,6667		1	0,9995	0,9979	0,9798	0,07815	0,06726
RAG 2015	3,23	2,597	2,867	2,041	1,676	1,172	1,125	1,041	0,8983	0,261		1	0,9997	0,9951	0,1741	0,1539
MIG 2015	4,33	3,697	4,146	3,396	2,881	2,416	2,37	2,246	2,103	1,506	1,205		1	1	0,7555	0,7213
HEVOO 2014	4,305	3,72	4,082	3,386	2,95	2,523	2,48	2,371	2,24	1,696	1,42	0,3203		1	0,9535	0,9416
LEVOO 2014	4,693	4,107	4,522	3,847	3,369	2,953	2,911	2,79	2,659	2,126	1,839	0,7392	0,3878		0,9934	0,9907
HEVOO 2015	7,312	6,635	7,745	7,204	6,106	5,734	5,683	5,418	5,263	4,74	4,289	2,982	2,34	1,892		1
LEVOO 2015	7,379	6,702	7,825	7,289	6,181	5,812	5,761	5,493	5,338	4,818	4,363	3,056	2,407	1,96	0,08212	

Table 13. Significant differences ($P < 0.05$) between the classes of oils are indicated in pink

TYROSOL																
	OO 2014	OO 2015	COR 2015	MOG 2015	MIG 2016	COR 2016	ASC 2015	RAG 2016	ASC 2016	RAG 2015	MOG 2016	MIG 2015	LEVOO 2014	HEVOO 2015	LEVOO 2015	HEVOO 2014
OO 2014		1	0,9976	0,9864	0,9707	0,962	0,9346	0,8477	0,8992	0,8152	0,6336	0,2481	0,1366	0,0006505	0,0003616	0,003431
OO 2015	0,384		0,9999	0,9989	0,9963	0,9942	0,987	0,9603	0,9763	0,9382	0,8271	0,4259	0,2517	0,002165	0,00124	0,008945
COR 2015	1,718	1,303		1	1	1	1	1	1	1	0,9988	0,9058	0,7143	0,01751	0,01018	0,05811
MOG 2015	2,038	1,603	0,26		1	1	1	1	1	1	0,9996	0,9308	0,7522	0,01357	0,007483	0,05711
MIG 2016	2,216	1,79	0,4976	0,2603		1	1	1	1	1	1	0,9775	0,8649	0,03636	0,02148	0,1068
COR 2016	2,284	1,869	0,6202	0,3979	0,143		1	1	1	1	1	0,9914	0,9209	0,07285	0,0458	0,1639
ASC 2015	2,443	2,028	0,7941	0,5823	0,3225	0,1739		1	1	1	1	0,9967	0,9526	0,1038	0,06697	0,2111
RAG 2016	2,753	2,296	0,966	0,7447	0,4423	0,2689	0,07347		1	1	1	0,9925	0,9184	0,0306	0,01687	0,1178
ASC 2016	2,589	2,163	0,9089	0,6984	0,4257	0,2683	0,08876	0,02409		1	1	0,9973	0,9553	0,08928	0,05592	0,2008
RAG 2015	2,84	2,425	1,229	1,044	0,7719	0,609	0,4351	0,4155	0,3606		1	0,9999	0,9912	0,2279	0,1578	0,3669
MOG 2016	3,235	2,809	1,62	1,456	1,162	0,9794	0,7999	0,8304	0,7361	0,3505		1	0,9981	0,3125	0,2216	0,4707
MIG 2015	4,059	3,644	2,565	2,461	2,152	1,945	1,771	1,917	1,74	1,336	1,029		1	0,8761	0,7911	0,9096
LEVOO 2014	4,433	4,049	3,071	2,989	2,702	2,505	2,346	2,515	2,329	1,949	1,683	0,7294		0,9988	0,9954	0,9981
HEVOO 2015	6,739	6,296	5,45	5,56	5,119	4,777	4,588	5,199	4,67	4,116	3,894	2,668	1,62		1	1
LEVOO 2015	6,948	6,505	5,681	5,809	5,36	5,009	4,82	5,466	4,911	4,348	4,135	2,899	1,829	0,2554		1
HEVOO 2014	6,119	5,735	4,892	4,9	4,573	4,326	4,167	4,518	4,199	3,77	3,554	2,55	1,686	0,3262	0,1177	

Table 14. Significant differences ($P < 0.05$) between the classes of oils are indicated in pink

CAFFEIC ACID																
	MIG 2015	MOG 2015	RAG 2015	HEVOO 2014	LEVOO 2014	OO 2014	OO 2015	LEVOO 2015	HEVOO 2015	ASC 2015	COR 2015	COR 2016	ASC 2016	RAG 2016	MIG 2016	MOG 2016
MIG 2015																
MOG 2015																
RAG 2015																
HEVOO 2014																
LEVOO 2014																
OO 2014																
OO 2015																
LEVOO 2015									0,9999	1	1	0,8331	0,5478	0,01714	0,0262	0,0004114
HEVOO 2015								0,7298		1	1	0,9765	0,8447	0,075	0,09437	0,002252
ASC 2015								0,6279	0,0344		1	0,9837	0,8895	0,1445	0,1572	0,006628
COR 2015								0,6279	0,0344	0		0,9837	0,8895	0,1445	0,1572	0,006628
COR 2016								2,176	1,514	1,427	1,427		1	0,7832	0,7523	0,1195
ASC 2016								2,83	2,142	1,996	1,996	0,5221		0,9329	0,9073	0,2186
RAG 2016								5,07	4,308	3,914	3,914	2,31	1,815		1	0,8363
MIG 2016								4,863	4,175	3,86	3,86	2,385	1,929	0,2979		0,9524
MOG 2016								6,662	5,974	5,508	5,508	4,034	3,635	2,167	1,706	

Table 15. Significant differences (P < 0.05) between the classes of oils are indicated in pink

VANILLIC ACID																
	OO 2014	OO 2015	LEVOO 2014	HEVOO 2014	LEVOO 2015	ASC 2016	COR 2015	RAG 2015	MOG 2016	COR 2016	ASC 2015	HEVOO 2015	RAG 2016	MOG 2015	MIG 2015	MIG 2016
OO 2014																
OO 2015			0,9884	0,9256	0,7688	0,5773	0,5357	0,3922	0,3311	0,3626	0,3272	0,09315	0,02855	0,02909	0,02255	0,005746
LEVOO 2014		1,933		1	1	1	0,9999	0,9986	0,9976	0,9978	0,9964	0,9364	0,7677	0,7244	0,6167	0,3579
HEVOO 2014		2,416	0,4832		1	1	1	1	1	1	0,9999	0,9939	0,946	0,9206	0,8485	0,6218
LEVOO 2015		2,887	0,655	0,09703		1	1	1	0,9999	0,9999	0,9998	0,9762	0,8229	0,7871	0,6787	0,3592
ASC 2016		3,283	1,139	0,6031	0,6127		1	1	1	1	1	0,9997	0,9876	0,9766	0,935	0,7378
COR 2015		3,365	1,277	0,7553	0,7896	0,1903		1	1	1	1	1	0,9974	0,9934	0,9743	0,8548
RAG 2015		3,657	1,569	1,047	1,136	0,5203	0,3196		1	1	1	1	0,9998	0,9992	0,9943	0,9422
MOG 2016		3,793	1,649	1,113	1,225	0,5813	0,3713	0,04126		1	1	1	0,9998	0,9992	0,9938	0,9344
COR 2016		3,721	1,634	1,112	1,213	0,5937	0,3906	0,07102	0,03209		1	1	0,9999	0,9996	0,9962	0,9549
ASC 2015		3,802	1,715	1,193	1,31	0,6854	0,4794	0,1598	0,1238	0,08878		1	1	0,9998	0,9978	0,9678
HEVOO 2015		4,597	2,365	1,807	2,095	1,362	1,111	0,7646	0,7493	0,6875	0,5912		1	1	0,9999	0,9955
RAG 2016		5,186	2,889	2,315	2,754	1,947	1,661	1,302	1,31	1,222	1,122	0,5663		1	1	0,9998
MOG 2015		5,177	2,986	2,438	2,844	2,091	1,82	1,481	1,493	1,406	1,312	0,8049	0,2888		1	1
MIG 2015		5,294	3,206	2,684	3,081	2,372	2,113	1,793	1,811	1,722	1,633	1,181	0,7134	0,4206		1
MIG 2016		5,876	3,732	3,196	3,729	2,956	2,666	2,336	2,375	2,262	2,171	1,754	1,292	0,9513	0,4837	

Table 16. Significant differences (P < 0.05) between the classes of oils are indicated in pink

ρ-COUMARIC ACID																
	OO 2015	OO 2014	RAG 2015	MIG 2015	MIG 2016	COR 2015	ASC 2015	HEVOO 2014	ASC 2016	MOG 2015	RAG 2016	COR 2016	HEVOO 2015	LEVOO 2015	MOG 2016	LEVOO 2014
OO 2015		1	0,9995	0,8014	0,7128	0,3424	0,3169	0,2996	0,1704	0,08541	0,02825	0,04548	0,005817	0,005817	0,0002499	0,001118
OO 2014	0,3079		1	0,9118	0,8547	0,5019	0,4719	0,4389	0,2857	0,1588	0,06067	0,0877	0,01373	0,01373	0,000658	0,002543
RAG 2015	1,508	1,176		0,9995	0,9981	0,913	0,8956	0,855	0,7326	0,5272	0,269	0,3364	0,07284	0,07284	0,003709	0,01361
MIG 2015	2,874	2,542	1,496		1	1	1	0,9998	0,9995	0,9946	0,9565	0,9532	0,674	0,674	0,1126	0,2028
MIG 2016	3,074	2,733	1,68	0,1344		1	1	0,9999	0,9998	0,9965	0,9644	0,962	0,6858	0,6858	0,1085	0,2055
COR 2015	3,824	3,492	2,537	1,041	0,9406		1	1	1	1	1	0,9999	0,9872	0,9872	0,5138	0,6298
ASC 2015	3,884	3,551	2,602	1,106	1,008	0,06505		1	1	1	1	1	0,9909	0,9909	0,5483	0,6597
HEVOO 2014	3,926	3,618	2,732	1,366	1,281	0,4157	0,3563		1	1	1	1	0,9998	0,9998	0,8584	0,8914
ASC 2016	4,302	3,96	3,032	1,487	1,4	0,4115	0,3443	0,05337		1	1	1	0,9984	0,9984	0,669	0,7665
MOG 2015	4,694	4,345	3,442	1,855	1,781	0,7513	0,6823	0,2424	0,3409		1	1	0,9999	0,9999	0,7844	0,8573
RAG 2016	5,236	4,87	4,003	2,321	2,266	1,152	1,078	0,5716	0,7333	0,3833		1	1	1	0,8546	0,9128
COR 2016	5,012	4,68	3,838	2,342	2,284	1,301	1,236	0,772	0,9322	0,6287	0,3107		1	1	0,9799	0,9853
HEVOO 2015	5,911	5,555	4,777	3,154	3,13	2,025	1,955	1,378	1,655	1,348	1,052	0,6139		1	0,9981	0,9985
LEVOO 2015	5,911	5,555	4,777	3,154	3,13	2,025	1,955	1,378	1,655	1,348	1,052	0,6139	0		0,9981	0,9985
MOG 2016	7,077	6,735	6,089	4,544	4,564	3,469	3,401	2,722	3,164	2,915	2,733	2,125	1,681	1,681		1
LEVOO 2014	6,543	6,235	5,558	4,193	4,184	3,242	3,183	2,617	2,957	2,725	2,538	2,055	1,644	1,644	0,1815	

Table 17. Significant differences (P < 0.05) between the classes of oils are indicated in pink

FERULIC ACID																
	OO 2014	OO 2015	MIG 2015	COR 2015	ASC 2015	LEVOO 2014	LEVOO 2015	HEVOO 2014	MIG 2016	COR 2016	RAG 2015	HEVOO 2015	ASC 2016	MOG 2016	RAG 2016	MOG 2015
OO 2014																
OO 2015																
MIG 2015				1	1	1	0,9989	0,9966	0,9558	0,9244	0,8835	0,5813	0,4582	0,06594	0,008833	0,003386
COR 2015			0,4695		1	1	1	0,9999	0,9949	0,9872	0,9746	0,8121	0,6994	0,1568	0,02908	0,01134
ASC 2015			0,4695	0		1	1	0,9999	0,9949	0,9872	0,9746	0,8121	0,6994	0,1568	0,02908	0,01134
LEVOO 2014			1,029	0,6	0,6		1	1	1	0,9999	0,9998	0,9903	0,9696	0,5679	0,2556	0,1233
LEVOO 2015			1,46	0,9505	0,9505	0,2138		1	1	1	0,9998	0,9819	0,9438	0,3704	0,08332	0,03307
HEVOO 2014			1,629	1,2	1,2	0,5555	0,4276		1	1	1	0,9999	0,9988	0,8461	0,5537	0,3209
MIG 2016			2,182	1,697	1,697	0,9244	0,8641	0,3081		1	1	1	0,9996	0,8405	0,4748	0,2461
COR 2016			2,347	1,878	1,878	1,114	1,086	0,5143	0,2424		1	1	1	0,934	0,6757	0,4081
RAG 2015			2,504	2,034	2,034	1,257	1,256	0,6571	0,404	0,1565		1	1	0,9617	0,7572	0,4884
HEVOO 2015			3,209	2,711	2,711	1,82	1,954	1,19	1,004	0,7192	0,5533		1	0,9946	0,9031	0,6674
ASC 2016			3,455	2,97	2,97	2,08	2,253	1,464	1,317	1,03	0,8687	0,3515		0,9996	0,9799	0,8547
MOG 2016			4,727	4,243	4,243	3,235	3,641	2,619	2,635	2,303	2,141	1,707	1,317		1	0,9998
RAG 2016			5,657	5,13	5,13	3,923	4,604	3,263	3,421	3,019	2,843	2,434	1,978	0,5345		1
MOG 2015			6,049	5,551	5,551	4,386	5,069	3,756	3,95	3,559	3,393	3,036	2,594	1,238	0,8115	

Table 18. Significant differences (P < 0.05) between the classes of oils are indicated in pink

LUTEOLIN																
	OO 2015	OO 2014	MOG 2015	HEVOO 2014	LEVOO 2014	MOG 2016	MIG 2015	ASC 2015	LEVOO 2015	MIG 2016	HEVOO 2015	ASC 2016	RAG 2015	COR 2015	COR 2016	RAG 2016
OO 2015		1	0,5333	0,2405	0,196	0,08315	0,08826	0,05603	0,01946	0,007698	0,001803	0,0002558	0,0003336	0,0001148	7.98E-03	1.20E-05
OO 2014	0,1385		0,6139	0,293	0,2421	0,1104	0,116	0,07522	0,02778	0,01115	0,002745	0,0003968	0,0005097	0,000178	1.28E-02	2.12E-05
MOG 2015	3,431	3,274		1	0,9999	0,999	0,9986	0,9929	0,963	0,8059	0,537	0,1559	0,1685	0,07889	0,008674	1.88E-02
HEVOO 2014	4,08	3,942	1,196		1	1	1	1	1	0,9999	0,9984	0,9117	0,9047	0,7754	0,3499	0,02324
LEVOO 2014	4,214	4,076	1,348	0,1341		1	1	1	1	1	0,9995	0,9442	0,9383	0,8336	0,417	0,03294
MOG 2016	4,708	4,554	1,586	0,1812	0,0324		1	1	1	0,9999	0,998	0,8683	0,8634	0,6895	0,2307	0,004852
MIG 2015	4,676	4,527	1,636	0,2691	0,1242	0,1048		1	1	1	0,9994	0,9229	0,9173	0,7816	0,3201	0,01156
ASC 2015	4,91	4,76	1,908	0,5027	0,3579	0,3692	0,256		1	1	1	0,9689	0,9648	0,8767	0,4376	0,02243
LEVOO 2015	5,403	5,244	2,277	0,692	0,5371	0,5812	0,4494	0,1717		1	1	0,9657	0,9621	0,8591	0,3736	0,008969
MIG 2016	5,797	5,643	2,863	1,27	1,121	1,242	1,095	0,8304	0,7276		1	0,9997	0,9995	0,9923	0,7872	0,09748
HEVOO 2015	6,365	6,205	3,423	1,654	1,499	1,692	1,518	1,241	1,178	0,3829		1	1	0,9985	0,8702	0,1209
ASC 2016	7,069	6,915	4,356	2,542	2,394	2,692	2,496	2,232	2,256	1,45	1,146		1	1	0,9995	0,7316
RAG 2015	6,976	6,827	4,309	2,569	2,424	2,707	2,52	2,264	2,283	1,507	1,215	0,1062		1	0,9998	0,8319
COR 2015	7,343	7,194	4,735	2,936	2,791	3,122	2,922	2,666	2,72	1,923	1,651	0,5217	0,4023		1	0,9529
COR 2016	8,215	8,065	5,748	3,808	3,663	4,108	3,876	3,62	3,755	2,909	2,686	1,507	1,357	0,9544		0,9999
RAG 2016	10,17	10,01	7,94	5,324	5,165	5,983	5,628	5,34	5,734	4,623	4,504	3,034	2,796	2,344	1,271	

Table 19. Significant differences (P < 0.05) between the classes of oils are indicated in pink

APIGENIN																
	OO 2015	OO 2014	MIG 2015	MIG 2016	ASC 2015	LEVOO 2014	ASC 2016	MOG 2016	HEVOO 2014	MOG 2015	RAG 2016	LEVOO 2015	HEVOO 2015	COR 2016	RAG 2015	COR 2015
OO 2015		1	0,4104	0,09715	0,000627	0,001971	5.91E-02	5.53E-02	0,0003898	2.18E-02	3.71E-05	4.94E-05	2.39E-05	4.82E-05	1,1E-08	1,01E-08
OO 2014	0,3987		0,628	0,2061	0,002021	0,005502	0,0002183	0,0002047	0,00118	8.60E-02	1.84E-04	2.33E-04	1.15E-04	2.06E-04	4.82E-05	4.43E-05
MIG 2015	3,677	3,246		1	0,5645	0,67	0,2086	0,2008	0,3534	0,1296	0,001285	0,001327	0,0006964	0,0007842	0,000206	0,0001904
MIG 2016	4,625	4,182	0,9344		0,9108	0,9431	0,5727	0,5597	0,7222	0,4309	0,009635	0,009452	0,005182	0,005186	0,001461	0,001356
ASC 2015	6,753	6,322	3,37	2,546		1	1	1	1	1	0,8643	0,8211	0,7195	0,5992	0,3649	0,3528
LEVOO 2014	6,332	5,933	3,162	2,4	0,08613		1	1	1	1	0,9565	0,9332	0,8782	0,7787	0,57	0,5573
ASC 2016	7,566	7,124	4,175	3,354	0,6943	0,5419		1	1	1	0,9868	0,975	0,9389	0,8603	0,6494	0,6354
MOG 2016	7,588	7,146	4,199	3,379	0,7187	0,564	0,02522		1	1	0,9885	0,9776	0,944	0,8683	0,6616	0,6476
HEVOO 2014	6,922	6,523	3,8	3,054	0,7235	0,5901	0,1128	0,09068		1	0,9988	0,9968	0,9897	0,961	0,8584	0,8499
MOG 2015	7,893	7,441	4,464	3,634	0,8896	0,7133	0,1831	0,1571	0,04421		0,9932	0,9853	0,9588	0,8925	0,6939	0,6799
RAG 2016	9,844	9,371	6,491	5,704	2,704	2,321	2,03	2,003	1,62	1,9		1	1	1	0,9993	0,9991
LEVOO 2015	9,76	9,3	6,48	5,712	2,825	2,449	2,177	2,15	1,768	2,054	0,2474		1	1	0,9999	0,9999
HEVOO 2015	9,972	9,512	6,715	5,957	3,06	2,661	2,422	2,395	1,98	2,306	0,5183	0,2594		1	1	1
COR 2016	9,767	9,337	6,672	5,956	3,302	2,928	2,716	2,692	2,291	2,613	1,007	0,7573	0,5219		1	1
RAG 2015	10,2	9,767	7,144	6,444	3,774	3,359	3,204	3,179	2,722	3,113	1,538	1,269	1,034	0,4718		1
COR 2015	10,22	9,792	7,171	6,472	3,801	3,384	3,231	3,207	2,746	3,142	1,568	1,298	1,063	0,4987	0,02696	

Table 20. Significant differences (P < 0.05) between the classes of oils are indicated in pink

PINORESINOL																
	OO 2015	OO 2014	RAG 2015	COR 2015	LEVOO 2015	LEVOO 2014	HEVOO 2015	RAG 2016	COR 2016	MIG 2015	MOG 2015	HEVOO 2014	MIG 2016	MOG 2016	ASC 2015	ASC 2016
OO 2015		1	0,9982	0,9782	0,9034	0,9358	0,7731	0,7052	0,7662	0,7136	0,3279	0,2616	0,05806	0,03717	0,01378	8.32E-05
OO 2014	0,1587		0,9995	0,9901	0,9447	0,9628	0,8457	0,7901	0,8356	0,7903	0,4097	0,3253	0,08183	0,05302	0,02042	1.50E-04
RAG 2015	1,678	1,507		1	1	1	0,9999	0,9998	0,9998	0,9994	0,9567	0,8763	0,5024	0,372	0,1864	1.76E-03
COR 2015	2,143	1,972	0,5092		1	1	1	1	1	1	0,9965	0,9722	0,7628	0,6261	0,3819	9.44E-03
LEVOO 2015	2,574	2,391	0,8664	0,3141		1	1	1	1	1	0,9988	0,9841	0,787	0,6459	0,3808	2.50E-03
LEVOO 2014	2,436	2,278	0,9532	0,4883	0,2394		1	1	1	1	1	0,999	0,9735	0,9269	0,7808	0,0004038
HEVOO 2015	2,941	2,758	1,275	0,7226	0,4502	0,1281		1	1	1	1	0,9978	0,9255	0,8296	0,5844	9.65E-03
RAG 2016	3,09	2,901	1,392	0,8195	0,5488	0,1951	0,0786		1	1	1	0,998	0,92	0,8179	0,5588	4.43E-03
COR 2016	2,957	2,786	1,401	0,8918	0,6532	0,3258	0,2447	0,1828		1	1	0,9998	0,986	0,9505	0,8128	0,0001548
MIG 2015	3,072	2,901	1,527	1,018	0,7897	0,4407	0,3812	0,3242	0,1259		1	0,9999	0,9926	0,9688	0,859	0,0002258
MOG 2015	3,858	3,678	2,32	1,78	1,615	1,096	1,177	1,15	0,834	0,7005		1	0,9999	0,9983	0,9742	0,0004783
HEVOO 2014	4,023	3,864	2,667	2,202	2,072	1,587	1,704	1,69	1,388	1,273	0,7036		1	1	1	0,03393
MIG 2016	4,893	4,717	3,491	2,965	2,909	2,19	2,484	2,508	2,044	1,914	1,312	0,4301		1	1	0,02038
MOG 2016	5,11	4,938	3,759	3,25	3,211	2,478	2,802	2,833	2,358	2,232	1,667	0,7644	0,3911		1	0,06833
ASC 2015	5,555	5,384	4,247	3,737	3,74	2,923	3,331	3,381	2,846	2,72	2,184	1,21	0,8949	0,4878		0,1691
ASC 2016	9,616	9,44	8,693	8,167	8,585	6,913	8,16	8,407	7,246	7,116	6,853	5,153	5,385	4,811	4,307	

Table 21. Significant differences (P < 0.05) between the classes of oils are indicated in pink

ACETOXYPINORESINOL																
	OO 2015	OO 2014	MOG 2015	COR 2016	ASC 2015	MIG 2015	COR 2015	ASC 2016	MOG 2016	MIG 2016	LEVOO 2015	LEVOO 2014	HEVOO 2015	RAG 2016	HEVOO 2014	RAG 2015
OO 2015		1	0,999	0,999	0,9448	0,9306	0,8642	0,8281	0,8159	0,6129	0,2248	0,02071	0,002589	1.54E-07	2.22E-05	7.54E-08
OO 2014	0,2182		0,9999	0,9999	0,9771	0,9695	0,929	0,906	0,8974	0,7319	0,3211	0,03348	0,00494	3.87E-07	4.67E-05	1.74E-07
MOG 2015	1,592	1,345		1	1	1	0,9998	0,9996	0,9995	0,9877	0,7538	0,1209	0,01592	4.43E-08	5.28E-05	4.94E-08
COR 2016	1,589	1,353	0,08378		1	1	1	0,9999	0,9999	0,9958	0,8665	0,195	0,04346	1.27E-06	3.18E-04	7.09E-07
ASC 2015	2,391	2,155	1,016	0,8785		1	1	1	1	1	0,9972	0,5409	0,2402	3,88E-08	4.36E-03	1.52E-05
MIG 2015	2,462	2,226	1,098	0,956	0,07753		1	1	1	1	0,9984	0,5773	0,2713	5.22E-05	5.47E-03	1.98E-05
COR 2015	2,704	2,469	1,38	1,222	0,3436	0,2661		1	1	1	0,9998	0,6994	0,3953	1.44E-04	1.18E-02	4.93E-05
ASC 2016	2,806	2,564	1,465	1,294	0,3863	0,3062	0,03138		1	1	0,9998	0,6723	0,345	4,23E-08	6,45E-06	1.84E-05
MOG 2016	2,838	2,596	1,502	1,329	0,4213	0,3413	0,06641	0,03626		1	0,9999	0,6879	0,3621	4.84E-05	7.13E-03	2.07E-05
MIG 2016	3,276	3,034	2,015	1,811	0,9032	0,8232	0,5483	0,5351	0,4988		1	0,8695	0,6236	3.05E-04	2.79E-02	1.07E-04
LEVOO 2015	4,126	3,874	2,985	2,698	1,745	1,661	1,372	1,391	1,353	0,8273		0,9833	0,9033	8,45E-07	9.28E-02	3.10E-04
LEVOO 2014	5,376	5,158	4,503	4,218	3,416	3,345	3,102	3,158	3,126	2,688	2,082		1	0,04415	0,09817	0,007485
HEVOO 2015	6,228	5,976	5,491	5,034	4,081	3,997	3,708	3,819	3,78	3,254	2,574	0,0204		0,002625	0,02383	0,0004402
RAG 2016	11,41	11,15	11,76	10,82	9,831	9,744	9,445	9,806	9,766	9,219	8,912	5,026	6,223		1	0,9994
HEVOO 2014	9,995	9,777	9,741	9,207	8,405	8,334	8,091	8,282	8,25	7,813	7,415	4,619	5,313	0,4617		1
RAG 2015	11,61	11,38	11,73	10,98	10,1	10,03	9,761	10,05	10,01	9,533	9,215	5,808	6,879	1,526	0,8194	

Table 22. Significant differences (P < 0.05) between the classes of oils are indicated in pink

SECOIRIDOID DERIVATIVES																
	OO 2015	OO 2014	LEVOO 2015	MOG 2015	LEVOO 2014	HEVOO 2015	MIG 2015	HEVOO 2014	ASC 2016	MOG 2016	MIG 2016	COR 2015	ASC 2015	RAG 2016	COR 2016	RAG 2015
OO 2015		1	0,01603	0,01354	0,05118	0,004643	0,003874	0,004503	7.87E-02	7,59E-05	5.55E-02	9.79E-02	8.63E-02	4.87E-03	4.28E-03	1.45E-04
OO 2014	0,2368		0,02959	0,02496	0,08159	0,009122	0,007346	0,008105	0,0001709	0,0001651	0,0001215	0,0002074	0,0001833	1.18E-02	9.62E-03	3.40E-04
LEVOO 2015	5,488	5,215		1	1	1	1	0,9996	0,8842	0,8798	0,8375	0,8691	0,8522	0,5565	0,3126	0,03631
MOG 2015	5,561	5,292	0,2082		1	1	1	0,9999	0,9464	0,9439	0,9177	0,9351	0,9244	0,7146	0,4387	0,06744
LEVOO 2014	4,955	4,718	0,233	0,0573		1	1	1	0,99	0,9894	0,9827	0,9859	0,9829	0,9258	0,7197	0,2368
HEVOO 2015	6	5,727	0,6274	0,4025	0,2793		1	1	0,9856	0,9846	0,9736	0,9803	0,9756	0,8556	0,5877	0,1113
MIG 2015	6,072	5,816	1,115	0,9002	0,7202	0,5453		1	0,9998	0,9998	0,9995	0,9997	0,9995	0,9934	0,9064	0,4029
HEVOO 2014	6,013	5,776	1,455	1,257	1,058	0,9423	0,4226		1	1	1	1	1	1	0,9925	0,7703
ASC 2016	7,471	7,208	2,641	2,382	1,974	2,05	1,36	0,8003		1	1	1	1	1	0,9999	0,9323
MOG 2016	7,483	7,22	2,656	2,396	1,986	2,064	1,373	0,8122	0,01357		1	1	1	1	0,9999	0,935
MIG 2016	7,587	7,324	2,781	2,518	2,09	2,19	1,488	0,9164	0,1325	0,1189		1	1	1	1	0,9558
COR 2015	7,397	7,141	2,69	2,44	2,046	2,12	1,452	0,9029	0,14	0,1269	0,01205		1	1	1	0,9678
ASC 2015	7,44	7,184	2,74	2,489	2,088	2,171	1,498	0,9453	0,188	0,1749	0,05999	0,04642		1	1	0,9732
RAG 2016	8,37	8,089	3,385	3,07	2,483	2,73	1,893	1,226	0,4307	0,4158	0,2856	0,261	0,2088		1	0,9657
COR 2016	8,411	8,155	3,894	3,618	3,059	3,325	2,563	1,917	1,287	1,274	1,159	1,111	1,064	0,9873		1
RAG 2015	9,442	9,187	5,12	4,816	4,091	4,55	3,692	2,948	2,454	2,441	2,326	2,24	2,194	2,257	1,13	

Table 23. Significant differences ($P < 0.05$) between the classes of oils are indicated in pink

"R" RATIO																
	COR	COR 2016	RAG 2016	ASC 2015	ASC 2016	MOG 2016	MOG 2015	MIG 2016	RAG 2015	MIG 2015	HEVOO 2014	LEVOO 2014	OO 2014	HEVOO 2015	LEVOO 2015	OO 2015
COR 2015		1	1	1	1	1	1	1	1	0,9668	0,8741	0,7342	0,6995	0,03887	0,001454	3.95E-09
COR 2016	0,1244		1	1	1	1	1	1	1	0,98	0,907	0,7845	0,7525	0,05141	0,002083	5.90E-09
RAG 2016	0,1926	0,05286		1	1	1	1	1	1	0,9545	0,8418	0,6715	0,631	0,0112	0,0001727	1.70E-10
ASC 2015	0,3061	0,1818	0,1514		1	1	1	1	1	0,9915	0,9441	0,849	0,8219	0,07596	0,003482	1.06E-08
ASC 2016	0,5026	0,3742	0,3692	0,1865		1	1	1	1	0,9956	0,9612	0,8803	0,8558	0,07868	0,003186	6.01E-09
MOG 2016	0,5026	0,3742	0,3692	0,1865	0		1	1	1	0,9956	0,9612	0,8803	0,8558	0,07868	0,003186	6.01E-09
MOG 2015	0,5705	0,4385	0,4442	0,2458	0,05627	0,05627		1	1	0,9958	0,9609	0,8773	0,8519	0,06559	0,002214	2.73E-09
MIG 2016	0,5545	0,4261	0,428	0,2384	0,05369	0,05369	0,001023		1	0,9968	0,9675	0,8942	0,8714	0,08759	0,003685	7.11E-09
RAG 2015	0,8992	0,7749	0,818	0,5931	0,4261	0,4261	0,3833	0,3742		0,9999	0,9947	0,9717	0,9624	0,2295	0,01671	7.36E-08
MIG 2015	2,248	2,124	2,334	1,942	1,819	1,819	1,814	1,767	1,349		1	1	1	0,8819	0,2674	5.66E-06
HEVOO 2014	2,674	2,56	2,769	2,394	2,29	2,29	2,292	2,243	1,853	0,6218		1	1	0,9976	0,7735	5.47E-04
LEVOO 2014	3,028	2,915	3,159	2,749	2,654	2,654	2,664	2,607	2,208	0,9763	0,3282		1	0,9999	0,9067	1.60E-03
OO 2014	3,102	2,988	3,24	2,822	2,73	2,73	2,741	2,682	2,281	1,05	0,3962	0,06791		0,9999	0,9258	2.00E-03
HEVOO 2015	5,087	4,952	5,641	4,755	4,737	4,737	4,831	4,68	4,112	2,649	1,719	1,34	1,261		0,9995	1.56E-03
LEVOO 2015	6,445	6,311	7,204	6,113	6,148	6,148	6,287	6,091	5,47	4,007	2,941	2,562	2,483	1,497		7.16E-02
OO 2015	12,44	12,33	13,51	12,16	12,32	12,32	12,55	12,28	11,62	10,39	9,044	8,715	8,648	8,724	7,502	

Table 24. Significant differences ($P < 0.05$) between the classes of oils are indicated in pink

2.3.5 Volatile substances

Volatile substances are key compounds affecting the sensorial properties of any food. In EVOOs they confer green, fruity notes, mainly deriving from compounds formed in the LOX cascade, as previously mentioned. However, also several degradation processes (lipid auto-oxidation, amino acid metabolism, sugar fermentation, etc.) lead to the production of volatile molecules that on the contrary can confer defects to the oil or in any case characterize an oil that is undergoing degradation. Volatile composition in the present study was determined by means of HS-SPME-GC-MS and in the figure below (Figure 24), an example of EVOO chromatogram is reported.

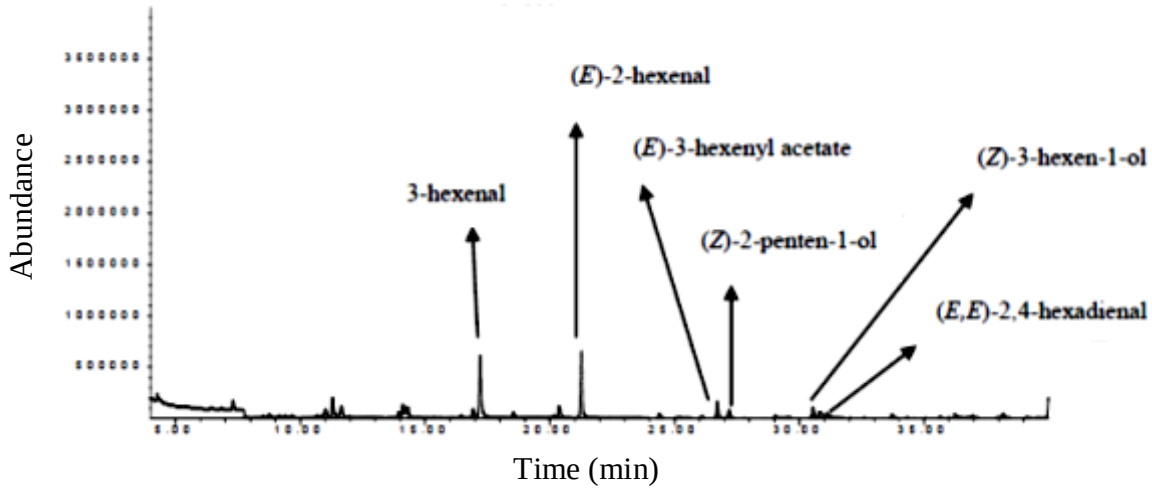


Figure 24. GC-MS chromatogram of an EVOO sample

The oil classes were compared in terms of relative percentage composition of some selected volatile molecules and in terms of their relative content. Twelve substances common found in all samples are selected and discussed. These compounds are: ethanol, 3-pentanone, 1-penten-3-one, 1-penten-3-ol, (*E*)-2-hexenal, acetic acid hexyl ester, hexanal, (*Z*)-3-hexen-1-ol-acetate, (*Z*)-2-penten-1-ol, 1-hexanol, (*Z*)-3-hexen-1-ol and (*E*)-2-hexen-1-ol.

Considering only the selected compounds, the average absolute area is calculated in order to have indications about the total content of VOCs in each category of oil analyzed (Figure 25).

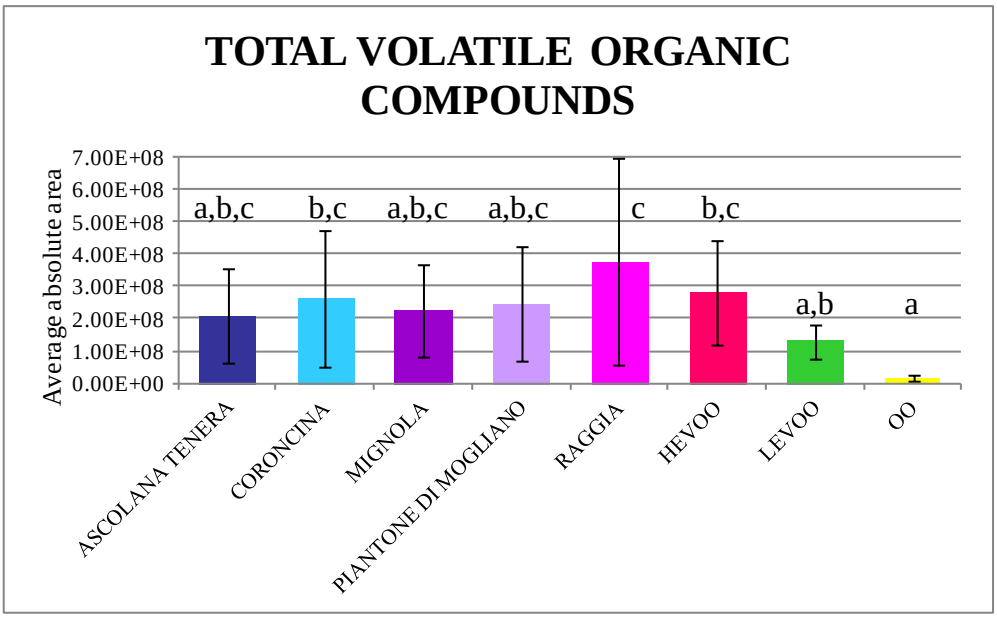
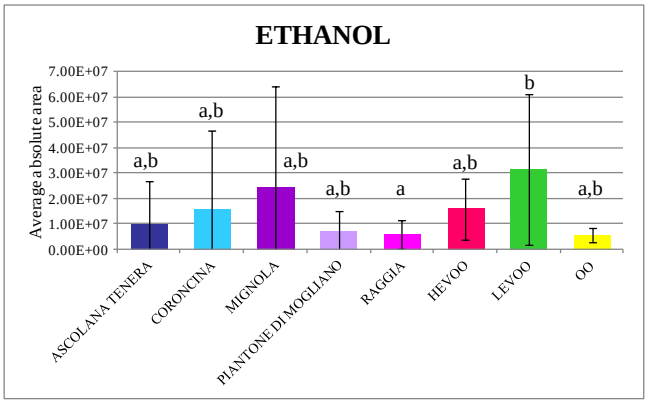
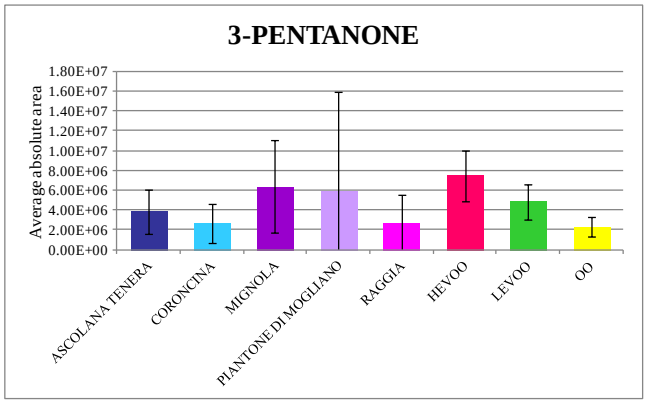


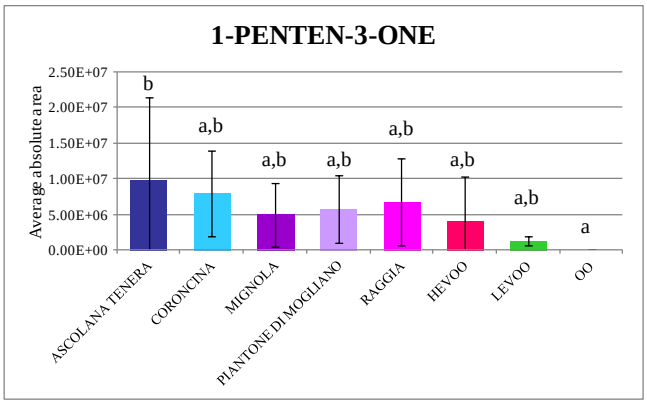
Figure 25. Total volatile organic compounds in each class of oils.
Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

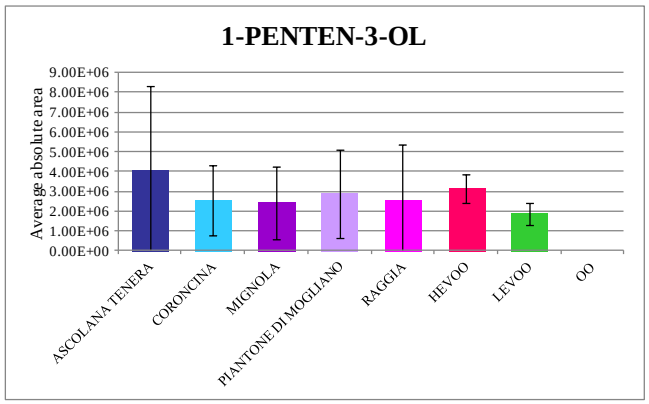
Considering the results obtained, Raggia and Ascolana Tenera are the cultivars with respectively the highest and the lowest content of volatile compounds. It is also interesting to see that the total content of volatile organic compounds decreases passing from year 2015 to year 2016 for all monovarietal oils. Significant differences ($P < 0.05$) between all categories analyzed are reported in Table 25. It is clear that in order to have a complete overview it is necessary to evaluate which kind of molecules compose the volatile fraction and in which terms they contribute to the *flavor* or *off-flavor*.

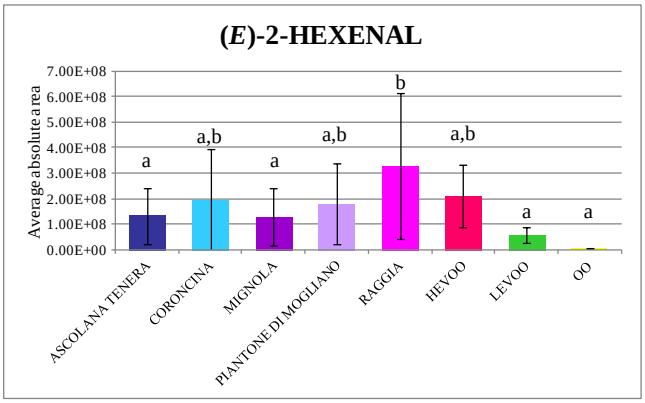
More in details, regarding the individual volatile compounds selected, some are known to be in relation with positive notes, like C6 compounds and some are known to be related with negative attributes, like ethanol (winey). In the figure below (Figure 26), the comparisons between the different classes of oils, for each detected compound are shown.

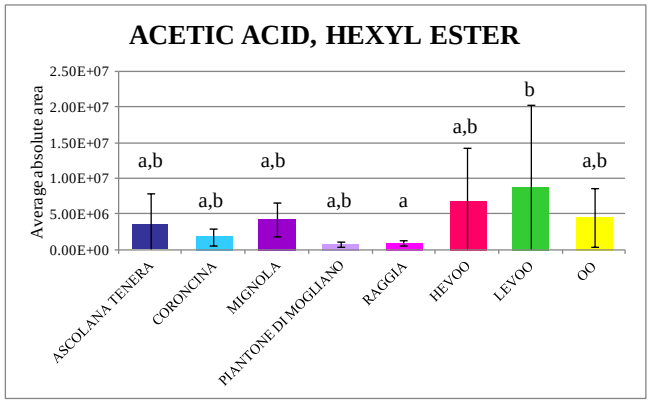

ETHANOL
Average absolute area
7.00E+07
6.00E+07
5.00E+07
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
a,b
a,b
a,b
a,b
a
a,b
b
a,b
ASCOLANA TENERA
CORONCINA
MIGNOLA
PIANTONE DI MOGLIANO
RAGGIA
HEVOO
LEVOO
OO

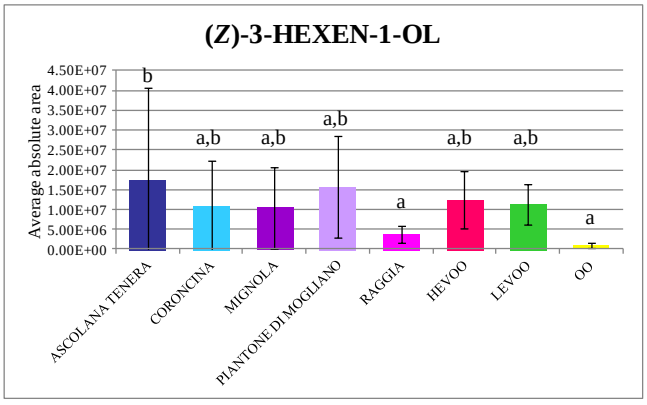

3-PENTANONE
Average absolute area
1.80E+07
1.60E+07
1.40E+07
1.20E+07
1.00E+07
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
ASCOLANA TENERA
CORONCINA
MIGNOLA
PIANTONE DI MOGLIANO
RAGGIA
HEVOO
LEVOO
OO

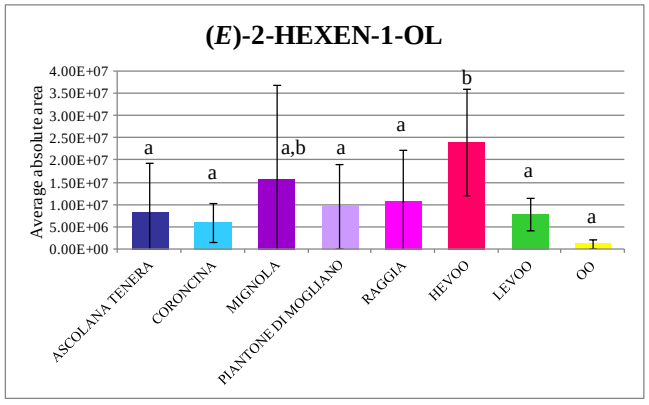

1-PENTEN-3-ONE
Average absolute area
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
b
a,b
a,b
a,b
a,b
a,b
a,b
a
ASCOLANA TENERA
CORONCINA
MIGNOLA
PIANTONE DI MOGLIANO
RAGGIA
HEVOO
LEVOO
OO


1-PENTEN-3-OL
Average absolute area
9.00E+06
8.00E+06
7.00E+06
6.00E+06
5.00E+06
4.00E+06
3.00E+06
2.00E+06
1.00E+06
0.00E+00
ASCOLANA TENERA
CORONCINA
MIGNOLA
PIANTONE DI MOGLIANO
RAGGIA
HEVOO
LEVOO
OO


(E)-2-HEXENAL
Average absolute area
7.00E+08
6.00E+08
5.00E+08
4.00E+08
3.00E+08
2.00E+08
1.00E+08
0.00E+00
a
a,b
a
a,b
b
a,b
a
a
ASCOLANA TENERA
CORONCINA
MIGNOLA
PIANTONE DI MOGLIANO
RAGGIA
HEVOO
LEVOO
OO


ACETIC ACID, HEXYL ESTER
Average absolute area
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
a,b
a,b
a,b
a,b
a
a,b
b
a,b
ASCOLANA TENERA
CORONCINA
MIGNOLA
PIANTONE DI MOGLIANO
RAGGIA
HEVOO
LEVOO
OO


(Z)-3-HEXEN-1-OL
Average absolute area
4.50E+07
4.00E+07
3.50E+07
3.00E+07
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
b
a,b
a,b
a,b
a
a,b
a,b
a
ASCOLANA TENERA
CORONCINA
MIGNOLA
PIANTONE DI MOGLIANO
RAGGIA
HEVOO
LEVOO
OO


(E)-2-HEXEN-1-OL
Average absolute area
4.00E+07
3.50E+07
3.00E+07
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
a
a
a,b
a
a
b
a
a
ASCOLANA TENERA
CORONCINA
MIGNOLA
PIANTONE DI MOGLIANO
RAGGIA
HEVOO
LEVOO
OO

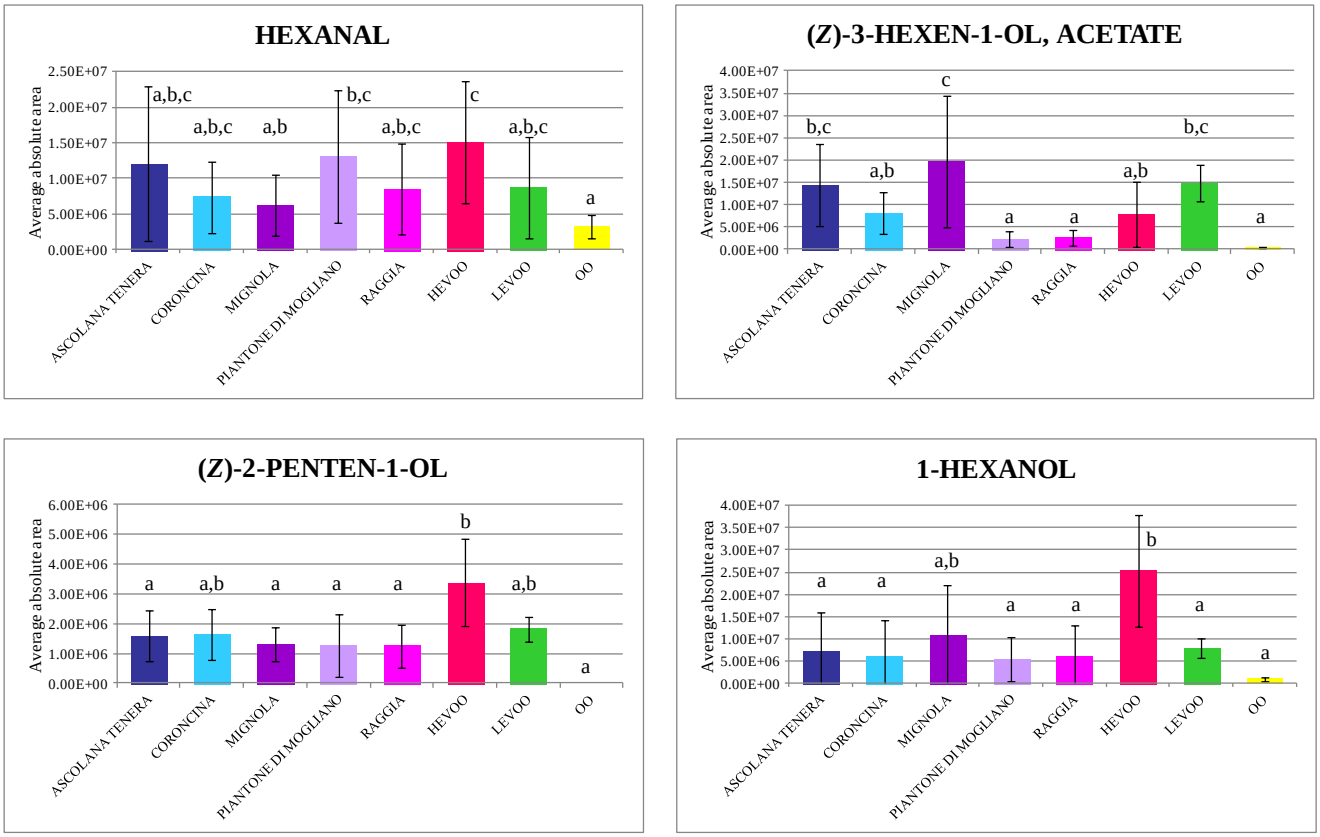


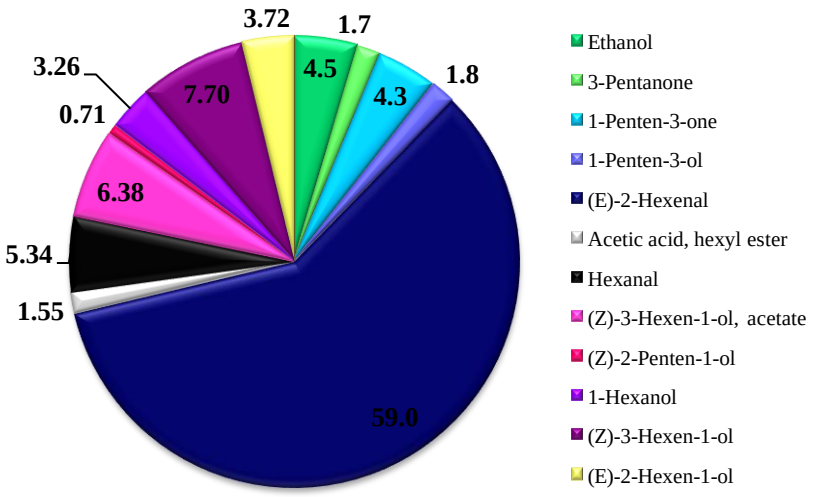
Figure 26. Average content of every detected compound in each class of oils. Different letters indicate significant differences ($P < 0.05$) between the olive oil classes under investigation

3-Pentanone together with hexanal, (*E*)-2-hexenal, 1-hexanol and (*E*)-3-hexen-1-ol, is produced by the lipoxygenase pathway, and they are responsible for green and fruity sensory attributes, which characterize EVOOs [48]. It is more abundant in HEVOOs and Mignola cultivar and less abundant in Raggia and Coroncina varieties. Moreover, it is interesting to observe the two years of production in which it would seem to be more abundant in all monovarietal oils of the year 2015 rather than in the year 2016. Also in the case of hexanal, it would seem to be more abundant in all monovarietal oils of the year 2015 rather than in the year 2016 and for commercial oils of year 2014 rather than in the year 2015. However, considering the different classes, HEVOOs, Ascolana Tenera and Piantone di Mogliano are the classes with the highest content of this molecule, while Mignola is the cultivar with the lowest content. 1-Penten-3-one has been shown to correlate with bitter and pungent sensations, even if the isolated molecule resulted to give sweet and strawberries notes [48]. It is more abundant in monovarietal oils than in commercial oils and between them, it shows an highest content in Ascolana Tenera cultivar and lowest content in Mignola cultivar. Considering the two years of production, it is more

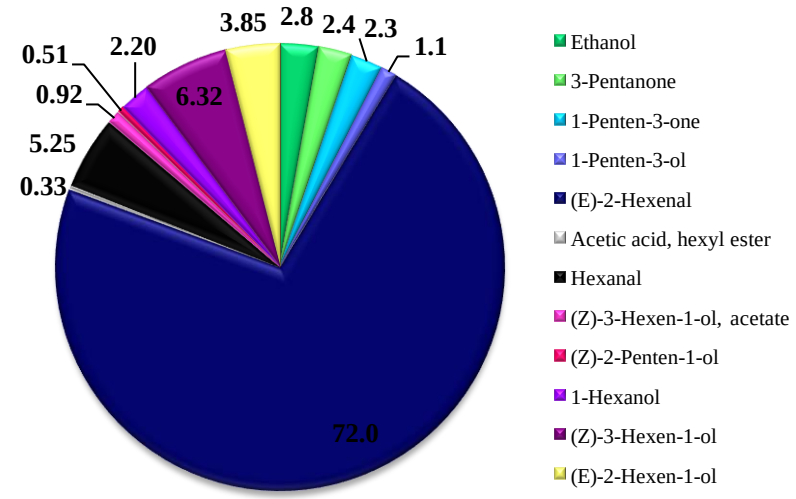
abundant in the cultivars of year 2015. The 1-penten-3-ol is originated from the homolitic breaking of the 13-hydroperoxide of linolenic acid. Butter and soft green are the sensory attributes associated to this compound [49]. The content of 1-penten-3-ol is more or less similar in each category of oils analyzed, except for Ascolana Tenera in which it shows the higher content. However, also in this case, it is more abundant in all monovarietal oils of the year 2015. 1-Hexanol derives from the reduction of hexanal carried out by alcohol dehydrogenase. The reaction is part of the lipoxygenase pathway and its precursor is the 13-hydroperoxide of linoleic acid, which is converted into hexanal by the hydroperoxide lyase action. This C6 alcohol gives the oil a fruity sensation and the typical banana smell. Particularly, significant differences are obtained between HEVOOs (that show the highest content) and all other categories of oils, even if this result can be considered anomalous and fortuitous. (*E*)-2-Hexenal originates from the lipoxygenase pathway; the isomerase action converts the (*Z*)-2-hexenal, whose precursor is the 13-hydroperoxide of linolenic acid, in (*E*)-2-hexenal. A positive *flavour* is generally associated to this molecule: the sensation of bitterness, the aroma of almond and a green note. However, several literature studies [50], show that this substance is also associated with *off-flavours* that give the oil the rancid defect. Given these considerations, we can not classify the sensorial attribute of this aldehyde as positive or negative because, most probably depends on its concentration in oil or the interaction with other substances that gives the typical aroma of grass, green apple. More in details, from Table 26 to Table 37, significant differences between all classes of oils for every detected compound ($P < 0.05$, one-way ANOVA and Tukey's test for pairwise comparison) are indicated in pink.

It is also important the percentage composition to highlight the differences between the different classes of oils. In this regard, pie charts, showing the composition of volatile substances common to all the cultivars, expressed in percentage area, are shown to evaluate the composition of the different cultivars.

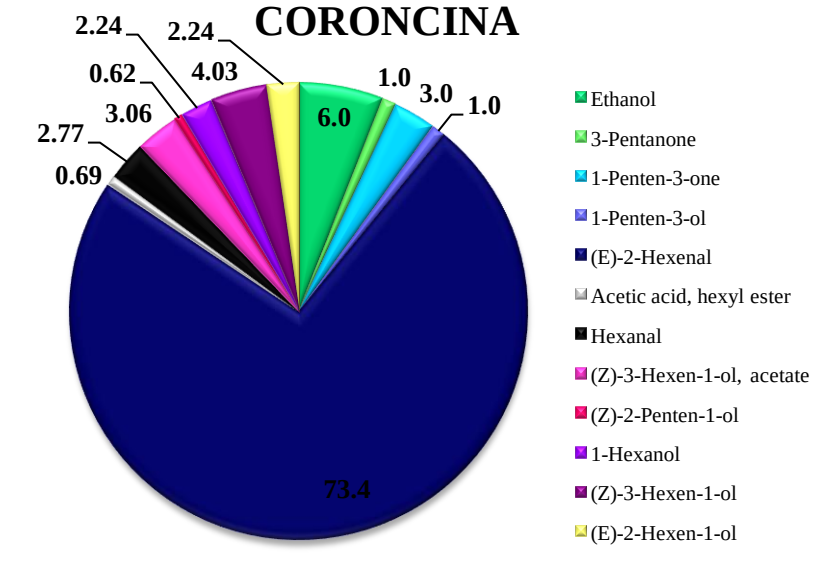
ASCOLANA TENERA



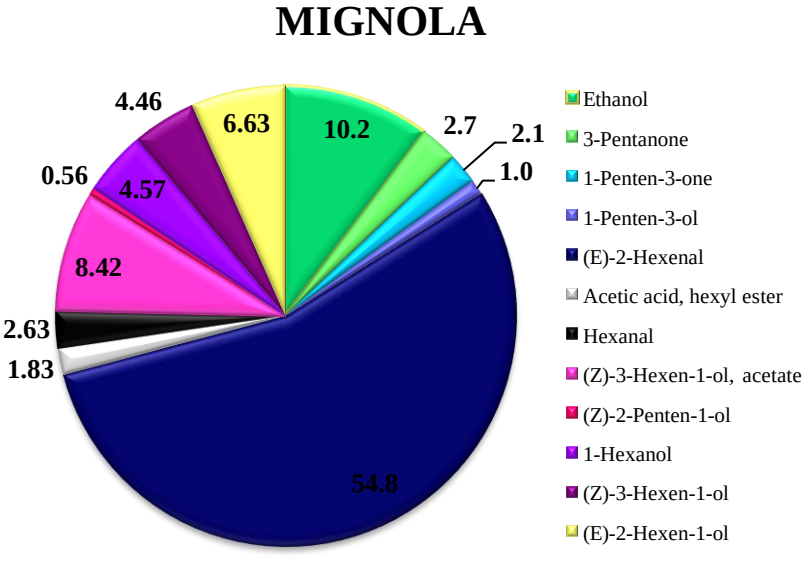
PIANTONE DI MOGLIANO

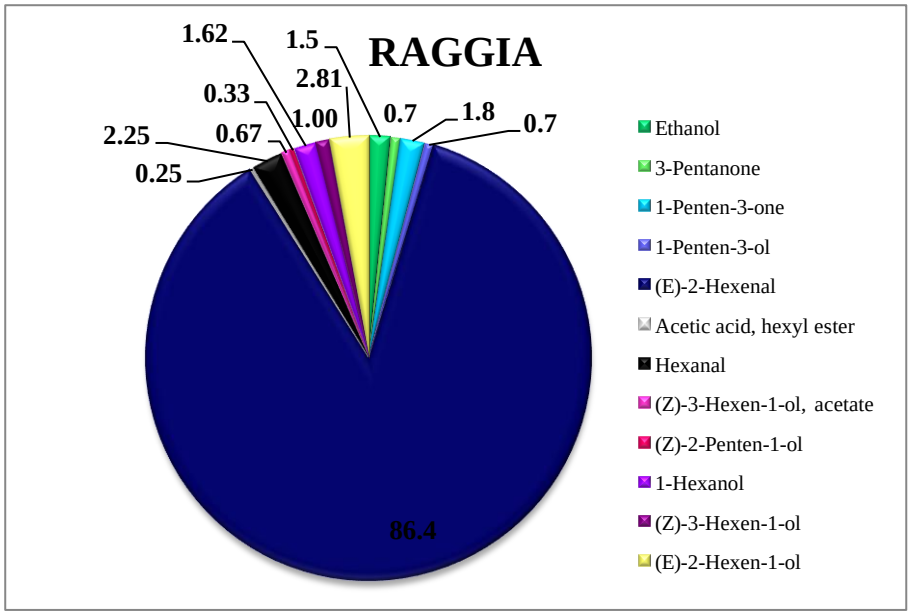


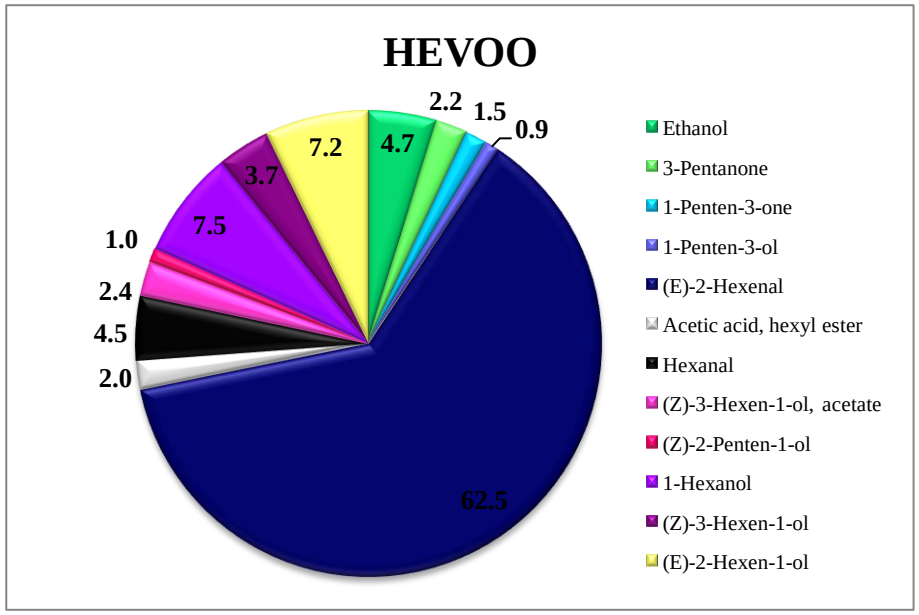
CORONCINA

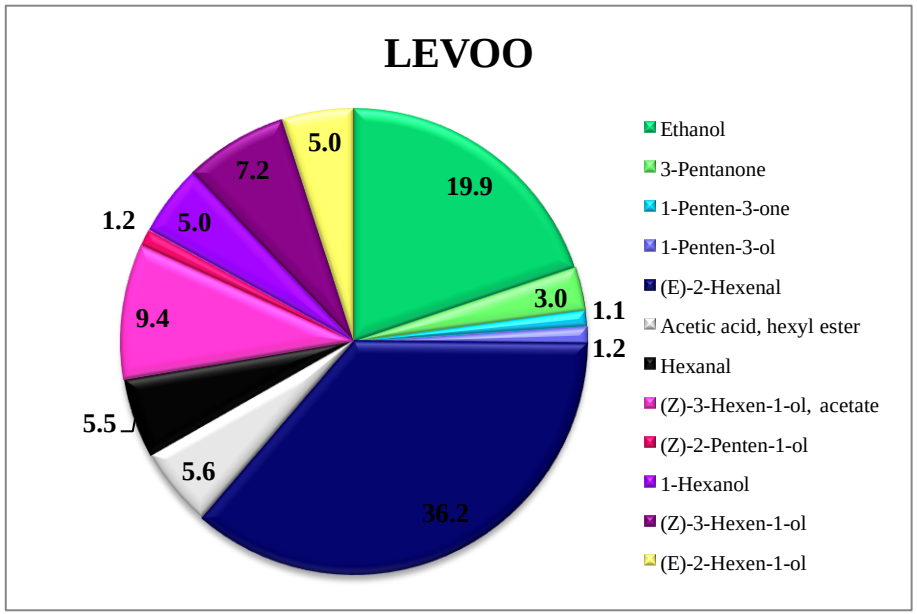


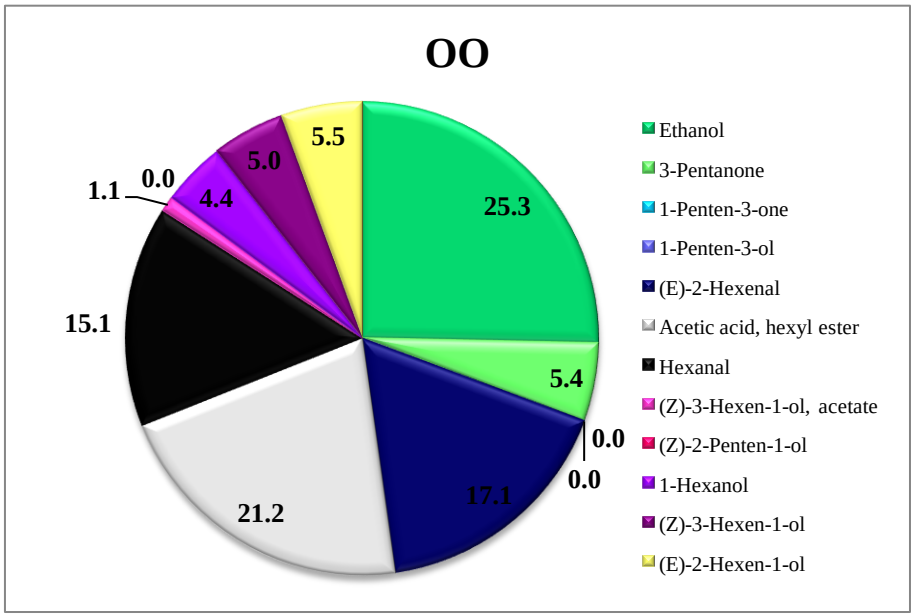
MIGNOLA




RAGGIA
1.62
1.5
2.81
0.33
1.00
0.7
1.8
0.7
2.25
0.67
0.25
86.4
Ethanol
3-Pentanone
1-Penten-3-one
1-Penten-3-ol
(E)-2-Hexenal
Acetic acid, hexyl ester
Hexanal
(Z)-3-Hexen-1-ol, acetate
(Z)-2-Penten-1-ol
1-Hexanol
(Z)-3-Hexen-1-ol
(E)-2-Hexen-1-ol


HEVOO
2.2
1.5
0.9
4.7
7.2
3.7
7.5
1.0
2.4
4.5
2.0
62.5
Ethanol
3-Pentanone
1-Penten-3-one
1-Penten-3-ol
(E)-2-Hexenal
Acetic acid, hexyl ester
Hexanal
(Z)-3-Hexen-1-ol, acetate
(Z)-2-Penten-1-ol
1-Hexanol
(Z)-3-Hexen-1-ol
(E)-2-Hexen-1-ol


LEVOO
5.0
19.9
7.2
1.2
5.0
3.0
1.1
9.4
1.2
5.5
5.6
36.2
Ethanol
3-Pentanone
1-Penten-3-one
1-Penten-3-ol
(E)-2-Hexenal
Acetic acid, hexyl ester
Hexanal
(Z)-3-Hexen-1-ol, acetate
(Z)-2-Penten-1-ol
1-Hexanol
(Z)-3-Hexen-1-ol
(E)-2-Hexen-1-ol


OO
5.5
5.0
1.1
0.0
4.4
25.3
15.1
5.4
0.0
0.0
17.1
21.2
Ethanol
3-Pentanone
1-Penten-3-one
1-Penten-3-ol
(E)-2-Hexenal
Acetic acid, hexyl ester
Hexanal
(Z)-3-Hexen-1-ol, acetate
(Z)-2-Penten-1-ol
1-Hexanol
(Z)-3-Hexen-1-ol
(E)-2-Hexen-1-ol

As it is possible to see from the graphs above, the percentage composition is similar for all different cultivars, except for LEVOO and OO categories that show another profile. In particular, the molecule found in highest percentage is (*E*)-2-hexenal (range value: 54.8% - 86.4%); in LEVOO and OO this compound is present respectively at 36.2% and 17.1%. Regarding the other compounds, excluding LEVOO and OO classes, they have a variable percentage from cultivar to cultivar. Anyway, ethanol (range value: 1.0% - 10.2%), hexanal (with a range values from 2.63% to 5.34%), 1-hexanol (with a range values from 1.62% to 4.57%, (*Z*)-3-hexen-1-ol (with a range values from 1.00% to 7.70%) and (*E*)-2-hexen-1-ol (with a range values from 2.81% to 10.31%) are the molecules present in greater quantity in each examined cultivar. On the contrary, 3-pentanone, 1-penten-3-one, 1-penten-3-ol, acetic acid, hexyl ester, (*Z*)-3-hexen-1-ol, acetate and (*Z*)-2-penten-1-ol are the compounds with the lowest percentage content in each category.

Considering only commercial oils, passing from HEVOOs to LEVOOs and then OOs, there is a gradual decrease of the percentage of compounds known to be associated with positive attributes (such as 2-(*E*)-hexenal) and an increase of the percentages of molecules known to be associated with negative notes (for example ethanol and acetic acid, hexyl ester) [24].

As previously mentioned, it is important to correlate these results with those obtained from sensory analysis (see the next paragraph).

TOTAL VOLATILE ORGANIC COMPOUNDS																
	OO 2015	OO 2014	LEVOO 2014	COR 2016	MOG 2016	MIG 2016	HEVOO 2014	ASC 2016	LEVOO 2015	ASC 2015	RAG 2016	MIG 2015	COR 2015	HEVOO 2015	MOG 2015	RAG 2015
OO 2015		1.00E+00	1.00E+00	0,9708	0,9848	0,9833	0,4798	0,8369	0,8646	0,1394	0,6548	0,007438	0,0007289	0,01194	0,001982	2.25E-09
OO 2014	0,1484		1.00E+00	0,9853	0,9933	0,9925	0,5555	0,8911	0,9143	0,1803	0,7397	0,01095	0,001131	0,01777	0,003076	3.92E-09
LEVOO 2014	1.13E+03	0,9855		1.00E+00	1.00E+00	1.00E+00	0,9426	0,9994	0,9998	0,6319	0,9949	0,104	0,01651	0,1691	0,04245	1.71E-07
COR 2016	2.22E+03	2.05E+03	0,9898		1.00E+00	1.00E+00	0,9989	1.00E+00	1.00E+00	0,9259	1.00E+00	0,2931	0,05552	0,4424	0,1363	1,69E-10
MOG 2016	2,062	1,897	0,8036	0,2347		1	0,9944	1.00E+00	1.00E+00	0,8369	1.00E+00	0,1695	0,024	0,2675	0,06327	1.94E-08
MIG 2016	2,082	1,918	0,8244	0,2118	0,02368		0,9951	1.00E+00	1	0,8448	1.00E+00	0,1759	0,02525	0,2771	0,06638	2.11E-08
HEVOO 2014	3,536	3,387	2,402	1.60E+03	1.86E+03	1,84		1.00E+00	0,9998	1.00E+00	1	0,9889	0,7978	0,9993	0,9562	1.23E-03
ASC 2016	2,783	2,618	1,525	0,5595	0,822	0,7984	1,14		1	0,9874	1	0,4919	0,1182	0,6805	0,2687	3.26E-07
LEVOO 2015	2,703	2,532	1,394	0,3729	0,6435	0,6185	1,38	0,223		0,9568	1	0,3092	0,05087	0,4636	0,1296	2.06E-08
ASC 2015	4,423	4,268	3,238	2,483	2,783	2,761	0,7297	2,022	2,319		0,9908	0,9999	0,9678	1	0,9988	3.59E-03
RAG 2016	3,193	3,016	1,845	0,8468	1,148	1,122	1,008	0,2479	0,5113	1.96E+03		0,4559	0,08814	0,6435	0,2149	2,26E-11
MIG 2015	5,813	5,653	4,588	3,942	4,306	4,283	1,994	3,512	3,903	1,304	3,584		1	1	1	8.21E-02
COR 2015	6,702	6,542	5,477	4,916	5,311	5,289	2,883	4,517	4,959	2,24	4,678	0,9737		0,9976	1	0,00131
HEVOO 2015	5,616	5,445	4,307	3,611	4,007	3,982	1,534	3,141	3,568	0,771	3,215	0,6647	1,721		1	1.43E-03
MOG 2015	6,332	6,164	5,047	4,435	4,85	4,826	2,323	4,004	4,465	1,62	4,156	0,2544	0,7783	0,992		4.52E-02
RAG 2015	12,62	12,46	11,4	11,4	12,01	11,99	8,805	11,22	12	8,473	11,97	7,461	6,487	8,757	7,659	

Table 25. Significant differences ($P < 0.05$) between the classes of oils are indicated in pink

ETHANOL																
	ASC 2016	MOG 2016	OO 2014	COR 2016	RAG 2016	OO 2015	MIG 2016	HEVOO 2014	RAG 2015	MOG 2015	ASC 2015	LEVOO 2014	HEVOO 2015	COR 2015	MIG 2015	LEVOO 2015
ASC 2016		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9994	0,9989	0,7905	0,7006	0,8746	0,243	0,003263	1
MOG 2016	0,1974		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9998	0,9996	0,8302	0,745	0,9065	0,2671	0,003219	1
OO 2014	0,5082	0,3534		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9849	0,9685	0,9973	0,7355	0,07721	1
COR 2016	0,3188	0,1319	0,2363		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9999	0,8931	0,8266	0,9528	0,3667	0,007156	1
RAG 2016	0,1913	0,02075	0,3871	0,1611		1.00E+00	1	1.00E+00	0,9995	0,999	0,7576	0,6548	0,839	0,1762	0,0009858	1
OO 2015	0,6093	0,4467	0,05702	0,3183	0,49		1	1.00E+00	1.00E+00	1	0,9799	0,9583	0,996	0,6706	0,04542	1
MIG 2016	0,4561	0,2737	0,1193	0,1373	0,3129	0,193		1	1	1	0,9251	0,8704	0,9722	0,4282	0,009918	1
HEVOO 2014	1,069	0,9276	0,4973	0,7974	0,9894	0,4672	0,6803		1	1	0,9993	0,9977	1	0,9258	0,2026	1
RAG 2015	1,518	1,37	0,7862	1.20E+03	1,487	0,7762	1,062	0,2252		1	0,9994	0,9976	1	0,8886	0,09294	1
MOG 2015	1,603	1,458	0,8587	1.28E+03	1,581	0,8538	1,147	0,2977	0,085		0,9997	0,9986	1	0,9109	0,1084	1
ASC 2015	2,899	2,798	2,055	2.61E+03	2,975	2,119	2,482	1,531	1,513	1,435		1	1	1	0,8495	0,9251
LEVOO 2014	3,099	3,004	2,23	2,808	3,193	2,305	2,682	1,706	1,713	1,636	0,1853		1	1	0,9101	0,8704
HEVOO 2015	2,669	2,559	1,736	2,34	2,774	1,797	2,198	1,162	1,102	1,014	0,5539	0,7594		0,9985	0,394	0,9722
COR 2015	4,081	4,015	3,024	3,775	4,292	3,161	3,643	2,479	2,623	2,541	0,947	0,7534	1,646		0,9944	0,4282
MIG 2015	6,182	6,187	4,764	5,863	6,643	5,034	5,726	4,203	4,664	4,579	2,745	2,545	3,716	1,858		0,009918
LEVOO 2015	0,4561	0,2737	0,1193	0,1373	0,3129	0,193	0	0,6803	1,062	1,147	2,482	2,682	2,198	3,643	5,726	

Table 26. Significant differences (P < 0.05) between the classes of oils are indicated in pink

3-PENTANONE																
	OO 2015	MOG 2016	MIG 2016	COR 2016	RAG 2016	ASC 2016	OO 2014	COR 2015	ASC 2015	RAG 2015	HEVOO 2014	LEVOO 2014	LEVOO 2015	MIG 2015	MOG 2015	HEVOO 2015
OO 2015																
MOG 2016			1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9996	0,9997	0,8657	0,9492	0,9979	0,1953	0,3884	0,454
MIG 2016		0,1574		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9999	0,9999	0,911	0,9723	0,9995	0,2516	0,4762	0,5472
COR 2016		0,1591	0,006878		1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9998	0,9999	0,8711	0,9542	0,9987	0,1672	0,3473	0,412
RAG 2016		0,01677	0,1608	0,1643		1.00E+00	1.00E+00	0,9999	0,9981	0,9986	0,7407	0,8732	0,9888	0,06369	0,1475	0,1839
ASC 2016		0,8236	0,6779	0,7153	0,8964		1	1.00E+00	1.00E+00	1.00E+00	0,998	0,9999	1	0,7497	0,9345	0,9601
OO 2014		0,474	0,308	0,334	0,5251	0,426		1.00E+00	1	1.00E+00	0,948	0,9883	0,9999	0,2798	0,5262	0,6021
COR 2015		1.08E+03	0,9088	0,9764	1.24E+03	0,09741	0,6275		1	1	0,9939	0,9996	1	0,487	0,7773	0,8423
ASC 2015		1,398	1,225	1,314	1.60E+03	0,3864	0,965	0,3539		1	0,9992	1	1	0,6635	0,9096	0,9471
RAG 2015		1,344	1,166	1,256	1.56E+03	0,3091	0,8956	0,2596	0,1076		0,9979	0,9999	1	0,5512	0,8381	0,8945
HEVOO 2014		2,628	2,47	2,611	2,948	1,609	2,296	1,797	1,481	1,62		1	0,9989	0,9998	1	1
LEVOO 2014		2,289	2,123	2,259	2,604	1,241	1,925	1,383	1,046	1,183	0,481		1	0,9885	0,9999	1
LEVOO 2015		1,614	1,426	1,549	1,917	0,4952	1,163	0,4979	0,1022	0,2286	1,526	1,06		0,5312	0,832	0,8927
MIG 2015		4,181	4,009	4,281	4,832	2,927	3,932	3,466	3,112	3,337	1,303	1,922	3,377		1	0,9999
MOG 2015		3,673	3,488	3,766	4,359	2,367	3,387	2,864	2,476	2,708	0,581	1,204	2,725	0,9331		1
HEVOO 2015		3,533	3,345	3,622	4,22	2,22	3,236	2,696	2,301	2,532	0,3927	1,013	2,539	1,179	0,2542	

Table 27. Significant differences (P < 0.05) between the classes of oils are indicated in pink

1-PENTEN-3-ONE																
	OO 2014	OO 2015	LEVOO 2014	LEVOO 2015	MIG 2016	MOG 2016	HEVOO 2015	COR 2016	HEVOO 2014	RAG 2016	MIG 2015	ASC 2016	MOG 2015	COR 2015	RAG 2015	ASC 2015
OO 2014																
OO 2015																
LEVOO 2014				1	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9501	1.00E+00	0,8935	0,9988	0,6022	0,0734	0,06997	0,001487
LEVOO 2015			0,1506		1.00E+00	1.00E+00	1.00E+00	0,9991	0,8127	0,9997	0,6847	0,9833	0,2214	0,007745	0,007211	4.45E-02
MIG 2016			0,5928	0,8548		1.00E+00	1.00E+00	1.00E+00	0,9941	1.00E+00	0,9792	1.00E+00	0,8269	0,149	0,1426	0,003286
MOG 2016			0,65	0,955	0,02149		1.00E+00	1.00E+00	0,9908	1.00E+00	0,9685	1.00E+00	0,7435	0,08863	0,0841	0,001181
HEVOO 2015			0,5453	0,8523	0,1167	0,1515		1.00E+00	0,9766	1.00E+00	0,9334	0,9999	0,5883	0,04287	0,04033	0,0003641
COR 2016			1,057	1,423	0,4668	0,4794	0,6493		0,9995	1.00E+00	0,9969	1.00E+00	0,9386	0,2412	0,2317	0,005899
HEVOO 2014			2,215	2,709	1,721	1,808	2.01E+03	1,336		0,9941	1	1	1.00E+00	0,9374	0,9323	0,2063
RAG 2016			0,8743	1,268	0,2219	0,2174	0,3956	0,3042	1,723		0,9759	1	0,7398	0,07176	0,06771	0,0006633
MIG 2015			2,468	3	1,985	2,088	2,305	1,609	0,2527	2,02		0,9997	1	0,9767	0,9742	0,3004
ASC 2016			1,464	1,934	0,8811	0,9284	1,13	0,4176	0,9934	0,7817	1,274		0,9844	0,3545	0,3421	0,01005
MOG 2015			3,168	4,025	2,671	2,874	3,195	2,278	0,6558	2,882	0,3693	1,918		0,9837	0,9814	0,235
COR 2015			4,677	5,725	4,278	4,576	4,951	3,966	2,285	4,689	2,012	3,679	1,929		1	0,9751
RAG 2015			4,703	5,755	4,304	4,604	4,981	3,994	2,31	4,72	2,037	3,708	1,958	0,02765		0,9777
ASC 2015			6,386	7,648	6,076	6,474	6,91	5,838	4,072	6,69	3,808	5,614	3,984	2,028	2,001	

Table 28. Significant differences (P < 0.05) between the classes of oils are indicated in pink

1-PENTEN-3-OL																
	OO 2014	OO 2015	MIG 2016	MOG 2016	COR 2016	LEVOO 2014	RAG 2016	ASC 2016	LEVOO 2015	HEVOO 2014	MIG 2015	COR 2015	HEVOO 2015	RAG 2015	MOG 2015	ASC 2015
OO 2014																
OO 2015																
MIG 2016				1.00E+00	1.00E+00	0,9999	1.00E+00	1.00E+00	1.00E+00	0,6592	0,4969	0,2746	0,6993	0,001699	0,05495	6.67E-03
MOG 2016			0,06136		1.00E+00	0,9996	1.00E+00	1.00E+00	1.00E+00	0,5061	0,3283	0,141	0,4989	0,0003184	0,01591	5.62E-04
COR 2016			0,298	0,3937		1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,6851	0,5044	0,2585	0,7102	0,0008891	0,03909	1.84E-03
LEVOO 2014			1.17E+03	1.32E+03	0,9627		0,9987	1.00E+00	1.00E+00	0,9866	0,9591	0,8474	0,9967	0,03039	0,4343	0,0002363
RAG 2016			0,0874	0,02228	0,4647	1,474		0,9998	1.00E+00	0,3392	0,1738	0,05081	0,2626	3.17E-02	0,00234	1.99E-05
ASC 2016			0,92	1.08E+03	0,6763	0,3749	1.25E+03		1.00E+00	0,8938	0,7689	0,5003	0,9294	0,002852	0,1086	6.02E-03
LEVOO 2015			0,609	0,7498	0,3228	0,7388	0,8877	0,4025		0,7243	0,5284	0,2553	0,7357	0,0005171	0,02932	5.84E-04
HEVOO 2014			3,053	3,359	2,999	1,886	3.72E+03	2,467	2.92E+03		1	1	1.00E+00	0,6112	0,9994	0,03158
MIG 2015			3,377	3,74	3,362	2,158	4.18E+03	2,816	3,314	0,1888		1	1	0,6333	0,9998	0,02835
COR 2015			3,875	4,312	3,918	2,614	4.87E+03	3,371	3,927	0,5775	0,4023		0,9996	0,7669	1	0,04366
HEVOO 2015			2,97	3,373	2,946	1,622	3,907	2,323	2,891	0,5556	0,8107	1,304		0,148	0,9296	0,0009233
RAG 2015			6,338	6,963	6,585	5,119	7,769	6,135	6,786	3,15	3,105	2,82	4,283		0,9571	0,9731
MOG 2015			4,829	5,416	4,998	3,505	6,214	4,464	5,136	1,367	1,23	0,8424	2,322	2,172		0,1186
ASC 2015			8,29	9,087	8,709	7,07	10,13	8,323	9,074	5,101	5,152	4,944	6,571	2,047	4,414	

Table 29. Significant differences (P < 0.05) between the classes of oils are indicated in pink

(E)-2-HEXENAL																
	OO 2015	OO 2014	LEVOO 2014	LEVOO 2015	MIG 2016	COR 2016	HEVOO 2014	MOG 2016	ASC 2015	ASC 2016	MIG 2015	RAG 2016	COR 2015	HEVOO 2015	MOG 2015	RAG 2015
OO 2015		1.00E+00	1.00E+00	1.00E+00	0,9987	0,9951	0,9335	0,9959	0,7778	0,9807	0,5872	0,7269	0,01707	0,2378	0,04234	2.02E-08
OO 2014	0,03359		1.00E+00	1.00E+00	0,999	0,996	0,9403	0,9967	0,792	0,9837	0,6058	0,7453	0,01854	0,2518	0,04581	2.29E-08
LEVOO 2014	0,05757	0,02398		1.00E+00	0,9992	0,9966	0,9448	0,9972	0,8018	0,9855	0,619	0,7581	0,01966	0,262	0,04845	2.51E-08
LEVOO 2015	1.18E+03	1.14E+03	1.11E+03		1.00E+00	1.00E+00	0,9985	1.00E+00	0,9721	1.00E+00	0,8847	0,9634	0,03208	0,4668	0,08126	7.55E-10
MIG 2016	1,627	1,589	1.56E+03	0,5914		1	1.00E+00	1.00E+00	0,9987	1.00E+00	0,9901	0,9994	0,1474	0,8426	0,3156	4.29E-08
COR 2016	1,838	1,802	1.78E+03	0,872	0,2883		1.00E+00	1	0,9999	1.00E+00	0,9987	1.00E+00	0,2813	0,9501	0,5222	4,32E-10
HEVOO 2014	2,448	2,414	2,39	1.65E+03	1,089	0,8052		1	1.00E+00	1	1.00E+00	1	0,8225	0,9999	0,9633	1.93E-04
MOG 2016	1,807	1,77	1.74E+03	0,8087	0,2062	0,08912	0,908		0,9997	1	0,9967	0,9999	0,2036	0,9098	0,4112	8.68E-08
ASC 2015	2,931	2,897	2.87E+03	2,203	1,625	1,327	0,483	1,444		1	1.00E+00	1	0,9598	1	0,9975	1.08E-03
ASC 2016	2,114	2,077	2,05	1,177	0,556	0,2488	0,6012	0,3498	1,137		0,9997	1	0,3301	0,9745	0,5925	2.86E-07
MIG 2015	3,326	3,29	3,264	2,639	1,971	1,629	0,6822	1,772	0,1605	1,434		1	0,953	1	0,9975	1.18E-04
RAG 2016	3,044	3,004	2,976	2,274	1,518	1,143	0,1359	1,293	0,438	0,9093	0,6878		0,5899	0,9997	0,8615	1.87E-07
COR 2015	5,464	5,428	5,402	5,18	4,391	3,972	2,821	4,191	2,299	3,853	2,343	3,321		0,9912	1	0,0001902
HEVOO 2015	4,089	4,05	4,022	3,562	2,767	2,36	1,263	2,549	0,705	2,181	0,5928	1,446	1,948		0,9999	5.74E-05
MOG 2015	5,049	5,011	4,984	4,722	3,888	3,452	2,274	3,675	1,726	3,315	1,724	2,712	0,7606	1,255		4.99E-03
RAG 2015	12,02	11,98	11,96	12,97	11,81	11,15	9,375	11,61	8,853	11,27	9,522	11,39	7,179	9,735	8,376	

Table 30. Significant differences (P < 0.05) between the classes of oils are indicated in pink

ACETIC ACID, HEXYL ESTER																
	OO 2015	MOG 2015	MOG 2016	RAG 2015	RAG 2016	COR 2015	COR 2016	OO 2014	HEVOO 2015	ASC 2016	MIG 2016	ASC 2015	LEVOO 2015	MIG 2015	HEVOO 2014	LEVOO 2014
OO 2015																
MOG 2015																
MOG 2016				1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9974	1.00E+00	0,9931	0,1352	0,01393
RAG 2015			0,1611		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9969	1.00E+00	0,9902	0,06273	0,003451
RAG 2016			0,04193	0,1714		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9997	0,9323	0,9989	0,8107	0,001452	1,29E-05
COR 2015			0,359	0,2099	0,4797		1.00E+00	1	1.00E+00	1.00E+00	1.00E+00	0,9968	1.00E+00	0,9868	0,02309	0,0005872
COR 2016			0,4039	0,2561	0,5775	0,04006		1	1.00E+00	1.00E+00	1.00E+00	0,9946	1.00E+00	0,9761	0,009962	0,0001526
OO 2014			0,6823	0,5802	0,9646	0,4276	0,4219		1.00E+00	1.00E+00	1.00E+00	0,9999	1.00E+00	0,9991	0,05482	0,001747
HEVOO 2015			0,2827	0,1093	0,4053	0,1485	0,2126	0,6423		1	1	0,9766	1.00E+00	0,9118	0,002515	2.21E-02
ASC 2016			0,5424	0,4171	0,7979	0,2298	0,2079	0,2321	0,4381		1	0,9983	1.00E+00	0,9907	0,01521	0,0002543
MIG 2016			0,8368	0,7592	1.27E+03	0,6329	0,6494	0,171	0,9171	0,4416		0,9999	1	0,9994	0,03562	0,0007336
ASC 2015			1,575	1,603	2.30E+03	1,609	1,698	1,182	2,007	1,508	1,105		0,9999	1	0,3586	0,02664
LEVOO 2015			0,8978	0,833	1,442	0,7213	0,7541	0,2275	1,065	0,5286	0,04967	1,137		0,999	0,01879	0,0002425
MIG 2015			1,745	1,814	2,711	1,876	2,011	1,414	2,394	1,803	1,362	0,1378	1,427		0,294	0,0146
HEVOO 2014			4,355	4,788	6,474	5,286	5,67	4,859	6,253	5,48	5,077	3,677	5,383	3,834		0,9979
LEVOO 2014			5,52	6,123	8,221	6,828	7,335	6,4	8,032	7,145	6,742	5,218	7,162	5,499	1,541	

Table 31. Significant differences (P < 0.05) between the classes of oils are indicated in pink

(Z)-3-HEXEN-1-OL																
	OO 2015	OO 2014	RAG 2016	HEVOO 2014	COR 2016	MOG 2016	MIG 2016	RAG 2015	LEVOO 2014	ASC 2016	LEVOO 2015	COR 2015	MIG 2015	HEVOO 2015	ASC 2015	MOG 2015
OO 2015		1.00E+00	1.00E+00	0,9999	1.00E+00	1.00E+00	1.00E+00	0,9999	0,9763	0,9829	0,8593	0,245	0,1992	0,5744	0,0002913	0,002791
OO 2014	0,07557		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9999	0,9829	0,9884	0,887	0,2754	0,2258	0,6191	0,000361	0,003478
RAG 2016	0,4567	0,3681		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9834	0,9869	0,8133	0,1407	0,1052	0,43	3.12E-02	0,0002074
HEVOO 2014	1.27E+03	1.20E+03	1.03E+03		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9999	0,8667	0,8176	0,9937	0,008435	0,07475
COR 2016	0,9422	0,8606	0,6316	0,4314		1.00E+00	1.00E+00	1	0,9998	1.00E+00	0,993	0,5357	0,46	0,8947	0,0008382	0,008225
MOG 2016	1,047	0,9653	0,7585	0,3266	0,1148		1.00E+00	1	0,9999	1.00E+00	0,9965	0,5947	0,5179	0,9263	0,001115	0,01101
MIG 2016	0,9362	0,8523	0,6185	0,4746	0,03478	0,1533		1	0,9996	0,9999	0,9878	0,4612	0,3867	0,8505	0,0004657	0,004419
RAG 2015	1,338	1.26E+03	1,111	0,03534	0,4338	0,3191	0,4828		1	1.00E+00	0,9997	0,7505	0,6799	0,9789	0,002416	0,02391
LEVOO 2014	2,163	2.09E+03	2,079	0,891	1,394	1,289	1,463	0,9977		1.00E+00	1	0,9974	0,9941	1	0,06042	0,373
ASC 2016	2,086	2.00E+03	2,029	0,675	1,232	1,113	1,311	0,7835	0,3134		1	0,9585	0,9304	0,9998	0,009376	0,08812
LEVOO 2015	2,718	2.63E+03	2,844	1,25	1,902	1,777	2,014	1,431	0,2212	0,6321		0,9957	0,9898	1	0,01946	0,1723
COR 2015	4,07	3.99E+03	4,419	2,696	3,426	3,311	3,573	2,992	1,734	2,307	1,814		1	1	0,4811	0,978
MIG 2015	4,206	4.13E+03	4,585	2,833	3,576	3,461	3,728	3,142	1,871	2,462	1,977	0,1497		0,9999	0,5507	0,9893
HEVOO 2015	3,351	3.26E+03	3,637	1,882	2,605	2,48	2,744	2,134	0,8537	1,362	0,7746	1,111	1,274		0,07336	0,476
ASC 2015	7,039	6.96E+03	7,798	5,768	6,661	6,556	6,873	6,265	4,877	5,724	5,41	3,534	3,397	4,777		0,9967
MOG 2015	6,21	6,124	7,158	4,768	5,778	5,656	6,03	5,318	3,757	4,681	4,298	2,144	1,985	3,544	1,772	

Table 32. Significant differences (P < 0.05) between the classes of oils are indicated in pink

(E)-2-HEXEN-1-OL																
	OO 2015	LEVOO 2014	OO 2014	ASC 2015	COR 2016	MIG 2016	COR 2015	MOG 2016	RAG 2016	HEVOO 2014	ASC 2016	LEVOO 2015	MOG 2015	RAG 2015	MIG 2015	HEVOO 2015
OO 2015		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9959	0,9975	0,9995	0,687	0,8681	0,9876	0,8982	0,2831	0,008757	0,01271
LEVOO 2014	0,5592		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9959	0,9998	1.00E+00	0,9999	0,9571	0,4375	0,5715
OO 2014	0,3094	0,3252		1.00E+00	1.00E+00	1.00E+00	0,9996	0,9998	1.00E+00	0,8232	0,9504	0,9984	0,9675	0,431	0,01906	0,02842
ASC 2015	0,567	0,1306	0,2575		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9066	0,9834	0,9998	0,9911	0,5712	0,03503	0,05294
COR 2016	0,7513	0,03482	0,417	0,1389		1.00E+00	1.00E+00	1.00E+00	1	0,8881	0,9797	0,9999	0,9887	0,4854	0,01661	0,0234
MIG 2016	1,073	0,1999	0,7384	0,4603	0,3521		1.00E+00	1.00E+00	1	0,9586	0,9966	1.00E+00	0,9987	0,6644	0,03601	0,05248
COR 2015	1,805	0,7348	1,471	1,193	1,154	0,8023		1.00E+00	1	0,9991	1.00E+00	1.00E+00	1	0,9479	0,1637	0,2416
MOG 2016	1,723	0,6508	1,38	1,094	1,048	0,6845	0,1442		1	0,9971	1.00E+00	1.00E+00	1	0,8977	0,09966	0,1467
RAG 2016	1,504	0,4356	1,136	0,8303	0,7582	0,3626	0,5392	0,3983		0,9709	0,9986	1	0,9996	0,6491	0,01984	0,0254
HEVOO 2014	3,127	1,805	2,818	2,56	2,627	2,305	1,573	1.75E+03	2,212		1	0,9989	1	1	0,9462	0,989
ASC 2016	2,691	1,382	2,357	2,079	2,125	1,773	0,9708	1.15E+03	1,63	0,6867		1	1	0,9998	0,5697	0,7343
LEVOO 2015	2,015	0,8207	1,657	1,36	1,347	0,9648	0,09454	0,2555	0,7077	1,596	0,9584		1	0,9268	0,1023	0,1478
MOG 2015	2,592	1,251	2,241	1,949	1,995	1,622	0,771	0,9532	1,463	0,9547	0,2587	0,7442		0,9972	0,3442	0,4826
RAG 2015	3,97	2,316	3,635	3,357	3,526	3,173	2,371	2.59E+03	3,204	0,5916	1,4	2,477	1,744		0,9906	0,9994
MIG 2015	5,759	3,622	5,425	5,146	5,485	5,133	4,331	4.62E+03	5,407	2,381	3,36	4,603	3,823	1,96		1
HEVOO 2015	5,601	3,357	5,244	4,947	5,333	4,951	4,081	4,397	5,296	1,99	3,028	4,392	3,531	1,509	0,617	

Table 33. Significant differences (P < 0.05) between the classes of oils are indicated in pink

HEXANAL																
	OO 2015	OO 2014	COR 2016	LEVOO 2014	MIG 2016	MOG 2016	MIG 2015	RAG 2016	ASC 2016	LEVOO 2015	COR 2015	HEVOO 2014	RAG 2015	ASC 2015	HEVOO 2015	MOG 2015
OO 2015		1.00E+00	1.00E+00	0,9953	1.00E+00	1.00E+00	0,9927	1.00E+00	0,9754	0,9985	0,8067	0,09971	0,1698	0,02745	0,4525	0,0006907
OO 2014	0,6159		1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9999	1.00E+00	0,9995	1.00E+00	0,9729	0,2721	0,4284	0,1005	0,7993	0,004412
COR 2016	0,7932	0,128		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9995	1.00E+00	0,9646	0,2046	0,3329	0,05964	0,7251	0,001207
LEVOO 2014	1.83E+03	1.22E+03	1.19E+03		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,8379	0,9557	0,5811	0,9996	0,09378
MIG 2016	0,985	0,3017	0,1876	1.05E+03		1.00E+00	1.00E+00	1.00E+00	0,9999	1.00E+00	0,9774	0,2232	0,36	0,06362	0,7642	0,001077
MOG 2016	1,181	0,4973	0,403	0,8513	0,223		1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9917	0,2958	0,4627	0,09445	0,8566	0,001993
MIG 2015	1,911	1,246	1,225	0,06699	1.08E+03	0,8619		1.00E+00	1.00E+00	1	1	0,7149	0,8899	0,3979	0,9976	0,02944
RAG 2016	1,183	0,4513	0,3476	0,993	0,1495	0,09482	1.03E+03		0,9999	1	0,9727	0,1722	0,2797	0,03802	0,6897	0,0002551
ASC 2016	2,172	1,489	1,495	0,14	1,353	1,13	0,23	1,333		1	1	0,772	0,927	0,4505	0,9993	0,03289
LEVOO 2015	1,646	0,935	0,8872	0,4687	0,717	0,4819	0,4413	0,635	0,7094		0,9991	0,4095	0,608	0,1422	0,9465	0,002825
COR 2015	2,861	2,196	2,265	0,8828	2,152	1,936	1,04	2,198	0,8446	1,57		0,9803	0,9991	0,8645	1	0,2286
HEVOO 2014	4,611	3,995	4,188	2,78	4,131	3,935	3,07	4,296	2,944	3,679	2,12		1	1	0,9974	0,9995
RAG 2015	4,305	3,639	3,847	2,326	3,785	3,57	2.62E+03	3,976	2,478	3,285	1,581	0,6761		1	1	0,9356
ASC 2015	5,25	4,607	4,88	3,338	4,847	4,641	3.70E+03	5,099	3,594	4,411	2,703	0,4341	1,184		0,9521	1
HEVOO 2015	3,59	2,879	3,048	1,476	2,962	2,727	1,72	3,122	1,536	2,381	0,591	1,734	1,124	2,349		0,3189
MOG 2015	6,721	6,023	6,517	4,645	6,56	6,33	5,218	7,074	5,167	6,197	4,115	1,493	2,437	1,081	3,879	

Table 34. Significant differences (P < 0.05) between the classes of oils are indicated in pink

(Z)-3-HEXEN-1-OL, ACETATE																
	LEVOO 2014	HEVOO 2014	OO 2014	OO 2015	MOG 2016	MOG 2015	RAG 2016	RAG 2015	COR 2015	COR 2016	HEVOO 2015	ASC 2016	MIG 2016	ASC 2015	LEVOO 2015	MIG 2015
LEVOO 2014																
HEVOO 2014																
OO 2014																
OO 2015					1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9684	0,7682	0,9215	0,5175	0,1089	0,01071	0,04551	3.51E-02
MOG 2016				0,3428		1.00E+00	1.00E+00	1.00E+00	0,9989	0,973	0,9971	0,8867	0,4592	0,1077	0,3234	0,002087
MOG 2015				0,6126	0,2103		1.00E+00	1.00E+00	0,9996	0,9787	0,9985	0,8904	0,4189	0,07927	0,2676	0,0008091
RAG 2016				0,4864	0,05001	0,2012		1.00E+00	0,993	0,869	0,9742	0,6266	0,1313	0,01116	0,04849	1.93E-02
RAG 2015				0,8329	0,3592	0,1447	0,3946		0,9996	0,9713	0,9982	0,8479	0,3023	0,03834	0,153	0,0001369
COR 2015				2,002	1,36	1,241	1.67E+03	1,226		1	1.00E+00	0,9995	0,8675	0,2983	0,7224	0,003562
COR 2016				2,739	1,962	1,903	2.47E+03	1,976	0,7043		1.00E+00	1	0,9837	0,5543	0,9403	0,01098
HEVOO 2015				2,275	1,512	1,411	1,95	1,436	0,06541	0,7265		0,999	0,7751	0,1739	0,5465	0,0006022
ASC 2016				3,267	2,409	2,396	3.05E+03	2,532	1,26	0,5782	1,354		0,9995	0,8096	0,9962	0,03745
MIG 2016				4,419	3,387	3,473	4.31E+03	3,745	2,472	1,84	2,723	1,262		0,9983	1	0,308
ASC 2015				5,582	4,426	4,6	5.56E+03	4,981	3,755	3,192	4,133	2,637	1,424		0,999	0,9449
LEVOO 2015				4,894	3,692	3,836	4.86E+03	4,214	2,844	2,185	3,208	1,558	0,1886	1,354		0,2601
MIG 2015				7,825	6,277	6,654	8.04E+03	7,329	6,056	5,571	6,769	4,993	3,73	2,16	3,857	

Table 35. Significant differences (P < 0.05) between the classes of oils are indicated in pink

(Z)-2-PENTEN-1-OL																
	LEVOO 2014	OO 2014	OO 2015	MIG 2015	COR 2015	ASC 2015	MOG 2015	RAG 2015	LEVOO 2015	HEVOO 2014	MIG 2016	MOG 2016	ASC 2016	HEVOO 2015	COR 2016	RAG 2016
LEVOO 2014																
OO 2014																
OO 2015																
MIG 2015					1.00E+00	1.00E+00	1.00E+00	1.00E+00	1	0,9595	0,9986	0,9956	0,9548	0,9998	0,9181	0,9997
COR 2015				0,3488		1.00E+00	1.00E+00	1.00E+00	1	0,9826	0,9999	0,9992	0,98	1.00E+00	0,9556	1.00E+00
ASC 2015				0,3909	0,04547		1.00E+00	1.00E+00	1.00E+00	0,986	0,9999	0,9995	0,984	1.00E+00	0,963	1.00E+00
MOG 2015				0,104	0,2987	0,3485		1.00E+00	1.00E+00	0,8965	0,9951	0,9843	0,867	0,9992	0,7812	0,9987
RAG 2015				0,351	0,02816	0,07798	0,3025		1.00E+00	0,9571	0,9994	0,997	0,9449	1.00E+00	0,8911	0,9999
LEVOO 2015				0,2663	0,154	0,2083	0,197	0,1412		0,8778	0,9951	0,9821	0,8284	0,9993	0,719	0,9987
HEVOO 2014				2,07	1,857	1,809	2,375	2,087	2,44		1	1	1	0,9988	1	0,9987
MIG 2016				1,398	1,114	1,063	1,602	1,288	1,602	0,9346		1	1	1	0,9998	1.00E+00
MOG 2016				1,585	1,32	1,268	1,833	1,519	1,863	0,7147	0,241		1	1	1	1.00E+00
ASC 2016				2,102	1,889	1,838	2,475	2,161	2,588	0,1049	0,9089	0,6679		0,9985	1	0,9983
HEVOO 2015				1,147	0,8265	0,7721	1,32	0,982	1,297	1,381	0,4248	0,6861	1,411		0,9927	1.00E+00
COR 2016				2,29	2,097	2,045	2,709	2,395	2,852	0,1172	1,152	0,9113	0,2433	1,675		0,9916
RAG 2016				1,194	0,8733	0,8176	1,394	1,045	1,39	1,393	0,4081	0,6789	1,43	0,03559	1,703	

Table 36. Significant differences (P < 0.05) between the classes of oils are indicated in pink

1-HEXANOL																
	OO 2015	OO 2014	COR 2016	MIG 2016	MOG 2016	LEVOO 2014	ASC 2016	RAG 2016	HEVOO 2014	LEVOO 2015	MOG 2015	COR 2015	ASC 2015	RAG 2015	MIG 2015	HEVOO 2015
OO 2015		1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9998	1.00E+00	1.00E+00	0,9709	0,9855	0,9608	0,8201	0,5862	0,6735	0,02196	0,0453
OO 2014	0,1266		1.00E+00	1.00E+00	1.00E+00	0,9999	1.00E+00	1.00E+00	0,9829	0,9929	0,9777	0,8688	0,6535	0,7387	0,02968	0,0609
COR 2016	0,5432	0,4064		1.00E+00	1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9948	0,9986	0,9929	0,9215	0,724	0,8065	0,02645	0,05402
MIG 2016	0,845	0,7082	0,3306		1.00E+00	1.00E+00	1.00E+00	1.00E+00	0,9993	0,9999	0,9992	0,9755	0,8546	0,9144	0,05312	0,1073
MOG 2016	0,8756	0,7351	0,3499	0,008383		1.00E+00	1.00E+00	1	0,9991	0,9999	0,9989	0,9685	0,8277	0,8946	0,03869	0,0784
LEVOO 2014	1,41	1.28E+03	0,9802	0,6784	0,6892		1.00E+00	1	1.00E+00	1.00E+00	1.00E+00	0,9999	0,9946	0,9986	0,34	0,5547
ASC 2016	1,136	0,9951	0,6363	0,2948	0,2965	0,4291		1	0,9999	1.00E+00	0,9999	0,9912	0,914	0,9568	0,06913	0,1382
RAG 2016	0,9269	0,7784	0,3762	0,01066	0,001755	0,7269	0,3173		0,9983	0,9997	0,9974	0,9449	0,7537	0,8351	0,01768	0,03463
HEVOO 2014	2,214	2,087	1,848	1,546	1,58	0,8034	1,32	1,669		1	1	1	1	1	0,7635	0,9305
LEVOO 2015	2,051	1,905	1,634	1,276	1,316	0,4224	1.00E+03	1,427	0,5053		1	1	0,9975	0,9996	0,2402	0,4315
MOG 2015	2,292	2,148	1,905	1,555	1,603	0,6924	1.30E+03	1,732	0,2185	0,3371		1	0,9998	1	0,4067	0,6514
COR 2015	2,827	2,69	2,501	2,171	2,234	1,303	1.95E+03	2,389	0,4355	1,079	0,7478		1	1	0,8416	0,9703
ASC 2015	3,328	3,195	3,05	2,733	2,807	1,855	2.53E+03	2,985	1,015	1,727	1,398	0,6469		1	0,9878	0,9997
RAG 2015	3,155	3,018	2,861	2,531	2,605	1,632	2.32E+03	2,787	0,764	1,469	1,13	0,3599	0,3012		0,9411	0,9954
MIG 2015	5,355	5,218	5,271	4,94	5,093	3,831	4.81E+03	5,451	2,963	4,083	3,685	2,769	2,014	2,409		1
HEVOO 2015	5,018	4,872	4,931	4,573	4,742	3,389	4,429	5,146	2,461	3,634	3,2	2,218	1,42	1,828	0,7852	

Table 37. Significant differences (P < 0.05) between the classes of oils are indicated in pink

2.3.6 Sensory analysis

A specific list was developed for the sensory descriptors of virgin olive oils. Some of positive attributes are:

- Fruity: it is one of the principle attribute of EVOOs, it is characteristic of the oils obtained from healthy, fresh olives, both ripe and unripe. The aroma of an oil obtained from unripe olives is generally characterized by an attribute of grass or leaves, while the oil obtained from ripe fruits is characterized by an aromatic flavor [51];
- Bitter: is the main flavor produced by diluted aqueous solutions of various substances such as quinine, caffeine and many alkaloids. It is the characteristic sensation of oils obtained from green olives or that are changing color. The presence of 1-penten-3-one is positively correlated with the bitterness in the mouth, while the (*Z*)-3-hexen-1-ol and the hexanal are negatively correlated [51]-[52];
- Spicy: characteristic sensation of the oils produced at the beginning of the harvest, mainly from unripe olives. A volatile compound positively correlated with this attribute is 1-penten-3-one, while the (*E*)-2-hexanal and the hexanal are negatively correlated [52].

Instead, the principal defects are:

- Moldy: *off-flavor* characteristic of the oil obtained from olives stored in thick piles or in jute bags for long periods before being subjected to the processing and subjected to an advanced stage of anaerobic fermentation [52]-[53]-[54];
- Moldy - moist: *off-flavor* characteristic of the oils obtained from fruits infested by fungi and yeasts as a result of preservation at low temperatures and high humidity. Fungi have the ability to oxidize free fatty acids to VOCs such as 2-heptanone and 2-nonanone. On the other hand, yeasts easily reduce carbonyl compounds [52]-[53]-[54]. Oils with these characteristics have a low concentration of (*E*)-2-hexenal and contain C8 VOCs and short chain fatty acids [54];
- Muddy sediment: *off-flavor* typically characteristic of oils left in contact with the sediment for a long time [52]-[53];
- Winey - acidic: odor due to the olives fermentation process which leads to the formation of acetic acid, ethyl acetate and ethanol. This smell is reminiscent of wine or vinegar [51]-[52]-[54];
- Metallic: *off-flavor* characteristic of oils left in contact with metal surfaces for a long time during the working or storage processes [53];

- Rancid: *off-flavor* characteristic of oils that have undergone oxidation. The main contributes are given by unsaturated aldehydes [52]-[53]-[54].

In the table below (Table 38), the selected common volatile substances with their sensorial attributes, olfactory threshold and literature references are reported.

Molecule	Sensorial attribute	Threshold (1g/Kg oil)	Literature references
Ethanol	Alcoholic	30000	Morales *et al*. (2005)
3-Pentanone	Sweet		Aparicio and Morales (1998)
1-penten-3-one	Green	50	Aparicio and Luna (2002)
	Spicy green	0,73	Reiners and Grosch (1998)
	Sweet, strawberry	-	Aparicio and Morales (1998)
1-Penten-3-ol	Wet ground	-	Aparicio and Morales (1998)
(*E*)-2-Hexenal	Green, apple	424	Reiners and Grosch (1998)
	Bitter, almond, green	420	Morales *et al*. (2005)
	Green astringent	1125	Aparicio and Luna (200)
Acetic acid, hexyl ester	Green, fruity, sweet	1040	Aparicio and Luna (2002) Baeten *et al*. (1998)
Hexanal	Green-sweet	75	Aparicio and Luna (2002)
	Green apple, grass	80	Morales *et al*. (2005)
	Green	300	Reiners and Grosch (1998)
(*Z*)-3-Hexen-1-ol acetate	-	-	-
(*Z*)-2-Penten-1-ol	Banana	-	Aparicio and Morales (1998)
1-Hexanol	Fruity, banana	400	Aparicio and Morales (1998)
	Undesirable	400	
(*Z*)-3-Hexen-1-ol	Banana	-	Aparicio and Morales (1998)
(*E*)-2-Hexen-ol	Green, leaves, grass	5000	Morales *et al*. (2005)
	Green, grass, sweet	8000	Aparicio and Morales (1998)

Table 38. Sensorial attribute associated to the volatile compound with their olfactory threshold and literature references

Anyway, in the table below (Table 39), the average sensorial values for each variety of oil are reported. In Figure 27 and Figure 28 are showed the total sensorial analysis score for all cultivar analyzed and for all cultivars in the two different years of production respectively.

VARIETY	VISUAL EXAM		OLFACTORY EXAM							GUSTATORY-TACTILE-RETROLFACTORY EXAM				
	Yellow	Green	Fruity	Leaves/Grass	Almond	Artichoke/Cardoon	Tomato	Apple	Berries	Bitter	Spicy	Sweet	Fluidity	Score
ASCOLANA TENERA 2015	4.9	3.4	5.3	2.9	2.1	2.3	2.6	0.0	0.0	4.8	4.7	2.6	5.2	7.8
ASCOLANA TENERA 2016	4.8	3.1	4.1	1.5	1.2	0.8	1.0	0.0	0.0	3.4	3.4	-	4.2	7.1
CORONCINA 2015	5.2	3.3	4.4	2.4	2.3	1.8	0.3	0.1	0.2	4.5	4.7	2.6	4.7	7.3
CORONCINA 2016	5.6	2.5	3.6	1.7	1.6	1.3	0.2	0.0	0.0	3.8	3.7	-	4.4	7.1
MIGNOLA 2015	6.7	1.5	4.2	1.2	1.4	0.6	0.0	0.0	2.0	4.2	3.8	2.9	4.7	7.1
MIGNOLA 2016	6.1	2.1	3.4	0.5	0.6	0.2	0.0	0.0	1.2	3.7	3.4	-	4.4	7.2
PIANTONE DI MOGLIANO 2015	5.0	3.1	4.2	2.1	1.8	1.3	0.2	0.1	0.2	3.7	4.1	3.0	4.6	7.1
PIANTONE DI MOGLIANO 2016	5.3	2.8	3.8	1.5	1.7	1.2	0.0	0.0	0.0	3.6	3.4	-	4.1	7.0
RAGGIA 2015	4.4	4.3	4.8	2.6	3.0	1.9	0.2	0.0	0.0	4.9	4.8	2.4	5.2	7.6
RAGGIA 2016	5.1	4.0	3.3	1.1	1.7	0.7	0.0	0.0	0.0	2.7	3.1	.	3.5	6.9

Table 39. Sensorial values for each cultivar

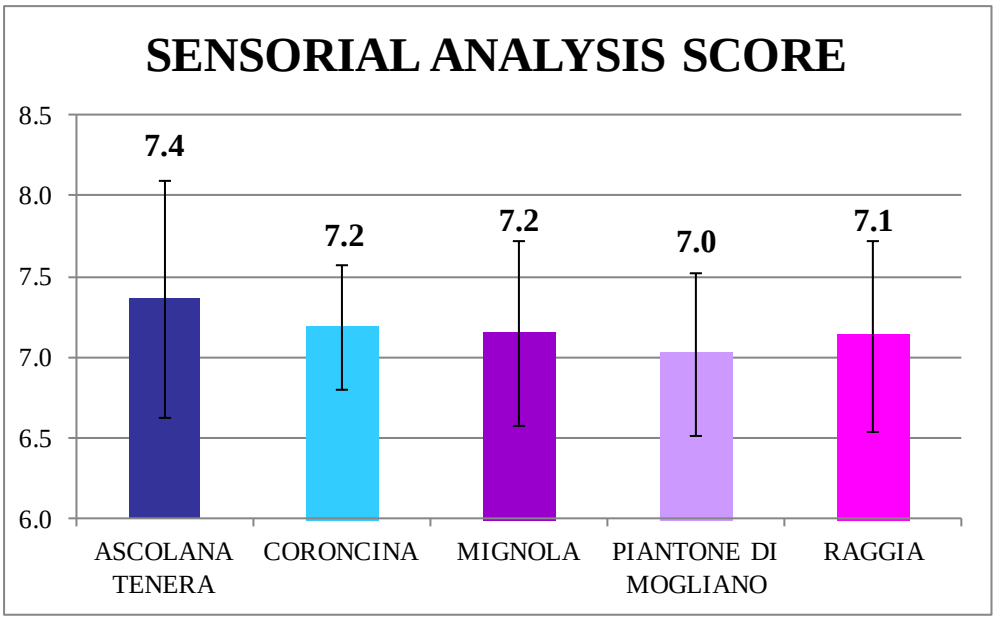


Figure 27. Average score for each cultivar examined.

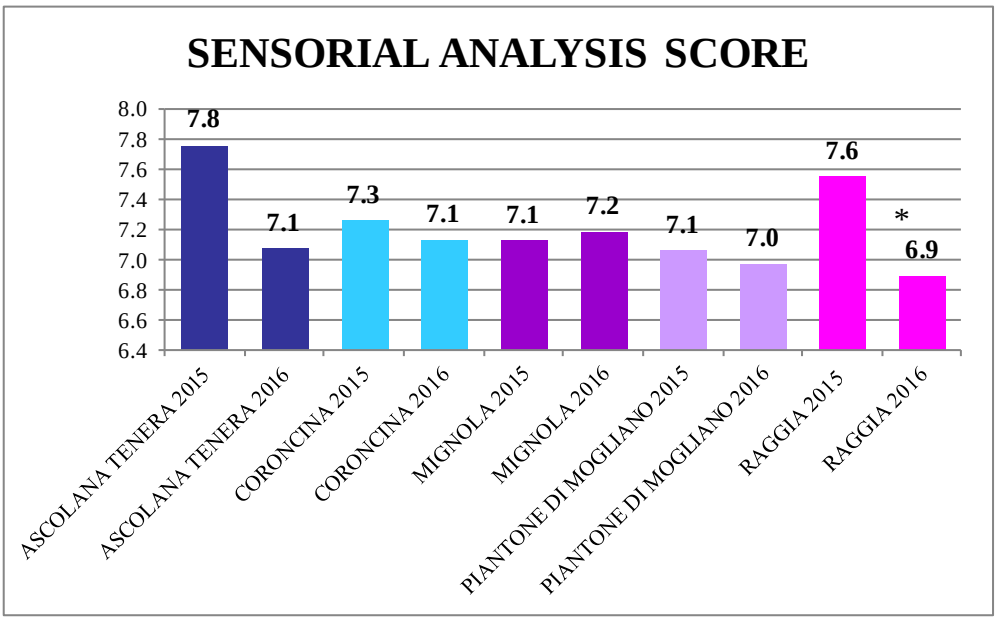


Figure 28. Average score for each cultivar examined in the two different year of production. Significant differences between the two examined years ($P < 0.05$, one-way ANOVA and Tukey's test for pairwise comparison) are indicated by "*"

Regarding the average total score obtained by the various cultivars in the sensory analysis, Ascolana Tenera has the highest score equal to 7.4, while Piantone di Mogliano is the cultivar with the lowest score equal to 7.0. Considering instead the two different years of production, it is possible to observe that all cultivars less than Mignola, received a lower score in 2016 than the previous year. This could be explained by worst pedo-climatic conditions that interferes with the

quality of olives. Anyway, considering Coroncina, Mignola and Piantone di Mogliano cultivars, this difference in scoring is minimal, while for the Ascolana Tenera and Raggia cultivars it is much more marked. In particular this difference is statistically significant ($P < 0.05$) for Raggia cultivar.

2.4 Conclusions

The results of the analyses showed that there are clear and important differences between MEVOOs and commercial oils. Furthermore, regarding commercial oils, significant differences in terms of chemical composition and sensory analysis have been highlighted between the two EVOOs classes investigated, indicating a generally lower level of quality of LEVOOs. Concerning MEVOO samples, significant differences ($P < 0.05$) were obtained in some case between the two considered years (2015 and 2016) of the same variety for the different analysis. Chemical and sensorial analyses, as a whole, have demonstrated and underline specific peculiarities of each cultivar analyzed. Considering fatty acids composition, oleic acid is a main strength of Piantone di Mogliano cultivar, which shows the highest average percentage content (77.55%). Stearic acid percentage resulted to be interesting, since it was significantly higher in LEVOOs thus seeming to be related, even if indirectly, to the oils quality. On the contrary, Piantone di Mogliano is the worst cultivar considering the vitamin E content; the chemical analysis of this parameter valorizes Mignola cultivar. Polar phenolic substances and volatile compounds show in both cases the superiority of Raggia cultivar respect the other varieties. Ascolana Tenera instead, is the best variety in terms of sensory analysis (also considering the two different years of production). In conclusion, it is possible to say that among all the cultivars investigated, overall, Raggia, Coroncina and Ascolana Tenera resulted to have a generally higher quality than Piantone di Mogliano and Mignola varieties. Principal Component Analysis (PCA) and other statistical elaborations of the overall results obtained are in progress to find and underline other possible correlations between the data obtained and the varieties examined.

Below, tables with the results related to every conducted analysis, for each single sample examined, are reported:

Table 40: Average acidity and peroxide value for commercial oils
Table 41: Average acidity and peroxide value for MEVOO oils
Table 42: Fatty acid composition for commercial oils
Table 43: Fatty acid composition for MEVOO oils
Table 44: Alkyl esters content in MEVOO oils
Table 45: α-tocopherol content for commercial oils
Table 46: α-tocopherol content for MEVOO oils
Table 47: Total polyphenols content for commercial oils
Table 48: Total polyphenols content for MEVOO oils
Table 49: Polyphenol compounds (in mg/kg) in commercial oils
Table 50: Polyphenol compounds (in mg/kg) in MEVOO oils
Table 51: Volatile compounds in commercial oils
Table 52: Volatile compounds in MEVOO oils
Table 53: Sensorial analysis results

Sample Name	Average acidity (%)	S.D.	R.S.D.%	Average peroxide value (mEq O_2/Kg)	S.D.	R.S.D.%
LEVOO.1-2014	0.38	0.00	0.00	8.60	0.00	0.00
LEVOO.2-2014	0.30	0.01	4.39	9.10	0.00	0.00
LEVOO.3-2014	0.26	0.01	3.65	9.60	0.28	2.95
LEVOO.4-2014	0.40	0.00	0.97	12.05	0.49	4.11
LEVOO.5-2014	0.48	0.00	0.19	12.75	0.21	1.66
HEVOO.1-2014	0.48	0.02	3.58	13.90	0.00	0.00
HEVOO.2-2014	0.27	0.00	1.62	9.60	0.00	0.00
HEVOO.3-2014	0.33	0.02	6.94	15.60	0.00	0.00
HEVOO.4-2014	0.52	0.01	1.40	10.80	0.00	0.00
HEVOO.5-2014	0.39	0.01	2.03	8.60	0.00	0.00
OO.1-2014	0.09	0.00	4.44	7.35	0.35	4.81
OO.2-2014	0.18	0.00	1.84	4.40	0.14	3.21
OO.3-2014	0.13	0.00	0.86	6.00	0.14	2.36
OO.4-2014	0.09	0.00	1.07	6.40	0.14	2.21
OO.5-2014	0.10	0.00	1.32	7.05	0.35	5.01
LEVOO.6-2015	0.56	0.00	0.00	25.56	0.17	0.66
LEVOO.7-2015	0.23	0.00	0.00	10.44	1.19	11.38
LEVOO.8-2015	0.25	0.00	0.00	9.96	0.85	8.52
LEVOO.9-2015	0.21	0.00	0.00	12.00	0.68	5.66
LEVOO.10-2015	0.54	0.02	3.72	19.56	0.17	0.87
HEVOO.6-2015	0.18	0.00	0.00	21.96	0.51	2.32
HEVOO.7-2015	0.18	0.00	0.00	14.04	0.17	1.21
HEVOO.8-2015	0.20	0.00	0.00	13.68	0.68	4.96
HEVOO.9-2015	0.26	0.01	3.82	22.80	1.70	7.44
HEVOO.10-2015	0.51	0.01	1.94	26.64	0.34	1.27
LEVOO.11-2015	0.42	0.03	6.53	13.00	0.00	0.00

LEVOO.12-2015	0.36	0.01	2.57	14.63	0.18	1.21
LEVOO.13-2015	0.43	0.01	2.11	11.50	0.35	3.07
LEVOO.14-2015	0.62	0.00	0.00	15.50	0.00	0.00
LEVOO.15-2015	0.52	0.00	0.00	10.00	0.00	0.00
HEVOO.11-2015	0.27	0.01	3.45	11.13	0.18	1.59
HEVOO.12-2015	0.45	0.01	2.05	10.75	0.35	3.29
HEVOO.13-2015	0.23	0.00	0.00	12.50	0.00	0.00
HEVOO.14-2015	0.50	0.01	1.84	5.88	0.18	3.01
HEVOO.15-2015	0.26	0.00	0.00	7.38	0.18	2.40
OO.6-2015	0.09	0.02	20.20	2.75	0.35	12.86
OO.7-2015	0.13	0.00	0.00	5.00	0.00	0.00
OO.8-2015	0.12	0.00	0.00	4.75	0.00	0.00
OO.9-2015	0.08	0.01	10.88	3.75	0.00	0.00
OO.10-2015	0.09	0.00	0.00	8.25	0.35	4.29

Table 40. Average acidity and peroxide value for commercial oils

Sample Name	Average acidity (%)	S.D.	R.S.D.%	Average peroxide value (mEq O_2/Kg)	S.D.	R.S.D.%
COR.102-2015	0.14	0.00	0.00	14.28	0.17	1.19
MOG.103-2015	0.14	0.00	0.00	12.36	0.17	1.37
MIG.104-2015	0.18	0.00	0.00	10.80	0.00	0.00
COR.105-2015	0.18	0.01	5.66	10.68	0.17	1.59
COR.106-2015	0.14	0.00	0.00	7.80	0.17	2.18
RAG.107-2015	0.21	0.00	0.00	12.00	0.00	0.00
RAG.108-2015	0.17	0.00	0.00	14.76	0.17	1.15
MOG.109-2015	0.14	0.00	0.00	13.80	0.17	1.23
MIG.110-2015	0.20	0.01	4.88	16.68	0.17	1.02
RAG.111-2015	0.21	0.00	0.00	8.76	0.51	5.81
MIG.112-2015	0.11	0.00	0.00	4.08	0.34	8.32
MOG.113-2015	0.13	0.01	7.44	4.44	0.17	3.82
RAG.114-2015	0.21	0.00	0.00	6.24	0.34	5.44
ASC.115-2015	0.28	0.00	0.00	10.80	0.34	3.14
ASC.116-2015	0.17	0.00	0.00	3.48	0.17	4.88
MIG.117-2015	0.23	0.00	0.00	8.04	0.17	2.11
COR.118-2015	0.16	0.01	6.15	15.60	0.34	2.18
COR.119-2015	0.20	0.00	0.00	14.40	0.00	0.00
MIG.120-2015	0.15	0.01	6.73	13.56	0.17	1.25
RAG.121-2015	0.21	0.00	0.00	6.24	0.00	0.00
MOG.122-2015	0.18	0.01	5.66	3.36	0.00	0.00
ASC.123-2015	0.28	0.00	0.00	5.52	0.00	0.00
ASC.124-2015	0.17	0.00	0.00	9.24	0.17	1.84
MIG.125-2015	0.18	0.01	5.66	10.92	0.17	1.55
RAG.126-2015	0.19	0.01	5.24	7.32	0.17	2.32
COR.127-2015	0.19	0.01	5.24	12.36	0.17	1.37
MOG.128-2015	0.17	0.00	0.00	10.80	0.34	3.14
MOG.129-2015	0.12	0.01	8.32	11.88	0.17	1.43

MOG.130-2015	0.14	0.00	0.00	16.92	0.17	1.00
MOG.131-2015	0.14	0.00	0.00	12.00	0.00	0.00
ASC.132-2015	0.14	0.00	0.00	9.60	0.00	0.00
RAG.136-2015	0.25	0.01	4.04	11.64	0.17	1.46
MOG.137-2015	0.20	0.01	4.88	8.52	0.17	1.99
MIG.138-2015	0.22	0.01	4.56	10.92	0.17	1.55
ASC.139-2015	0.20	0.01	4.88	7.32	0.17	2.32
COR.140-2015	0.20	0.00	0.00	11.64	0.17	1.46
ASC.141-2015	0.55	0.02	3.63	9.24	0.51	5.51
ASC. 143-2016	0.19	0.01	4.56	8.00	0.35	4.42
MIG. 144-2016	0.16	0.00	0.00	16.75	1.41	8.44
MOG. 145-2016	0.22	0.01	3.82	9.50	0.71	7.44
COR. 146-2016	0.25	0.01	3.45	9.00	0.00	0.00
ASC. 147-2016	0.38	0.01	2.24	14.00	0.00	0.00
ASC. 148-2016	0.30	0.00	0.00	26.38	0.18	0.67
ASC. 149-2016	0.59	0.01	1.46	21.13	0.88	4.18
ASC. 150-2016	0.44	0.00	0.00	30.50	0.35	1.16
RAG. 151-2016	0.19	0.00	0.00	11.38	0.18	1.55
COR. 152-2016	0.13	0.01	6.73	13.38	0.18	1.32
RAG. 153-2016	0.33	0.01	2.57	22.00	0.35	1.61
MIG. 154-2016	0.40	0.00	0.00	15.75	0.00	0.00
MOG. 155-2016	0.31	0.01	2.77	17.38	0.18	1.02
COR.156-2016	0.27	0.00	0.00	12.38	0.18	1.43
MIG. 157-2016	0.27	0.00	0.00	20.50	0.35	1.72
MOG.158-2016	0.37	0.01	2.32	13.63	0.18	1.30
RAG.159-2016	0.32	0.01	2.67	11.00	0.35	3.21
RAG.160-2016	0.27	0.00	0.00	20.50	0.35	1.72
RAG. 161-2016	0.17	0.00	0.00	28.38	0.18	0.62
MIG. 162-2016	0.19	0.01	4.56	6.25	0.00	0.00
RAG. 163-2016	0.36	0.00	0.00	14.50	0.00	0.00

RAG. 164-2016	0.49	0.00	0.00	23.00	0.00	0.00
RAG. 165-2016	0.95	0.00	0.00	23.88	0.18	0.74
RAG. 166-2016	0.24	0.00	0.00	19.75	0.00	0.00
ASC. 167-2016	0.21	0.01	4.04	15.13	0.18	1.17
COR. 168-2016	0.19	0.01	4.56	15.50	0.35	2.28
RAG. 169-2016	0.25	0.01	3.45	13.00	0.35	2.72
MOG. 170-2016	0.14	0.01	6.15	17.75	0.35	1.99
MIG. 171-2016	0.33	0.00	0.00	18.75	0.00	0.00
COR. 173-2016	0.18	0.00	0.00	12.38	0.18	1.43
ASC. 174-2016	0.24	0.01	3.63	21.63	0.18	0.82
RAG. 176-2016	0.30	0.00	0.00	30.75	0.00	0.00
MIG. 177-2016	0.31	0.01	2.77	11.13	0.18	1.59
MIG. 178-2016	0.17	0.02	10.10	35.25	0.00	0.00
COR. 180-2016	0.24	0.01	3.63	20.13	0.18	0.88
MOG. 181-2016	0.29	0.00	0.00	10.13	0.18	1.75
ASC. 182-2016	0.40	0.00	0.00	12.25	0.00	0.00
MOG. 186-2016	0.24	0.00	0.00	11.00	0.00	0.00
RAG. 187-2016	0.27	0.01	3.14	12.13	0.18	1.46
COR. 188-2016	0.27	0.00	0.00	18.75	0.00	0.00
MOG. 191-2016	0.16	0.00	0.00	6.00	0.35	5.89
MOG. 192-2016	0.24	0.00	0.00	12.63	0.18	1.40
MIG. 193-2016	0.19	0.00	0.00	22.38	0.18	0.79

Table 41. Average acidity and peroxide value in MEVOOs

SAMPLE NAME	AREA PERCENTAGE						
	PALMITIC ACID	PALMITOLEIC ACID	STEARIC ACID	OLEIC ACID	LINOLEIC ACID	LINOLENIC ACID	OLEIC/LINOLEIC
LEVOO.1-2014	13.88	1.00	2.63	74.43	7.48	0.58	9.95
LEVOO.2-2014	12.17	0.98	2.49	77.25	6.61	0.50	11.68
LEVOO.3-2014	12.02	0.64	3.29	77.99	5.47	0.59	14.25
LEVOO.4-2014	14.81	1.13	2.34	73.40	7.74	0.59	9.49
LEVOO.5-2014	16.69	1.75	2.67	65.64	12.63	0.62	5.20
HEVOO.1-2014	14.69	0.93	2.33	73.36	8.03	0.65	9.13
HEVOO.2-2014	14.73	0.91	2.05	74.45	7.22	0.64	10.31
HEVOO.3-2014	16.66	1.30	2.10	73.04	6.24	0.67	11.71
HEVOO.4-2014	12.09	0.53	2.01	78.19	6.57	0.62	11.90
HEVOO.5-2014	12.99	0.53	2.11	76.34	7.36	0.67	10.37
OO.1-2014	15.52	1.51	3.04	68.60	10.75	0.57	6.38
OO.2-2014	13.73	1.01	3.15	73.49	8.08	0.55	9.10
OO.3-2014	14.06	1.31	2.71	71.04	10.24	0.64	6.94
OO.4-2014	16.26	1.58	2.75	68.10	10.73	0.58	6.35
OO.5-2014	18.65	2.12	2.75	61.47	14.45	0.57	4.25
LEVOO.6-2015	12.43	0.81	3.04	74.97	8.02	0.73	9.34
LEVOO.7-2015	11.36	0.76	3.42	77.12	6.69	0.64	11.52
LEVOO.8-2015	11.58	0.76	3.24	78.25	5.50	0.67	14.23
LEVOO.9-2015	10.62	0.47	2.43	79.46	6.36	0.65	12.49
LEVOO.10-2015	13.66	1.09	2.63	69.07	12.93	0.63	5.34
HEVOO.6-2015	13.75	0.85	2.32	75.91	6.60	0.57	11.50
HEVOO.7-2015	13.52	1.03	2.14	74.03	8.80	0.47	8.41
HEVOO.8-2015	13.97	0.97	2.70	73.17	8.56	0.63	8.54
HEVOO.9-2015	12.12	0.74	2.58	77.20	6.73	0.63	11.46

HEVOO.10-2015	14.04	0.91	2.27	72.19	9.79	0.80	7.38
LEVOO.11-2015	11.04	0.83	2.96	77.16	7.27	0.74	10.62
LEVOO.12-2015	12.20	1.02	2.43	74.53	9.14	0.67	8.15
LEVOO.13-2015	12.33	1.06	2.65	74.59	8.64	0.73	8.64
LEVOO.14-2015	11.91	0.95	2.38	75.83	8.21	0.73	9.23
LEVOO.15-2015	12.99	1.08	2.14	73.84	9.20	0.75	8.03
HEVOO.11-2015	12.72	0.87	2.27	75.39	8.04	0.71	9.38
HEVOO.12-2015	12.16	0.92	2.77	76.00	7.52	0.63	10.11
HEVOO.13-2015	12.18	1.10	2.25	74.71	9.03	0.74	8.27
HEVOO.14-2015	11.14	0.72	2.76	73.50	11.19	0.69	6.57
HEVOO.15-2015	10.95	0.51	2.03	78.09	7.71	0.71	10.12
OO.6-2015	13.00	1.35	2.47	72.36	10.15	0.67	7.13
OO.7-2015	12.38	1.25	2.65	72.74	10.32	0.66	7.05
OO.8-2015	14.04	1.53	2.58	69.04	12.13	0.67	5.69
OO.9-2015	13.43	1.62	2.27	71.19	10.87	0.62	6.55
OO.10-2015	13.32	1.24	2.52	71.99	10.30	0.62	6.99

Table 42. Fatty acid composition for commercial oils

SAMPLE NAME	AREA PERCENTAGE						
	PALMITIC ACID	PALMITOLEIC ACID	STEARIC ACID	OLEIC ACID	LINOLEIC ACID	LINOLENIC ACID	OLEIC/LINOLEIC
COR.102-2015	11.79	0.85	2.21	76.52	8.00	0.63	9.57
MOG.103-2015	12.45	1.07	2.35	74.89	8.60	0.64	8.70
MIG.104-2015	13.19	1.31	2.23	73.51	9.20	0.57	7.99
COR.105-2015	12.54	0.82	2.36	76.24	7.41	0.63	10.29
COR.106-2015	11.84	0.63	2.23	76.53	8.18	0.58	9.36
RAG.107-2015	14.43	1.20	2.26	73.42	8.05	0.63	9.12
RAG.108-2015	12.31	0.70	2.14	77.20	7.02	0.64	11.00
MOG.109-2015	12.97	0.84	2.25	76.12	7.23	0.59	10.53
MIG.110-2015	14.10	1.40	2.13	72.11	9.53	0.73	7.57
RAG.111-2015	13.23	0.93	2.46	76.00	6.79	0.59	11.19
MIG.112-2015	14.78	1.94	1.79	71.23	9.59	0.68	7.43
MOG.113-2015	11.26	0.63	2.15	78.44	7.07	0.44	11.09
RAG.114-2015	10.62	0.29	2.66	80.17	5.54	0.72	14.48
ASC.115-2015	13.66	1.01	2.22	76.11	6.31	0.69	12.05
ASC.116-2015	13.91	0.97	2.24	75.30	6.98	0.61	10.79
MIG.117-2015	13.59	1.24	2.08	74.93	7.60	0.56	9.86
COR.118-2015	12.43	0.80	2.23	76.72	7.18	0.65	10.69
COR.119-2015	12.48	0.73	2.42	73.90	9.82	0.65	7.52
MIG.120-2015	13.85	1.34	2.09	72.95	9.17	0.59	7.96
RAG.121-2015	13.96	0.89	2.45	74.54	7.56	0.61	9.85
MOG.122-2015	12.26	0.76	2.56	77.13	6.60	0.70	11.69
ASC.123-2015	14.88	1.26	2.22	75.44	5.49	0.71	13.74
ASC.124-2015	13.54	0.81	2.53	76.05	6.43	0.64	11.82
MIG.125-2015	13.05	1.38	2.22	73.21	9.65	0.49	7.58
RAG.126-2015	12.88	0.94	2.36	75.16	8.04	0.63	9.35

COR.127-2015	12.78	0.74	2.37	76.89	6.62	0.61	11.62
MOG.128-2015	12.36	0.72	2.42	77.52	6.41	0.57	12.09
MOG.129-2015	12.71	0.71	2.51	76.91	6.52	0.64	11.80
MOG.130-2015	12.03	0.77	2.37	77.83	6.27	0.72	12.41
MOG.131-2015	10.69	0.64	2.49	79.07	6.48	0.64	12.21
ASC.132-2015	14.57	1.20	2.39	74.23	6.99	0.62	10.62
RAG.136-2015	13.07	0.76	2.50	76.44	6.67	0.56	11.46
MOG.137-2015	11.10	0.58	2.67	79.35	5.76	0.55	13.79
MIG.138-2015	15.08	1.59	2.15	71.19	9.46	0.53	7.53
ASC.139-2015	13.81	1.17	2.11	76.45	5.93	0.53	12.90
COR.140-2015	12.22	0.71	2.53	76.09	7.89	0.56	9.64
ASC.141-2016	14.64	1.31	2.15	75.24	5.85	0.80	12.87
ASC.143-2016	16.12	1.15	1.97	73.10	6.87	0.78	10.64
MIG.144-2016	15.94	2.01	1.47	69.24	10.80	0.53	6.41
MOG.145-2016	12.07	0.76	1.97	78.31	6.39	0.49	12.25
COR.146-2016	11.86	0.63	1.97	77.61	7.43	0.50	10.45
ASC.147-2016	14.44	1.05	1.79	75.35	6.68	0.70	11.29
ASC.148-2016	12.93	1.14	1.63	77.07	6.43	0.80	11.99
ASC.149-2016	13.12	1.41	1.62	76.34	6.67	0.84	11.45
ASC.150-2016	14.34	0.90	1.71	74.43	7.96	0.66	9.35
RAG.151-2016	12.79	0.76	1.91	77.81	6.18	0.56	12.60
COR.152-2016	13.81	0.91	1.97	74.29	8.39	0.62	8.86
RAG.153-2016	13.81	1.04	1.82	75.71	6.99	0.64	10.83
MIG.154-2016	14.83	1.37	1.70	72.65	8.92	0.53	8.14
MOG.155-2016	12.71	0.84	1.72	78.09	6.04	0.60	12.92
COR.156-2016	14.07	0.96	2.20	73.43	8.73	0.61	8.41
MIG.157-2016	15.57	1.61	1.84	71.49	8.95	0.53	7.99
MOG.158-2016	13.13	0.87	2.01	77.23	6.24	0.51	12.37

RAG.159-2016	14.94	1.12	1.81	74.47	7.11	0.54	10.48
RAG.160-2016	11.54	0.33	2.02	79.18	6.25	0.68	12.67
RAG.161-2016	13.86	0.80	2.37	75.43	6.86	0.68	11.00
MIG.162-2016	15.30	1.67	1.78	70.91	9.93	0.42	7.14
RAG.163-2016	13.36	0.91	1.81	75.94	7.37	0.61	10.31
RAG.164-2016	13.98	0.99	1.88	75.28	7.22	0.65	10.43
RAG.165-2016	14.04	1.05	1.80	74.44	8.04	0.64	9.26
RAG.166-2016	13.05	0.94	1.87	77.20	6.40	0.55	12.07
ASC.167-2016	14.15	1.19	1.83	75.24	7.02	0.58	10.72
COR.168-2016	14.31	1.02	1.83	73.38	8.82	0.64	8.32
RAG.169-2016	14.35	1.12	1.66	74.21	8.00	0.66	9.28
MOG.170-2016	13.11	1.01	1.72	76.00	7.46	0.69	10.19
MIG.171-2016	15.29	1.58	1.65	70.78	10.06	0.64	7.04
COR.173-2016	13.59	0.94	1.69	73.58	9.56	0.64	7.69
ASC.174-2016	14.22	1.24	1.78	75.07	7.11	0.57	10.56
RAG.176-2016	13.97	0.98	1.92	74.57	8.08	0.47	9.23
MIG.177-2016	15.42	1.74	1.70	71.26	9.37	0.52	7.61
MIG.178-2016	15.24	1.64	1.72	71.77	9.12	0.52	7.87
COR.180-2016	14.40	0.94	2.09	72.68	9.26	0.64	7.85
MOG.181-2016	12.37	0.85	1.80	77.97	6.51	0.50	11.97
ASC.182-2016	12.93	0.99	1.69	77.84	6.06	0.48	12.86
MOG.186-2016	12.15	0.74	1.84	78.26	6.45	0.56	12.14
RAG.187-2016	12.69	0.83	1.98	77.63	6.31	0.55	12.29
COR.188-2016	13.62	0.99	1.91	74.72	8.19	0.57	9.12
MOG.191-2016	10.60	0.22	2.34	80.66	5.53	0.65	14.59
MOG.192-2016	13.66	0.73	2.73	74.51	7.77	0.60	9.59
MIG.193-2016	14.67	1.65	1.89	73.02	8.38	0.39	8.71

Table 43. Fatty acid composition in MEVOOs

SAMPLE NAME	mg kg^{-1}		
	FAMEs	**FAEEs**	**Σ (FAME + FAEE)**
COR.102-2015	4	3	6
MOG.103-2015	3	3	6
MIG.104-2015	6	10	15
COR.105-2015	4	4	7
COR.106-2015	2	3	5
RAG.107-2015	5	4	9
RAG.108-2015	3	3	6
MOG.109-2015	3	2	5
MIG.110-2015	3	6	9
RAG.111-2015	2	2	4
MIG.112-2015	1	2	3
MOG.113-2015	2	2	4
RAG.114-2015	9	3	12
ASC.115-2015	11	7	19
ASC.116-2015	3	2	5
MIG.117-2015	6	7	14
COR.118-2015	3	2	5
COR.119-2015	5	2	7
MIG.120-2015	2	2	5
RAG.121-2015	2	2	4
MOG.122-2015	2	2	3
ASC.123-2015	3	2	5
ASC.124-2015	7	2	9
MIG.125-2015	2	2	4
RAG.126-2015	3	2	5

COR.127-2015	6	5	10
MOG.128-2015	4	4	8
MOG.129-2015	3	1	4
MOG.130-2015	5	2	6
MOG.131-2015	5	3	8
ASC.132-2015	2	1	2
RAG.136-2015	3	1	4
MOG.137-2015	2	1	3
MIG.138-2015	4	1	5
ASC.139-2015	3	2	5
COR.140-2015	7	4	12
ASC.141-2015	66	613	679
ASC.143-2016	2	1	3
MIG.144-2016	2	2	4
MOG.145-2016	5	3	8
COR.146-2016	10	4	13
ASC.147-2016	6	5	11
ASC.148-2016	6	3	8
ASC.149-2016	8	7	15
ASC.150-2016	18	9	27
RAG.151-2016	2	4	5
COR.152-2016	2	1	4
RAG.153-2016	4	9	13
MIG.154-2016	6	10	16
MOG.155-2016	11	21	32
COR.156-2016	10	7	17
MIG.157-2016	7	10	17
MOG.158-2016	16	17	33

RAG.159-2016	6	7	13
RAG.160-2016	5	5	10
RAG.161-2016	8	2	10
MIG.162-2016	3	4	6
RAG.163-2016	7	7	14
RAG.164-2016	9	14	23
RAG.165-2016	25	15	40
RAG.166-2016	3	4	8
ASC.167-2016	4	2	6
COR.168-2016	3	2	5
RAG.169-2016	8	5	13
MOG.170-2016	3	2	5
MIG.171-2016	9	9	18
COR.173-2016	4	1	5
ASC.174-2016	3	5	8
RAG.176-2016	4	4	8
MIG.177-2016	7	10	17
MIG.178-2016	4	7	10
COR.180-2016	10	8	18
MOG.181-2016	6	7	12
ASC.182-2016	7	10	17
MOG.186-2016	6	6	12
RAG.187-2016	6	8	14
COR.188-2016	12	7	19
MOG.191-2016	2	2	4
MOG.192-2016	13	9	22
MIG.193-2016	3	3	6

Table 44. Alkyl esters content in MEVOOs

SAMPLE NAME	mg kg^{-1}
LEVOO.1-2014	159.21
LEVOO.2-2014	211.56
LEVOO.3-2014	134.98
LEVOO.4-2014	213.73
LEVOO.5-2014	230.69
HEVOO.1-2014	190.02
HEVOO.2-2014	190.62
HEVOO.3-2014	149.61
HEVOO.4-2014	158.69
HEVOO.5-2014	169.51
OO.1-2014	144.67
OO.2-2014	122.95
OO.3-2014	124.08
OO.4-2014	128.40
OO.5-2014	181.71
LEVOO.6-2015	161.74
LEVOO.7-2015	243.97
LEVOO.8-2015	262.39
LEVOO.9-2015	291.14
LEVOO.10-2015	307.06
HEVOO.6-2015	257.16
HEVOO.7-2015	218.08
HEVOO.8-2015	251.99
HEVOO.9-2015	255.70

HEVOO.10-2015	267.86
LEVOO.11-2015	216.13
LEVOO.12-2015	237.65
LEVOO.13-2015	222.51
LEVOO.14-2015	219.60
LEVOO.15-2015	239.05
HEVOO.11-2015	223.49
HEVOO.12-2015	223.55
HEVOO.13-2015	247.07
HEVOO.14-2015	181.97
HEVOO.15-2015	236.01
OO.6-2015	147.57
OO.7-2015	108.19
OO.8-2015	125.33
OO.9-2015	132.14
OO.10-2015	179.24

Table 45. α-tocopherol content for commercial oils

SAMPLE NAME	mg kg^{-1}
COR.102-2015	232.51
MOG.103-2015	227.05
MIG.104-2015	245.31
COR.105-2015	219.44
COR.106-2015	248.95
RAG.107-2015	240.21
RAG.108-2015	223.59
MOG.109-2015	202.22
MIG.110-2015	309.78
RAG.111-2015	177.64
MIG.112-2015	309.16
MOG.113-2015	122.11
RAG.114-2015	282.44
ASC.115-2015	261.06
ASC.116-2015	280.71
MIG.117-2015	286.24
COR.118-2015	229.74
COR.119-2015	249.99
MIG.120-2015	299.75
RAG.121-2015	225.67
MOG.122-2015	235.43
ASC.123-2015	314.89
ASC.124-2015	259.16
MIG.125-2015	308.48
RAG.126-2015	235.02

COR.127-2015	207.93
MOG.128-2015	168.30
MOG.129-2015	234.76
MOG.130-2015	231.38
MOG.131-2015	175.22
ASC.132-2015	296.54
RAG.136-2015	148.22
MOG.137-2015	174.79
MIG.138-2015	287.72
ASC.139-2015	209.66
COR.140-2015	238.48
ASC.141-2015	474.46
ASC. 143-2016	408.33
MIG. 144-2016	390.74
MOG. 145-2016	262.89
COR. 146-2016	316.07
ASC. 147-2016	397.46
ASC. 148-2016	418.04
ASC. 149-2016	483.42
ASC. 150-2016	336.98
RAG. 151-2016	267.54
COR. 152-2016	387.67
RAG. 153-2016	265.46
MIG. 154-2016	372.08
MOG. 155-2016	215.10
COR.156-2016	421.11
MIG. 157-2016	438.12
MOG.158-2016	256.01

RAG.159-2016	231.70
RAG.160-2016	313.83
RAG. 161-2016	473.13
MIG. 162-2016	376.56
RAG. 163-2016	282.22
RAG. 164-2016	301.80
RAG. 165-2016	284.38
RAG. 166-2016	255.51
ASC. 167-2016	348.51
COR. 168-2016	330.09
RAG. 169-2016	318.81
MOG. 170-2016	308.44
MIG. 171-2016	371.83
COR. 173-2016	358.55
ASC. 174-2016	329.85
RAG. 176-2016	223.73
MIG. 177-2016	414.97
MIG. 178-2016	459.94
COR. 180-2016	396.63
MOG. 181-2016	208.22
ASC. 182-2016	279.32
MOG. 186-2016	223.65
RAG. 187-2016	221.82
COR. 188-2016	336.23
MOG. 191-2016	317.82
MOG. 192-2016	233.03
MIG. 193-2016	353.82

Table 46. α-tocopherol content in MEVOOs

SAMPLE NAME	Concentration (ppm) in to the oil (µg of gallic acid/g of oil)
LEVOO.1-2014	68.27
LEVOO.2-2014	119.64
LEVOO.3-2014	79.37
LEVOO.4-2014	77.35
LEVOO.5-2014	67.26
HEVOO.1-2014	99.33
HEVOO.2-2014	73.10
HEVOO.3-2014	56.40
HEVOO.4-2014	75.20
HEVOO.5-2014	99.60
OO.1-2014	16.15
OO.2-2014	9.43
OO.3-2014	18.38
OO.4-2014	11.66
OO.5-2014	5.12
LEVOO.6-2015	85.59
LEVOO.7-2015	147.76
LEVOO.8-2015	158.23
LEVOO.9-2015	226.05
LEVOO.10-2015	180.27
HEVOO.6-2015	153.67
HEVOO.7-2015	112.20
HEVOO.8-2015	182.31
HEVOO.9-2015	150.45

HEVOO.10-2015	116.25
LEVOO.11-2015	133.29
LEVOO.12-2015	128.84
LEVOO.13-2015	169.98
LEVOO.14-2015	93.31
LEVOO.15-2015	164.79
HEVOO.11-2015	152.66
HEVOO.12-2015	122.17
HEVOO.13-2015	180.60
HEVOO.14-2015	167.44
HEVOO.15-2015	159.15
OO.6-2015	42.54
OO.7-2015	48.93
OO.8-2015	43.51
OO.9-2015	21.89
OO.10-2015	28.98

Table 47. Total polyphenols content for commercial oils

SAMPLE NAME	Concentration (ppm) in to the oil (µg of gallic acid/g of oil)
COR.102-2015	171.49
MOG.103-2015	147.22
MIG.104-2015	223.13
COR.105-2015	235.03
COR.106-2015	156.32
RAG.107-2015	162.97
RAG.108-2015	105.75
MOG.109-2015	165.68
MIG.110-2015	129.51
RAG.111-2015	202.49
MIG.112-2015	159.57
MOG.113-2015	204.51
RAG.114-2015	154.80
ASC.115-2015	191.46
ASC.116-2015	265.26
MIG.117-2015	185.10
COR.118-2015	172.95
COR.119-2015	214.78
MIG.120-2015	182.02
RAG.121-2015	217.89
MOG.122-2015	250.29
ASC.123-2015	170.44
ASC.124-2015	214.11
MIG.125-2015	207.88
RAG.126-2015	198.09

COR.127-2015	198.29
MOG.128-2015	142.06
MOG.129-2015	106.43
MOG.130-2015	95.54
MOG.131-2015	108.78
ASC.132-2015	189.76
RAG.136-2015	304.23
MOG.137-2015	253.71
MIG.138-2015	406.92
ASC.139-2015	313.24
COR.140-2015	297.78
ASC.141-2015	107.36
ASC.143-2016	469.35
MIG.144-2016	239.03
MOG.145-2016	450.65
COR.146-2016	360.32
ASC.147-2016	312.58
ASC.148-2016	256.45
ASC.149-2016	343.55
ASC.150-2016	326.13
RAG.151-2016	374.52
COR.152-2016	96.45
RAG.153-2016	367.42
MIG.154-2016	219.68
MOG.155-2016	282.26
COR.156-2016	482.26
MIG.157-2016	524.19
MOG.158-2016	364.19

RAG.159-2016	452.58
RAG.160-2016	649.35
RAG.161-2016	453.23
MIG.162-2016	630.65
RAG.163-2016	417.74
RAG.164-2016	248.06
RAG.165-2016	184.19
RAG.166-2016	395.81
ASC.167-2016	535.16
COR.168-2016	181.61
RAG.169-2016	329.35
MOG.170-2016	255.81
MIG.171-2016	173.87
COR.173-2016	183.55
ASC.174-2016	360.97
RAG.176-2016	250.65
MIG.177-2016	253.87
MIG.178-2016	390.00
COR.180-2016	411.29
MOG.181-2016	351.94
ASC.182-2016	338.39
MOG.186-2016	439.68
RAG.187-2016	490.65
COR.188-2016	294.52
MOG.191-2016	310.00
MOG.192-2016	319.68
MIG.193-2016	336.45

Table 48. Total polyphenols content in MEVOOs

SAMPLE NAME	HYDROXYTYROSOL R.T.=5.3 min (280λ)	TYROSOL R.T.=7.6 min (280λ)	CAFFEIC ACID R.T.=9.9 min (325λ)	VANILLIC ACID R.T.=10.7min (260λ)	*p*-COUMARIC ACID R.T.=15.5min (310λ)	FERULIC ACID R.T.=17.25min (325λ)	LUTEOLIN R.T.=34.8min (350λ)	APIGENIN R.T.=41.55min (338λ)	SECOIRIDOID DERIVATIVES R.T.=20-40min (280λ)	PINORESINOL R.T.=33.5min (280λ)	ACETOXYPINORESINOL R.T.=34.5min (280λ)
LEVOO.1-2014	6.57	8.14	0.00	0.15	0.14	0.00	1.59	0.72	238.28	0.10	18.01
LEVOO.2-2014	13.52	10.82	0.00	0.23	0.19	0.04	2.77	0.89	315.22	8.11	25.86
LEVOO.3-2014	10.09	12.06	0.00	0.19	0.19	0.04	1.92	0.85	244.26	6.52	9.63
LEVOO.4-2014	11.81	13.81	0.00	0.28	0.16	0.05	2.17	1.01	258.24	8.24	23.98
LEVOO.5-2014	8.36	8.50	0.00	0.18	0.26	0.05	1.77	0.83	197.04	5.26	13.14
HEVOO.1-2014	9.04	10.85	0.00	0.39	0.17	0.05	2.19	0.78	308.23	9.31	28.41
HEVOO.2-2014	7.41	12.43	0.00	0.19	0.10	0.04	2.20	1.22	279.73	8.20	31.52
HEVOO.3-2014	6.53	7.46	0.00	0.27	0.14	0.06	2.89	0.92	201.97	9.40	31.83
HEVOO.4-2014	6.24	15.92	0.00	0.17	0.08	0.02	1.07	0.81	322.50	8.65	32.74
HEVOO.5-2014	17.39	24.45	0.00	0.24	0.11	0.04	1.56	0.94	390.82	8.97	40.64
OO.1-2014	0.92	1.27	0.00	0.00	0.03	0.00	0.14	0.15	20.26	0.55	1.10
OO.2-2014	0.68	0.75	0.00	0.00	0.03	0.00	0.10	0.07	21.54	1.31	1.43
OO.3-2014	1.86	2.09	0.00	0.00	0.03	0.00	0.28	0.17	38.18	1.30	2.47
OO.4-2014	0.94	1.19	0.00	0.00	0.00	0.00	0.18	0.13	32.97	1.05	1.70
OO.5-2014	0.69	1.27	0.00	0.00	0.04	0.00	0.10	0.06	24.31	0.62	0.71
LEVOO.6-2015	12.46	12.80	0.00	0.15	0.09	0.02	1.25	0.63	101.08	2.61	5.39
LEVOO.7-2015	7.37	8.77	0.00	0.18	0.18	0.03	2.14	0.74	216.24	3.18	5.99
LEVOO.8-2015	7.85	6.36	0.00	0.19	0.16	0.04	1.97	0.82	248.76	7.29	7.42
LEVOO.9-2015	5.40	6.32	0.00	0.25	0.12	0.03	2.16	1.48	402.18	2.18	17.75
LEVOO.10-2015	11.35	11.49	0.00	0.11	0.15	0.04	2.29	1.20	266.18	3.20	10.03
HEVOO.6-2015	4.24	6.54	0.00	0.36	0.05	0.06	3.97	1.89	274.22	3.30	28.31
HEVOO.7-2015	4.60	5.78	0.00	0.24	0.11	0.04	2.29	0.84	152.67	3.87	19.77
HEVOO.8-2015	18.69	17.51	0.00	0.26	0.20	0.07	3.37	1.25	338.62	2.89	14.39
HEVOO.9-2015	9.25	12.13	0.00	0.47	0.13	0.09	2.65	1.21	237.26	3.60	14.47
HEVOO.10-2015	15.84	21.18	0.07	0.53	0.25	0.07	3.37	1.25	151.82	10.31	6.73

LEVOO.11-2015	18.43	17.55	0.00	0.26	0.14	0.03	2.10	1.02	242.05	6.03	11.49
LEVOO.12-2015	12.88	13.35	0.00	0.41	0.16	0.05	2.39	1.17	235.70	6.14	15.95
LEVOO.13-2015	13.81	14.57	0.00	0.27	0.19	0.04	1.99	1.10	209.62	6.26	12.56
LEVOO.14-2015	25.40	34.94	0.00	0.40	0.15	0.05	3.06	1.62	146.80	7.96	15.83
LEVOO.15-2015	18.48	13.92	0.01	0.38	0.17	0.05	3.24	1.48	342.04	7.35	20.66
HEVOO.11-2015	19.93	17.84	0.02	0.71	0.13	0.06	2.78	1.15	257.15	5.65	18.45
HEVOO.12-2015	8.59	7.82	0.00	0.34	0.15	0.03	1.78	0.73	279.84	5.77	15.51
HEVOO.13-2015	12.82	13.53	0.00	0.55	0.22	0.04	3.08	1.31	322.03	6.94	20.95
HEVOO.14-2015	34.95	28.73	0.03	0.25	0.20	0.08	1.59	0.71	240.63	11.30	16.59
HEVOO.15-2015	3.40	5.20	0.00	0.30	0.07	0.03	1.56	1.15	366.25	5.11	26.64
OO.6-2015	1.58	1.76	0.00	0.02	0.01	0.00	0.04	0.03	15.88	0.47	0.66
OO.7-2015	1.44	1.83	0.00	0.03	0.02	0.00	0.08	0.09	4.75	0.97	0.93
OO.8-2015	3.52	3.51	0.00	0.03	0.03	0.00	0.21	0.11	25.80	0.83	1.02
OO.9-2015	1.35	1.34	0.00	0.01	0.01	0.00	0.05	0.03	17.33	0.33	0.44
OO.10-2015	2.85	2.18	0.00	0.02	0.02	0.00	0.10	0.07	17.50	0.60	0.84

Table 49. Polyphenol compounds (in mg/kg) in commercial oils

SAMPLE NAME	HYDROXYTYROSOL R.T.=5.3 min (280λ)	TYROSOL R.T.=7.6 min (280λ)	CAFFEIC ACID R.T.=9.9 min (325λ)	VANILLIC ACID R.T.=10.7min (260λ)	*p*-COUMARIC ACID R.T.=15.5min (310λ)	FERULIC ACID R.T.=17.25min (325λ)	LUTEOLIN R.T.=34.8min (350λ)	APIGENIN R.T.=41.55min (338λ)	SECOIRIDOID DERIVATIVES R.T.=20-40min (280λ)	PINORESINOL R.T.=33.5min (280λ)	ACETOXYPINORESINOL R.T.=34.5min (280λ)
COR.102-2015	3.18	4.50	0.00	0.38	0.08	0.04	3.67	1.21	173.83	4.69	8.20
MOG.103-2015	5.29	7.01	0.00	0.33	0.13	0.04	3.44	1.15	228.77	5.86	6.49
MIG.104-2015	3.40	21.28	0.00	0.54	0.12	0.03	2.33	0.44	237.92	6.97	9.03
COR.105-2015	2.72	2.56	0.08	0.56	0.16	0.05	4.69	1.82	370.81	6.45	2.77
COR.106-2015	4.85	5.95	0.00	0.11	0.08	0.01	2.05	1.07	264.85	3.47	3.63
RAG.107-2015	5.93	5.95	0.00	0.12	0.03	0.02	4.28	1.33	347.41	4.30	25.23
RAG.108-2015	11.71	12.64	0.00	0.33	0.05	0.05	0.74	1.33	175.13	4.59	26.77
MOG.109-2015	1.80	6.48	0.00	0.24	0.08	0.06	1.67	0.95	231.18	5.16	4.49
MIG.110-2015	9.28	8.00	0.00	0.39	0.08	0.05	2.46	0.64	258.12	7.30	10.52
RAG.111-2015	4.74	5.34	0.00	0.31	0.04	0.04	4.69	1.51	598.14	4.00	42.85
MIG.112-2015	8.71	4.90	0.00	0.40	0.05	0.02	2.43	0.47	268.83	7.45	6.01
MOG.113-2015	2.53	3.28	0.00	0.30	0.11	0.08	2.41	1.46	369.92	7.91	2.44
RAG.114-2015	9.79	6.73	0.00	0.43	0.09	0.06	2.05	1.03	583.59	3.57	25.44
ASC.115-2015	4.69	5.47	0.00	0.45	0.12	0.04	1.81	0.62	258.81	23.56	2.44
ASC.116-2015	4.56	5.80	0.00	0.39	0.07	0.05	2.22	1.25	380.04	9.50	5.86
MIG.117-2015	9.78	9.33	0.00	0.96	0.10	0.06	1.67	0.44	220.31	5.29	8.82
COR.118-2015	2.52	3.61	0.00	0.08	0.08	0.00	2.37	1.13	266.56	4.24	3.68
COR.119-2015	7.12	6.31	0.00	0.45	0.15	0.04	4.71	1.44	409.85	3.75	5.76
MIG.120-2015	6.93	6.17	0.00	0.39	0.09	0.02	2.28	0.55	228.82	4.49	8.63
RAG.121-2015	5.07	5.77	0.00	0.58	0.07	0.07	3.10	1.15	445.68	3.63	44.95
MOG.122-2015	2.85	3.71	0.00	0.62	0.13	0.09	1.36	1.10	400.72	5.39	5.83
ASC.123-2015	5.12	5.68	0.00	0.10	0.11	0.01	1.52	0.38	334.64	9.44	0.00
ASC.124-2015	5.40	4.16	0.00	0.29	0.13	0.03	3.08	1.15	282.25	9.07	3.74
MIG.125-2015	4.14	2.67	0.00	0.23	0.04	0.00	1.21	0.24	224.20	5.04	5.81
RAG.126-2015	5.08	5.91	0.00	0.38	0.05	0.06	2.29	1.04	264.39	3.81	33.11
COR.127-2015	3.39	5.78	0.00	0.37	0.06	0.07	2.03	0.82	405.11	7.26	26.43

MOG.128-2015	1.42	4.37	0.00	0.25	0.07	0.05	0.96	0.79	259.21	10.36	16.09
MOG.129-2015	1.11	5.64	0.00	0.69	0.14	0.12	0.76	0.82	149.71	7.99	2.60
MOG.130-2015	0.76	5.61	0.00	0.78	0.15	0.09	0.22	0.34	106.45	6.96	1.88
MOG.131-2015	1.37	5.00	0.00	0.33	0.13	0.08	1.22	0.87	117.01	12.16	3.92
ASC.132-2015	3.41	3.19	0.00	0.23	0.04	0.03	2.82	0.99	305.49	12.05	2.96
RAG.136-2015	5.18	5.68	0.00	0.26	0.05	0.04	4.42	1.36	593.92	2.93	49.98
MOG.137-2015	4.28	4.85	0.00	0.57	0.19	0.09	1.41	0.97	370.99	6.89	4.04
MIG.138-2015	19.01	12.34	0.00	0.51	0.13	0.00	2.30	0.67	536.82	8.85	8.10
ASC.139-2015	9.60	11.69	0.00	0.53	0.11	0.00	2.34	1.04	558.15	11.34	20.87
COR.140-2015	4.60	3.97	0.00	0.28	0.16	0.00	3.15	1.28	490.32	3.16	11.53
ASC.141-2015	2.84	6.60	0.08	0.51	0.20	0.05	1.59	0.52	274.94	3.49	19.57
ASC.143-2016	4.99	3.28	0.00	0.48	0.12	0.05	3.75	0.94	460.31	10.34	1.95
MIG.144-2016	7.25	4.48	0.02	0.55	0.05	0.03	1.67	0.36	285.75	10.92	7.13
MOG.145-2016	5.46	6.74	0.21	0.39	0.21	0.09	2.11	0.75	570.23	9.78	14.91
COR.146-2016	8.39	9.38	0.04	0.22	0.15	0.04	3.61	0.94	560.56	5.83	15.26
ASC.147-2016	6.17	6.83	0.02	0.32	0.11	0.08	2.91	1.03	421.67	18.95	7.80
ASC.148-2016	1.28	5.62	0.08	0.09	0.03	0.02	1.65	0.65	177.45	20.65	3.55
ASC.149-2016	3.76	4.84	0.03	0.17	0.11	0.05	1.46	0.47	315.80	43.74	1.59
ASC.150-2016	10.97	15.20	0.02	0.62	0.17	0.07	3.52	0.99	305.63	16.56	7.08
RAG.151-2016	2.06	3.90	0.15	0.71	0.06	0.09	5.81	1.41	354.87	6.96	45.44
COR.152-2016	3.56	3.42	0.06	0.45	0.14	0.06	4.17	1.40	354.30	7.41	1.66
RAG.153-2016	7.72	15.06	0.09	0.19	0.04	0.04	4.15	1.20	305.84	7.71	31.92
MIG.154-2016	4.19	2.99	0.08	0.13	0.11	0.06	2.94	1.19	236.83	12.84	20.99
MOG.155-2016	1.09	3.24	0.15	0.05	0.05	0.00	0.71	0.52	206.76	16.70	1.68
COR.156-2016	4.74	4.72	0.11	0.22	0.14	0.04	3.56	1.13	540.82	4.96	5.41
MIG.157-2016	5.46	5.43	0.23	0.61	0.18	0.05	3.73	0.63	405.69	8.42	13.45
MOG.158-2016	2.85	6.79	0.21	0.30	0.35	0.06	1.96	1.12	375.02	14.71	4.44
RAG.159-2016	4.19	6.37	0.06	0.57	0.19	0.09	4.93	1.26	400.31	6.50	48.48

RAG.160-2016	7.83	9.55	0.06	0.46	0.16	0.08	2.36	0.78	714.81	3.49	20.99
RAG.161-2016	4.19	4.45	0.03	0.48	0.04	0.06	1.10	0.38	289.30	3.96	26.90
MIG.162-2016	5.81	5.36	0.00	0.74	0.08	0.05	2.27	0.51	570.16	9.36	9.42
RAG.163-2016	2.62	4.39	0.10	0.77	0.20	0.10	3.94	1.01	365.76	8.24	34.63
RAG.164-2016	1.86	2.20	0.06	0.07	0.23	0.02	4.47	1.70	290.29	0.72	4.53
RAG.165-2016	3.90	6.80	0.14	0.78	0.31	0.15	2.97	0.83	370.97	9.08	26.27
RAG.166-2016	2.60	2.92	0.12	0.22	0.15	0.06	9.53	1.73	404.01	5.33	39.59
ASC.167-2016	5.34	4.78	0.02	0.33	0.12	0.05	4.20	1.11	463.37	3.31	25.78
COR.168-2016	3.79	3.09	0.04	0.07	0.01	0.00	4.37	1.37	263.24	5.23	4.93
RAG.169-2016	4.60	4.04	0.04	0.19	0.04	0.04	2.29	0.98	248.21	6.33	22.13
MOG.170-2016	4.79	4.60	0.09	0.32	0.14	0.07	3.04	1.08	238.22	1.59	15.26
MIG.171-2016	4.23	3.40	0.00	0.00	0.10	0.03	2.07	0.51	282.58	9.75	4.92
COR.173-2016	4.43	3.80	0.00	0.25	0.18	0.06	3.45	1.06	312.62	7.45	1.39
ASC.174-2016	4.77	4.52	0.08	0.09	0.16	0.05	3.24	0.97	288.88	2.16	20.77
RAG.176-2016	2.01	4.93	0.04	0.20	0.05	0.04	3.65	0.69	208.87	6.42	39.25
MIG.177-2016	5.85	4.97	0.07	0.21	0.05	0.02	2.26	0.73	280.72	10.30	7.24
MIG.178-2016	9.87	10.66	0.15	1.50	0.12	0.08	2.39	0.31	332.43	9.01	10.77
COR.180-2016	8.47	9.68	0.01	0.84	0.21	0.08	4.04	1.09	418.96	5.15	6.01
MOG.181-2016	1.61	3.55	0.15	0.28	0.08	0.06	2.63	1.32	277.28	15.31	8.59
ASC.182-2016	2.80	4.83	0.12	0.33	0.13	0.08	3.60	1.21	245.05	31.88	3.00
MOG.186-2016	2.38	6.00	0.02	0.54	0.21	0.12	1.70	1.05	316.72	12.68	4.60
RAG.187-2016	2.32	9.80	0.00	0.61	0.12	0.09	3.44	1.29	240.43	7.06	41.16
COR.188-2016	3.87	6.33	0.00	0.40	0.14	0.05	2.08	1.41	241.62	7.83	4.01
MOG.191-2016	17.94	23.58	0.01	0.35	0.07	0.04	2.73	1.03	380.20	2.27	18.74
MOG.192-2016	3.37	5.22	0.02	0.55	0.36	0.10	1.58	0.52	317.79	9.26	4.04
MIG.193-2016	8.33	6.93	0.08	0.47	0.03	0.04	2.76	0.47	323.63	7.02	8.52

Table 50. Polyphenol compounds (in mg/kg) in MEVOOs

	Ethanol		3-Pentanone		1-Penten-3-one		1-Penten-3-ol		(*E*)-2-Hexenal		Acetic acid, hexyl ester		Hexanal		(*Z*)-3-Hexen-1-ol, acetate		(*Z*)-2-Penten-1-ol		1-Hexanol		(*Z*)-3-Hexen-1-ol		(*E*)-2-Hexen-1-ol	
SAMPLE NAME	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**
LEVOO.1-2014	1.35E+07	56.79	6.18E+06	26.08	2.10E+06	8.86	1.96E+06	8.27		0.00		0.00		0.00		0.00		0.00		0.00		0.00		0.00
LEVOO.2-2014	1.20E+07	14.33	5.44E+06	6.50		0.00	1.72E+06	2.06	2.63E+07	31.39		0.00	1.12E+07	13.41		0.00		0.00	5.63E+06	6.73	1.75E+07	20.89	3.93E+06	4.69
LEVOO.3-2014	5.69E+07	43.12	4.73E+06	3.58	1.11E+06	0.84	1.33E+06	1.01		0.00	3.47E+07	26.29		0.00		0.00		0.00	8.41E+06	6.37	2.11E+07	16.02	3.65E+06	2.76
LEVOO.4-2014	1.85E+07	28.18	8.41E+06	12.83	2.54E+06	3.88	2.87E+06	4.37		0.00	3.24E+07	49.39		0.00		0.00		0.00		0.00	8.88E+05	1.35		0.00
LEVOO.5-2014	2.18E+07	22.40	5.22E+06	5.36	1.56E+06	1.60	1.80E+06	1.85		0.00	1.58E+07	16.27	2.76E+07	28.37		0.00		0.00	1.29E+07	13.22	1.06E+07	10.93		0.00
HEVOO.1-2014	2.03E+06	1.76	7.04E+06	6.10	8.39E+06	7.27	3.75E+06	3.25		0.00	1.77E+07	15.35	3.58E+07	31.03		0.00		0.00	1.36E+07	11.76	1.59E+07	13.78	1.12E+07	9.70
HEVOO.2-2014		0.00	7.38E+06	73.81		0.00	2.62E+06	26.19		0.00		0.00		0.00		0.00		0.00		0.00		0.00		0.00
HEVOO.3-2014	2.01E+07	8.29		0.00		0.00	3.43E+06	1.42	1.24E+08	51.09	1.72E+07	7.08	2.29E+07	9.43		0.00	3.82E+06	1.58	1.54E+07	6.36	9.67E+06	3.99	2.61E+07	10.76
HEVOO.4-2014	5.44E+06	1.16	5.31E+06	1.13	2.36E+07	5.03	2.30E+06	0.49	3.41E+08	72.85	1.16E+07	2.47	2.14E+07	4.58		0.00		0.00	1.26E+07	2.69	5.30E+06	1.13	3.96E+07	8.46
HEVOO.5-2014	1.13E+07	3.84	8.17E+06	2.76	2.41E+06	0.81	4.83E+06	1.64	2.43E+08	82.23	1.97E+07	6.67		0.00		0.00	6.06E+06	2.05		0.00		0.00		0.00
OO.1-2014	4.65E+06	22.77	1.72E+06	8.42		0.00		0.00		0.00	8.10E+06	39.68	2.11E+06	10.31		0.00		0.00	1.22E+06	5.97	1.18E+06	5.77	1.45E+06	7.08
OO.2-2014	6.28E+06	35.85	1.86E+06	10.63		0.00		0.00	2.70E+06	15.44		0.00	3.56E+06	20.32		0.00		0.00	7.63E+05	4.35	1.73E+06	9.88	6.18E+05	3.53
OO.3-2014	7.51E+06	23.80	3.41E+06	10.81		0.00		0.00	1.09E+07	34.54		0.00	7.04E+06	22.32		0.00		0.00		0.00		0.00	2.69E+06	8.52
OO.4-2014		0.00	3.23E+06	18.17		0.00		0.00		0.00	5.45E+06	30.64	3.29E+06	18.53		0.00		0.00	1.53E+06	8.58	2.16E+06	12.15	2.12E+06	11.93
OO.5-2014	2.21E+06	14.04	1.29E+06	8.20		0.00		0.00	5.86E+06	37.29		0.00	4.74E+06	30.12		0.00		0.00		0.00		0.00	1.63E+06	10.34
LEVOO.6-2015	5.68E+07	35.17	3.46E+06	2.14		0.00	1.29E+06	0.80	3.97E+07	24.57	2.20E+06	1.36	1.70E+07	10.51	1.15E+07	7.11		0.00	7.31E+06	4.53	1.60E+07	9.89	6.33E+06	3.92
LEVOO.7-2015	1.20E+08	57.64	2.19E+06	1.05		0.00		0.00	3.11E+07	14.89	3.06E+06	1.46	3.66E+06	1.75	2.39E+07	11.42		0.00	5.58E+06	2.67	1.28E+07	6.13	6.23E+06	2.98
LEVOO.8-2015	4.97E+07	33.06	2.28E+06	1.52		0.00		0.00	5.76E+07	38.35	3.18E+06	2.11	2.90E+06	1.93	1.82E+07	12.12		0.00		0.00	1.28E+07	8.55	3.55E+06	2.36
LEVOO.9-2015	1.68E+07	7.71	2.30E+06	1.06	2.50E+06	1.15	1.70E+06	0.78	1.35E+08	62.19	4.41E+06	2.03	4.82E+06	2.22	1.91E+07	8.80		0.00	9.92E+06	4.56	6.28E+06	2.89	1.44E+07	6.61
LEVOO.10-2015	2.81E+07	15.93	3.88E+06	2.20	1.40E+06	0.80	1.08E+06	0.61	9.49E+07	53.78	3.36E+06	1.90	6.22E+06	3.53	1.17E+07	6.66		0.00	8.60E+06	4.88	4.98E+06	2.82	1.22E+07	6.89
HEVOO.6-2015		0.00	4.17E+06	0.80	2.84E+06	0.55	2.14E+06	0.41	4.23E+08	81.29		0.00	1.66E+07	3.19		0.00		0.00	3.46E+07	6.65	8.74E+06	1.68	2.83E+07	5.43
HEVOO.7-2015		0.00	6.34E+06	1.82		0.00	2.02E+06	0.58	2.01E+08	57.69	3.73E+06	1.07	1.35E+07	3.89	2.51E+07	7.21		0.00	3.73E+07	10.72	2.82E+07	8.10	3.10E+07	8.92
HEVOO.8-2015	1.70E+07	4.39	5.46E+06	1.41	6.42E+06	1.65	2.83E+06	0.73	2.85E+08	73.25	1.01E+06	0.26	1.27E+07	3.26	4.47E+06	1.15		0.00	1.91E+07	4.92	7.87E+06	2.02	2.71E+07	6.96
HEVOO.9-2015	4.18E+07	9.32	8.53E+06	1.90	2.88E+06	0.64	3.40E+06	0.76	2.60E+08	57.98	1.66E+06	0.37	1.47E+07	3.27	6.12E+06	1.37		0.00	4.62E+07	10.30	1.88E+07	4.20	4.44E+07	9.91

HEVOO.10-2015	2.65E+07	5.20	6.21E+06	1.22	2.92E+06	0.57	3.71E+06	0.73	3.58E+08	70.28	3.50E+06	0.69	1.97E+07	3.87	1.44E+07	2.83		0.00	2.38E+07	4.68	1.32E+07	2.58	3.74E+07	7.34
LEVOO.11-2015	2.82E+07	26.41	4906289	4.60	925947.5	0.87	1.55E+06	1.45	3.08E+07	28.91	2.62E+06	2.46	3.27E+06	3.07	1.27E+07	11.92	1.23E+06	1.15	5.04E+06	4.72	9.88E+06	9.27	5.52E+06	5.18
LEVOO.12-2015	1.26E+07	9.98	4830556	3.82	2326252	1.84	2.02E+06	1.59	5.37E+07	42.45	3.01E+06	2.38	6.20E+06	4.90	1.28E+07	10.13	1.93E+06	1.52	7.90E+06	6.25	1.18E+07	9.33	7.34E+06	5.80
LEVOO.13-2015	1.61E+07	14.42	5238111.5	4.68	1290800.5	1.15	1.82E+06	1.62	3.70E+07	33.10	2.90E+06	2.59	6.62E+06	5.92	1.20E+07	10.76	1.82E+06	1.62	6.41E+06	5.73	1.27E+07	11.40	7.82E+06	6.99
LEVOO.14-2015	4.61E+06	3.94	5820320	4.97	882532.5	0.75	1.86E+06	1.59	4.74E+07	40.52	3.81E+06	3.25	8.23E+06	7.03	1.38E+07	11.78	1.80E+06	1.54	7.14E+06	6.10	1.04E+07	8.92	1.12E+07	9.60
LEVOO.15-2015	1.59E+07	9.85	7445820	4.60	2105556	1.30	3.12E+06	1.93	7.59E+07	46.95	2.82E+06	1.74	6.62E+06	4.09	1.31E+07	8.13	2.38E+06	1.47	9.33E+06	5.77	1.08E+07	6.70	1.21E+07	7.47
HEVOO.11-2015	6.83E+05	0.49	9305091	6.64	1704629	1.22	3.38E+06	2.41	8.95E+07	63.82	1.48E+06	1.06	5.85E+06	4.17	5.48E+06	3.91	2.29E+06	1.64		0.00	7.47E+06	5.33	1.31E+07	9.32
HEVOO.12-2015	2.27E+07	19.95	6618215	5.81	1291836	1.13	2.77E+06	2.43	5.29E+07	46.42	1.44E+06	1.27	7.93E+06	6.96	5.72E+06	5.02	1.76E+06	1.54		0.00	5.05E+06	4.43	5.75E+06	5.05
HEVOO.13-2015	1.81E+07	11.18	6672803	4.13	2121452	1.31	2.82E+06	1.75	9.16E+07	56.68	7.75E+05	0.48	6.03E+06	3.73	5.25E+06	3.25	2.48E+06	1.54		0.00	1.10E+07	6.80	1.48E+07	9.17
HEVOO.14-2015	2.08E+07	13.31	14918837.5	9.52	2192592.5	1.40	3.33E+06	2.13	5.48E+07	35.01	1.45E+06	0.93	5.26E+06	3.36	4.13E+06	2.63	4.20E+06	2.68		0.00	2.40E+07	15.33	2.14E+07	13.69
HEVOO.15-2015	3.87E+06	1.36	8587598	3.03	3809989.5	1.34	3.76E+06	1.33	2.24E+08	79.09		0.00	1.33E+07	4.69	9.96E+05	0.35	3.03E+06	1.07		0.00	7.74E+06	2.73	1.42E+07	5.00
OO.6-2015	8.32E+06	69.98		0.00		0.00		0.00	1.08E+06	9.09		0.00	1.49E+06	12.55	3.65E+05	3.07		0.00		0.00	6.31E+05	5.30		0.00
OO.7-2015	1.58E+06	18.08		0.00		0.00		0.00	2.83E+06	32.44		0.00	3.06E+06	35.02	3.75E+05	4.29		0.00		0.00	6.28E+05	7.19	2.59E+05	2.97
OO.8-2015	6.19E+06	46.21		0.00		0.00		0.00	2.00E+06	14.95	1.14E+05	0.85	2.33E+06	17.38	8.60E+05	6.42		0.00	5.63E+05	4.20	9.77E+05	7.29	3.64E+05	2.71
OO.9-2015	2.64E+06	39.40		0.00		0.00		0.00	1.26E+06	18.80		0.00	1.97E+06	29.47	3.14E+05	4.69		0.00		0.00	5.12E+05	7.65		0.00
OO.10-2015	9.56E+06	55.76		0.00		0.00		0.00	2.71E+06	15.80		0.00	2.74E+06	15.98	4.29E+05	2.50		0.00	6.39E+05	3.73	6.92E+05	4.04	3.75E+05	2.19

Table 51. Volatile compounds in commercial oils

	Ethanol		3-Pentanone		1-Penten-3-one		1-Penten-3-ol		(*E*)-2-Hexenal		Acetic acid, hexyl ester		Hexanal		(*Z*)-3-Hexen-1-ol, acetate		(*Z*)-2-Penten-1-ol		1-Hexanol		(*Z*)-3-Hexen-1-ol		(*E*)-2-Hexen-1-ol	
SAMPLE NAME	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**	**AREA**	**%**
COR.102-2015	1.51E+07	5.21	4.67E+06	1.61	5.02E+06	1.73	2.98E+06	1.03	1.88E+08	64.79	2.92E+06	1.01	1.27E+07	4.39	1.02E+07	3.53		0.00	1.82E+07	6.28	1.98E+07	6.83	1.04E+07	3.60
MOG.103-2015	9.13E+06	2.65	8.55E+06	2.48	4.46E+06	1.29	3.47E+06	1.01	2.55E+08	73.88	9.50E+05	0.28	1.59E+07	4.60	5.14E+06	1.49		0.00	1.31E+07	3.79	1.35E+07	3.91	1.59E+07	4.61
MIG.104-2015	1.52E+08	51.42	8.43E+06	2.85		0.00	2.13E+06	0.72	2.84E+07	9.60	6.45E+06	2.18	5.84E+06	1.98	3.41E+07	11.54		0.00	2.34E+07	7.92	1.98E+07	6.69	1.51E+07	5.11
COR.105-2015	1.02E+07	4.27	1.13E+06	0.47	2.00E+07	8.33	5.06E+06	2.11	1.61E+08	66.98	2.75E+06	1.15	1.41E+07	5.87	7.52E+06	3.14	1.20E+06	0.50	3.26E+06	1.36	1.23E+07	5.15	1.60E+06	0.67
COR.106-2015	2.09E+07	8.36	2.52E+06	1.01	8.28E+06	3.31	2.19E+06	0.88	1.84E+08	73.51	1.10E+06	0.44	4.09E+06	1.64	5.92E+06	2.37		0.00	6.62E+06	2.65	7.54E+06	3.02	7.04E+06	2.82
RAG.107-2015	5.92E+06	1.13	2.84E+06	0.54	2.13E+07	4.06	4.54E+06	0.87	4.56E+08	86.99		0.00	1.19E+07	2.28	2.37E+06	0.45	9.23E+05	0.18	5.95E+06	1.14	5.28E+06	1.01	7.10E+06	1.35
RAG.108-2015	1.87E+07	2.81	1.19E+07	1.79	6.00E+06	0.90	4.79E+06	0.72	5.33E+08	79.98	1.95E+06	0.29	1.49E+07	2.23	7.56E+06	1.13	1.15E+06	0.17	1.98E+07	2.97	6.47E+06	0.97	4.01E+07	6.02
MOG.109-2015		0.00	3.72E+07	15.10	4.38E+06	1.78	5.56E+06	2.26	1.14E+08	46.14		0.00	9.98E+06	4.05	3.59E+06	1.46	8.07E+05	0.33	6.97E+06	2.83	3.40E+07	13.82	3.01E+07	12.23
MIG.110-2015	5.70E+07	14.70	1.23E+07	3.17	4.32E+06	1.11	5.34E+06	1.37	1.94E+08	49.99	5.81E+06	1.50	1.50E+07	3.86	2.23E+07	5.74	7.72E+05	0.20	2.42E+07	6.23	1.17E+07	3.02	3.54E+07	9.11
RAG.111-2015	8112052	0.91	2898023	0.33	1.8E+07	2.04	5816968	0.66	8.1E+08	91.51		0.00	1.8E+07	2.00	2186310	0.25	1229038	0.14	3040762	0.34	8734622	0.98	7475391	0.84
MIG.112-2015	4.99E+06	1.02	5.98E+06	1.22	1.63E+07	3.32	4.47E+06	0.91	4.10E+08	83.32	2.64E+06	0.54	9.41E+06	1.91	1.06E+07	2.16	6.84E+05	0.14	5.08E+06	1.03	1.40E+07	2.85	7.83E+06	1.59
MOG.113-2015	6.63E+06	3.61	2.64E+06	1.44	9.09E+06	4.95	3.15E+06	1.71	1.12E+08	60.71		0.00	2.52E+07	13.74	8.32E+05	0.45		0.00	2.31E+06	1.25	2.06E+07	11.23	1.68E+06	0.92
RAG.114-2015	1.11E+07	0.90	4.86E+06	0.39	1.78E+07	1.45	1.09E+07	0.88	1.09E+09	88.34		0.00	2.28E+07	1.85		0.00	1.30E+06	0.11	2.41E+07	1.95	7.22E+06	0.59	4.37E+07	3.55
ASC.115-2015	2.50E+07	18.13	6.11E+06	4.44	3.66E+07	26.57	7.84E+06	5.69		0.00		0.00	2.69E+07	19.54	2.56E+07	18.58		0.00	9.70E+06	7.05		0.00		0.00
ASC.116-2015	1.34E+07	2.14	7.66E+06	1.23	3.68E+07	5.89	1.14E+07	1.83	4.43E+08	70.94	3.46E+06	0.56	2.99E+07	4.79	3.09E+07	4.95	1.80E+06	0.29	1.18E+07	1.89	3.03E+07	4.86	3.92E+06	0.63
MIG.117-2015	2.90E+07	11.25	1.43E+07	5.55		0.00	1.74E+06	0.67	4.75E+07	18.39	4.86E+06	1.88	1.97E+06	0.76	1.42E+07	5.52		0.00	3.77E+07	14.62	2.59E+07	10.03	8.09E+07	31.33
COR.118-2015		0.00		0.00	1.10E+07	3.85	2.02E+06	0.71	2.46E+08	86.11		0.00	6.18E+06	2.16	4.10E+06	1.44		0.00	3.23E+06	1.13	1.06E+07	3.72	2.52E+06	0.88
COR.119-2015	1.62E+07	5.23	2.59E+06	0.84	1.27E+07	4.11	2.97E+06	0.96	2.34E+08	75.67	5.86E+05	0.19	4.15E+06	1.34	3.27E+06	1.06	7.01E+05	0.23	3.39E+06	1.10	1.47E+07	4.74	1.40E+07	4.54
MIG.120-2015	3.01E+07	8.30	8.80E+06	2.43	4.48E+06	1.24	1.85E+06	0.51	2.35E+08	64.99	3.62E+06	1.00	4.61E+06	1.27	1.38E+07	3.80		0.00	2.09E+07	5.78	1.02E+07	2.80	2.86E+07	7.88
RAG.121-2015	1.46E+07	2.29	2.58E+06	0.40	8.24E+06	1.29	2.21E+06	0.35	5.65E+08	88.33		0.00	6.28E+06	0.98	1.68E+06	0.26	6.04E+05	0.09	1.66E+07	2.59	5.53E+06	0.86	1.63E+07	2.55
MOG.122-2015	1.58E+07	5.59	1.30E+06	0.46	8.13E+06	2.87	3.61E+06	1.27	2.10E+08	74.19		0.00	1.43E+07	5.03		0.00	7.80E+05	0.28	6.27E+06	2.21	1.94E+07	6.83	3.62E+06	1.28
ASC.123-2015	1.91E+06	1.46	3.39E+06	2.59	8.51E+06	6.49	1.56E+06	1.19	7.57E+07	57.74	9.13E+05	0.70	4.58E+06	3.49	1.16E+07	8.84	6.32E+05	0.48	1.81E+06	1.38	1.87E+07	14.31	1.74E+06	1.33
ASC.124-2015	1.81E+07	6.35	1.12E+06	0.39	8.40E+06	2.95	1.42E+06	0.50	2.09E+08	73.33	2.15E+06	0.75	2.31E+06	0.81	2.42E+07	8.51		0.00	4.94E+06	1.73	1.03E+07	3.62	3.01E+06	1.06
MIG.125-2015	1.96E+07	16.15		0.00	2.09E+06	1.72		0.00	4.65E+07	38.19	8.75E+06	7.19	1.40E+06	1.15	3.76E+07	30.92		0.00	2.15E+06	1.77	2.72E+06	2.24	8.20E+05	0.67
RAG .126-2015	1.63E+07	4.90	5.93E+05	0.18	3.35E+06	1.01		0.00	2.91E+08	87.48	9.73E+05	0.29	5.44E+06	1.64	2.09E+06	0.63		0.00	3.34E+06	1.01	3.68E+06	1.11	5.84E+06	1.76

COR.127-2015	3.30E+06	0.35	2.34E+06	0.25	2.03E+07	2.17	5.40E+06	0.58	8.63E+08	92.52		0.00	1.85E+07	1.98		0.00	1.26E+06	0.13	3.04E+06	0.33	6.56E+06	0.70	9.12E+06	0.98
MOG.128-2015	7.43E+06	0.95	1.30E+06	0.17	2.00E+07	2.56	4.85E+06	0.62	7.17E+08	91.54		0.00	1.62E+07	2.07		0.00	1.03E+06	0.13	3.35E+06	0.43	6.62E+06	0.85	5.44E+06	0.69
MOG.129-2015		0.00	4.76E+06	1.69	9.16E+06	3.25	4.43E+06	1.58	1.83E+08	65.13		0.00	2.55E+07	9.04		0.00	8.66E+05	0.31	6.46E+06	2.29	3.86E+07	13.71	8.42E+06	2.99
MOG.130-2015	5.75E+06	1.13	1.21E+07	2.38	6.89E+06	1.35	5.73E+06	1.12	3.74E+08	73.26		0.00	2.38E+07	4.66		0.00	7.10E+05	0.14	1.84E+07	3.61	3.58E+07	7.01	2.73E+07	5.35
MOG.131-2015	3.22E+07	12.41	1.89E+06	0.73	2.62E+06	1.01	6.40E+05	0.25	1.66E+08	64.21		0.00	1.49E+07	5.73		0.00		0.00	7.92E+06	3.06	2.61E+07	10.08	6.53E+06	2.52
ASC.132-2015		0.00	2.05E+06	2.41	2.22E+06	2.61	1.41E+07	16.60	5.06E+06	5.94	1.66E+07	19.49	4.14E+06	4.86	1.04E+06	1.22	8.15E+05	0.96	3.42E+07	40.11	3.98E+06	4.68	9.58E+05	1.13
RAG.136-2015	6.09E+06	0.79	1.58E+06	0.20	9.98E+06	1.29	5.97E+06	0.77	7.10E+08	91.87	8.51E+05	0.11	2.00E+07	2.59	3.19E+06	0.41	6.36E+05	0.08	3.48E+06	0.45	5.32E+06	0.69	5.75E+06	0.74
MOG.137-2015	8.00E+06	2.99	2.57E+06	0.96	9.92E+06	3.71	7.38E+06	2.76	1.73E+08	64.82		0.00	3.36E+07	12.55	1.15E+06	0.43	8.19E+05	0.31	3.77E+06	1.41	2.42E+07	9.07	2.65E+06	0.99
MIG.138-2015	1.42E+07	3.13	7.35E+06	1.63	1.03E+07	2.28	5.62E+06	1.25	2.82E+08	62.41	6.70E+06	1.48	1.48E+07	3.27	4.31E+07	9.55	8.75E+05	0.19	1.49E+07	3.30	3.81E+07	8.44	1.39E+07	3.08
ASC.139-2015	5.67E+07	16.20	4.12E+06	1.18	5.96E+06	1.70	6.53E+06	1.86	1.13E+08	32.17	3.23E+06	0.92	3.57E+07	10.20	1.85E+07	5.30	8.08E+05	0.23	8.76E+06	2.50	9.20E+07	26.27	5.13E+06	1.46
COR.140-2015	1.17E+08	28.37	7.48E+06	1.81	7.07E+06	1.72	5.96E+06	1.45	1.58E+08	38.45	1.56E+06	0.38	1.16E+07	2.82	1.09E+07	2.64	7.92E+05	0.19	3.08E+07	7.48	4.71E+07	11.42	1.35E+07	3.27
ASC.143-2016	5.70E+05	0.79		0.00	1.05E+07	14.52	2.57E+06	3.56	3.54E+07	49.06	2.36E+06	3.27	5.84E+06	8.09	8.42E+06	11.67		0.00	1.28E+06	1.77	5.25E+06	7.27		0.00
MIG.144-2016	3.23E+06	1.45	1.82E+06	0.82	2.88E+06	1.30		0.00	1.35E+08	60.55	7.17E+06	3.23	5.68E+06	2.55	4.74E+07	21.31	1.47E+06	0.66	3.29E+06	1.48	6.97E+06	3.13	7.82E+06	3.52
MOG.145-2016	2.78E+06	8.48	1.71E+06	5.23	4.89E+06	14.94	1.61E+06	4.93	1.39E+06	4.24	1.13E+06	3.45	3.48E+06	10.64	1.62E+06	4.96	4.50E+06	13.74	3.06E+05	0.93	3.67E+05	1.12	8.96E+06	27.34
COR.146-2016	1.87E+06	1.08	2.92E+06	1.69	3.70E+06	2.14	1.64E+06	0.95	1.46E+08	84.38	2.21E+06	1.28	2.83E+06	1.63	6.31E+06	3.64	2.84E+06	1.64	1.27E+05	0.07	7.38E+05	0.43	1.87E+06	1.08
ASC.147-2016	1.10E+06	0.46		0.00	8.50E+06	3.52	2.20E+06	0.91	1.78E+08	73.73	1.43E+06	0.59	1.35E+07	5.60	1.25E+07	5.17	2.76E+06	1.14	3.96E+06	1.64	1.12E+07	4.64	6.29E+06	2.60
ASC.148-2016		0.00		0.00	1.95E+06	0.89	2.51E+06	1.15	1.53E+08	70.11		0.00	1.12E+07	5.12	2.34E+06	1.08	2.29E+06	1.05	5.61E+06	2.58	1.00E+07	4.61	2.92E+07	13.40
ASC.149-2016	1.51E+06	0.72	4.22E+06	2.02	4.70E+06	2.24	2.19E+06	1.05	1.51E+08	71.82	1.73E+06	0.82	1.17E+07	5.59	8.48E+06	4.05	2.27E+06	1.08	3.19E+06	1.52	1.01E+07	4.81	8.98E+06	4.29
ASC.150-2016	2.52E+06	1.07	4.72E+06	2.01	2.98E+06	1.27	1.87E+06	0.80	1.23E+08	52.31	6.28E+06	2.68	9.91E+06	4.22	2.21E+07	9.42	2.80E+06	1.19	1.30E+07	5.52	1.29E+07	5.49	3.29E+07	14.01
RAG.151-2016	1.83E+06	0.59		0.00	4.53E+06	1.45	1.38E+06	0.44	2.95E+08	94.63	9.69E+05	0.31	4.42E+06	1.42	2.89E+05	0.09	4.64E+05	0.15		0.00		0.00	2.85E+06	0.91
COR.152-2016	3.21E+05	0.25	9.65E+05	0.74	6.08E+06	4.65	1.56E+06	1.19	9.73E+07	74.44	1.15E+06	0.88	3.65E+06	2.79	9.52E+06	7.28	2.47E+06	1.89	8.24E+05	0.63	5.79E+06	4.42	1.09E+06	0.83
RAG.153-2016		0.00	2.26E+06	1.96		0.00	7.67E+05	0.66	8.25E+07	71.31	6.81E+05	0.59	6.28E+06	5.43	2.07E+06	1.79	1.09E+06	0.94	4.61E+06	3.99	1.94E+06	1.68	1.35E+07	11.65
MIG.154-2016	6.29E+05	0.35	2.02E+06	1.12	4.10E+06	2.28	8.78E+05	0.49	1.47E+08	81.83		0.00	5.25E+06	2.93	5.66E+06	3.15	1.19E+06	0.66	3.34E+06	1.86	5.16E+06	2.87	4.39E+06	2.45
MOG.155-2016	1.68E+06	1.02	1.75E+06	1.07	2.98E+06	1.82	6.55E+05	0.40	1.33E+08	80.76		0.00	6.42E+06	3.91	7.33E+05	0.45	9.91E+05	0.60	3.85E+06	2.35	6.84E+06	4.17	5.68E+06	3.46
COR.156-2016	3.48E+06	2.63	2.06E+06	1.56	6.48E+06	4.90	2.12E+06	1.60	9.07E+07	68.60	5.75E+05	0.43	7.32E+06	5.53	3.76E+06	2.84	2.94E+06	2.22	3.75E+06	2.84	6.49E+06	4.91	2.56E+06	1.94
MIG.157-2016	4.11E+06	2.65		0.00	4.62E+06	2.98	1.76E+06	1.13	1.09E+08	70.24	2.67E+06	1.72	6.82E+06	4.40	1.05E+07	6.73	2.56E+06	1.65	3.82E+06	2.46	5.79E+06	3.73	3.57E+06	2.30
MOG.158-2016	1.67E+06	1.37		0.00	6.27E+06	5.13	1.83E+06	1.50	9.78E+07	80.02		0.00	9.87E+06	8.07		0.00	2.20E+06	1.80	1.36E+06	1.11		0.00	1.22E+06	1.00
RAG.159-2016	2.56E+06	1.42	3.29E+06	1.83	5.59E+06	3.11	2.01E+06	1.12	1.37E+08	76.26	1.15E+06	0.64	1.46E+07	8.12		0.00	3.11E+06	1.73	2.82E+06	1.57	2.81E+06	1.56	4.75E+06	2.64

RAG.160-2016	3.82E+06	2.05	8.37E+05	0.45	5.45E+06	2.93	1.53E+06	0.82	1.60E+08	85.80		0.00	2.51E+06	1.35		0.00	3.06E+06	1.65	1.77E+06	0.95	1.07E+06	0.57	6.36E+06	3.42
RAG.161-2016	3.05E+05	0.17	6.24E+05	0.35	1.24E+06	0.70	7.55E+05	0.42	1.62E+08	90.94		0.00	3.74E+06	2.11		0.00	1.16E+06	0.65	1.51E+06	0.85	3.68E+06	2.07	3.08E+06	1.74
MIG.162-2016	6.49E+06	6.93		0.00		0.00	1.25E+06	1.33	6.08E+07	64.84	1.73E+06	1.85	4.45E+06	4.75	9.37E+06	9.99	1.77E+06	1.89	1.92E+06	2.05	3.44E+06	3.67	2.54E+06	2.71
RAG.163-2016	4.13E+06	2.35		0.00	3.97E+06	2.26	9.08E+05	0.52	1.47E+08	83.55		0.00	5.73E+06	3.26		0.00	1.44E+06	0.82	3.29E+06	1.87	3.21E+06	1.83	6.23E+06	3.54
RAG.164-2016	1.80E+06	1.10	9.26E+05	0.56	3.02E+06	1.84	7.08E+05	0.43	1.42E+08	86.40	7.88E+05	0.48	6.42E+06	3.91		0.00	9.60E+05	0.58	1.55E+06	0.94	2.09E+06	1.27	4.09E+06	2.49
RAG.165-2016	2.29E+06	1.16		0.00	3.02E+06	1.52	1.08E+06	0.54	1.69E+08	85.45	9.64E+05	0.49	5.75E+06	2.91	1.69E+06	0.85	1.42E+06	0.72	2.38E+06	1.20	2.89E+06	1.46	7.31E+06	3.70
RAG.166-2016	9.50E+05	0.61	1.05E+06	0.67	3.09E+06	1.98	6.81E+05	0.44	1.35E+08	86.30	5.42E+05	0.35	4.27E+06	2.73	2.13E+06	1.36	1.07E+06	0.68	1.98E+06	1.26	1.62E+06	1.04	4.05E+06	2.59
ASC.167-2016	3.99E+05	0.48		0.00	4.63E+06	5.61	9.64E+05	1.17	6.23E+07	75.45	1.64E+06	1.99	3.91E+06	4.73		0.00	1.76E+06	2.13	1.03E+06	1.25	5.22E+06	6.32	7.27E+05	0.88
COR.168-2016	5.87E+05	0.53	6.10E+05	0.55	2.25E+06	2.02	4.59E+05	0.41	8.79E+07	78.63	1.80E+06	1.61	3.05E+06	2.73	9.23E+06	8.26	7.63E+05	0.68	1.31E+06	1.17	1.76E+06	1.57	2.06E+06	1.84
RAG.169-2016	1.11E+06	0.84	5.97E+05	0.45	2.50E+06	1.90	4.96E+05	0.38	1.14E+08	86.65	5.92E+05	0.45	2.50E+06	1.90	2.01E+06	1.53	7.75E+05	0.59	1.76E+06	1.34	1.78E+06	1.35	3.45E+06	2.62
MOG.170-2016	9.80E+05	0.68		0.00	2.06E+06	1.44	5.76E+05	0.40	1.20E+08	83.84	3.78E+05	0.26	2.70E+06	1.88	3.09E+06	2.15	9.17E+05	0.64	2.57E+06	1.79	4.64E+06	3.23	5.27E+06	3.68
MIG.171-2016	1.61E+06	1.53		0.00	1.93E+06	1.84	5.13E+05	0.49	7.30E+07	69.27	3.25E+06	3.09	2.83E+06	2.69	1.29E+07	12.25	7.74E+05	0.74	2.01E+06	1.91	3.03E+06	2.88	3.51E+06	3.33
COR.173-2016	5.97E+05	0.42		0.00	2.84E+06	1.98	7.57E+05	0.53	9.85E+07	68.80	4.62E+06	3.22	2.15E+06	1.50	2.10E+07	14.64	1.40E+06	0.98	2.51E+06	1.75	4.67E+06	3.26	4.17E+06	2.91
ASC.174-2016	4.39E+05	0.45	7.98E+05	0.82	2.37E+06	2.42	6.39E+05	0.65	6.88E+07	70.22	1.17E+06	1.20	3.14E+06	3.21	9.23E+06	9.42	8.86E+05	0.90	1.49E+06	1.52	6.80E+06	6.95	2.20E+06	2.25
RAG.176-2016	6.24E+05	0.47		0.00	1.99E+06	1.51	5.48E+05	0.42	1.12E+08	85.24	1.31E+06	1.00	3.04E+06	2.31	1.95E+06	1.48	7.78E+05	0.59	2.21E+06	1.68	1.32E+06	1.00	5.65E+06	4.30
MIG.177-2016	3.19E+06	2.70	1.80E+06	1.52	2.43E+06	2.05		0.00	7.24E+07	61.26	3.20E+06	2.71	8.04E+06	6.80	1.44E+07	12.21	1.49E+06	1.26	2.22E+06	1.88	3.94E+06	3.33	5.04E+06	4.26
MIG.178-2016	1.01E+07	7.92	1.06E+06	0.83	1.14E+06	0.90	7.91E+05	0.62	8.37E+07	65.79	3.27E+06	2.57	6.15E+06	4.84		0.00	1.54E+06	1.21	5.07E+06	3.99	6.14E+06	4.83	8.27E+06	6.51
COR.180-2016	9.66E+06	7.85		0.00	1.33E+06	1.08	7.18E+05	0.58	7.73E+07	62.83	1.61E+06	1.31	8.38E+06	6.81	6.84E+06	5.56	1.35E+06	1.10	3.56E+06	2.89	5.71E+06	4.64	6.56E+06	5.34
MOG.181-2016	4.15E+05	0.37		0.00	1.05E+06	0.93		0.00	8.97E+07	79.87		0.00	9.13E+06	8.13		0.00	4.92E+05	0.44	1.68E+06	1.49	5.52E+06	4.92	4.33E+06	3.86
ASC.182-2016	7.15E+05	0.46		0.00	1.89E+06	1.23	4.66E+05	0.30	1.14E+08	74.16	1.10E+06	0.71	5.88E+06	3.81	1.22E+07	7.89	7.25E+05	0.47	2.25E+06	1.46	8.92E+06	5.78	5.76E+06	3.73
MOG.186-2016	8.25E+06	6.99	9.78E+05	0.83	3.26E+06	2.77	8.36E+05	0.71	8.34E+07	70.68		0.00	6.16E+06	5.22		0.00	1.49E+06	1.26	1.92E+06	1.63	7.25E+06	6.14	4.45E+06	3.78
RAG.187-2016	5.16E+06	3.06		0.00	1.74E+06	1.03	9.19E+05	0.54	1.22E+08	72.21	8.01E+05	0.48	4.06E+06	2.41	3.86E+06	2.29	1.33E+06	0.79	1.05E+07	6.26	3.64E+06	2.16	1.48E+07	8.78
COR.188-2016	6.19E+06	4.45	1.75E+06	1.26	4.30E+06	3.09	1.50E+06	1.08	9.58E+07	68.75	1.08E+06	0.77	4.12E+06	2.95	6.98E+06	5.01	2.45E+06	1.76	2.45E+06	1.76	6.00E+06	4.31	6.72E+06	4.83
MOG.191-2016	1.69E+06	0.99		0.00	3.99E+05	0.23	4.86E+05	0.28	1.22E+08	70.85		0.00	3.18E+06	1.86		0.00	9.19E+05	0.54	1.17E+07	6.84	4.78E+06	2.78	2.68E+07	15.63
MOG.192-2016	1.41E+06	1.07	9.11E+05	0.69	2.25E+06	1.70	9.70E+05	0.74	1.12E+08	84.88		0.00	2.90E+06	2.20		0.00		0.00	1.58E+06	1.20	4.69E+06	3.56	5.20E+06	3.94
MIG.193-2016		0.00		0.00		0.00		0.00	4.00E+06	78.44	8.19E+04	1.60	4.29E+05	8.41	4.32E+05	8.46		0.00		0.00	1.57E+05	3.08		0.00

Table 52. Volatile compounds in MEVOOs

SAMPLE NAME	VISUAL EXAM		OLFACTORY EXAM							GUSTATORY-TACTILE-RETROLFACTORY EXAM					
	Yellow	Green	Fruity	Leaves/Grass	Almond	Artichoke/Cardoon	Tomato	Apple	Berries	Bitter	Spicy	Sweet	Fluidity	Score	Defects
COR.102-2015	6.7	1.5	3.8	1.3	1.3	0.0	0.0	1.0	1.6	3.6	4.2	3.6	3.9	6.7	
MOG.103-2015	6.3	1.0	4.1	1.2	1.9	0.0	0.0	0.0	2.1	3.7	4.2	3.3	3.9	6.6	
MIG.104-2015	6.2	2.0	3.4	0.0	1.4	0.0	0.0	0.0	0.0	4.2	3.5	3.4	3.6	5.9	fusty
COR.105-2015	6.3	2.3	5.0	2.9	2.4	2.9	1.9	0.0	0.0	4.5	5.2	2.0	5.3	7.8	
COR.106-2015	4.6	4.5	4.0	2.2	1.7	2.2	0.0	0.0	0.0	3.9	4.0	2.1	4.2	7.0	
RAG.107-2015	4.0	5.3	5.1	3.3	2.8	2.9	1.4	0.0	0.0	4.8	5.0	1.6	4.8	7.7	
RAG.108-2015	4.8	3.6	4.4	1.9	3.4	2.0	0.0	0.0	0.0	3.7	4.0	3.1	4.2	7.2	
MOG.109-2015	4.9	3.7	4.0	2.6	1.8	1.9	0.0	0.0	0.0	3.8	4.8	2.6	4.9	7.3	
MIG.110-2015	7.0	1.8	4.2	1.3	1.2	0.0	0.0	0.0	3.1	4.1	3.2	3.0	4.7	7.0	
RAG.111-2015	5.9	2.4	5.1	3.5	3.0	2.5	0.0	0.0	0.0	5.9	5.4	1.8	5.5	7.8	
MIG.112-2015	7.1	0.4	4.8	2.0	2.1	1.5	0.0	0.0	1.6	3.2	3.6	2.7	4.2	7.5	
MOG.113-2015	7.3	0.9	4.6	2.6	2.0	1.9	0.0	0.7	0.0	3.9	4.4	2.3	5.3	7.6	
RAG.114-2015	2.2	6.3	5.5	3.6	3.4	3.0	0.0	0.0	0.0	6.0	5.1	1.5	5.5	7.9	
ASC.115-2015	4.8	3.8	5.4	3.4	1.9	3.0	2.6	0.0	0.0	5.0	4.2	2.2	5.1	7.8	
ASC.116-2015	6.3	1.9	5.5	3.7	1.7	3.6	2.9	0.0	0.0	5.1	5.0	2.0	5.2	8.0	
MIG.117-2015	8.0	0.9	4.0	1.4	0.9	0.0	0.0	0.0	2.5	3.8	3.3	2.7	5.2	6.6	
COR.118-2015	3.1	5.5	4.3	2.5	2.4	2.0	0.0	0.0	0.0	4.3	4.6	2.4	4.2	7.2	
COR.119-2015	5.1	3.4	4.6	2.7	2.3	2.3	0.0	0.0	0.0	5.3	4.4	2.3	5.0	7.6	
MIG.120-2015	7.1	1.2	3.9	1.5	1.5	1.0	0.0	0.0	2.3	4.8	4.1	2.7	5.1	7.4	
RAG.121-2015	3.8	4.7	4.7	2.4	3.1	1.2	0.0	0.0	0.0	4.9	5.0	2.8	5.4	7.3	
MOG.122-2015	2.2	5.3	4.3	3.0	2.6	2.3	0.0	0.0	0.0	5.0	4.9	2.7	5.0	7.5	
ASC.123-2015	2.1	6.5	5.3	3.4		2.6	2.0	0.0	0.0	4.0	4.5	3.0	4.7	7.5	
ASC.124-2015	5.7	2.3	5.2	1.9	3.1	0.9	2.6	0.0	0.0	4.5	4.6	3.2	4.9	7.4	
MIG.125-2015	5.4	1.6	4.5	0.0	0.0	0.0	0.0	0.0	3.4	4.5	3.8	3.5	4.2	7.6	

RAG.126-2015	5.4	2.7	4.0	1.3	2.2	0.0	0.0	0.0	0.0	4.5	4.4	2.4	5.2	7.4	
COR.127-2015	4.9	2.7	4.7	2.8	3.3	1.6	0.0	0.0	0.0	5.5	4.8	2.8	4.9	7.5	
MOG.128-2015	3.9	4.1	5.0	3.1	3.3	2.0	0.0	0.0	0.0	4.2	4.8	3.1	4.7	7.5	
MOG.129-2015	4.9	3.2	4.1	2.4	1.5	0.0	0.0	0.0	0.0	2.9	3.1	3.1	4.0	6.6	
MOG.130-2015	4.1	4.3	4.3	1.8	2.3	2.0	0.0	0.0	0.0	3.2	3.5	3.8	4.2	6.9	
MOG.131-2015	5.9	1.3	2.9	0.0	0.0	0.0	0.0	0.0	0.0	2.2	2.2	3.6	3.8	6.2	fusty, winey
ASC.132-2015	2.8	4.8	4.9	2.9	2.2	2.1	2.2	0.0	0.0	4.9	4.7	2.8	4.9	7.9	
RAG.136-2015	4.4	4.9	4.8	2.3	3.4	1.7	0.0	0.0	0.0	4.8	5.0	3.2	5.8	7.6	
MOG.137-2015	5.3	4.1	4.5	2.5	1.1	1.8	1.7	0.0	0.0	4.7	4.7	2.7	5.2	7.4	
MIG.138-2015	6.0	2.6	4.8	2.1	2.4	1.5	0.0	0.0	0.8	4.5	5.0	2.6	5.6	7.8	
ASC.139-2015	7.7	1.2	5.8	2.3	1.4	1.6	3.3	0.0	0.0	5.0	5.0	2.2	6.5	7.9	
COR.140-2015	6.0	3.1	4.8	2.2	2.6	2.0	0.0	0.0	0.0	4.8	5.3	3.3	5.4	7.0	
ASC.143-2016	5.9	1.9	5.9	3.9	2.0	2.0	2.3	0.0	0.0	5.5	4.3		5.0	8.0	
MIG.144-2016	6.5	1.3	3.9	0.2	0.0	0.0	0.0	0.0	2.9	4.0	3.6		4.0	7.6	
MOG.145-2016	5.5	1.6	3.3	1.7	1.7	0.0	0.0	0.0	0.0	3.9	3.3		3.7	6.8	
COR.146-2016	8.0	0.0	2.6	0.0	1.0	0.0	0.0	0.0	0.0	2.6	3.9		2.6	6.6	
ASC.147-2016	6.2	1.1	4.5	1.9	2.7	1.0	0.0	0.0	0.0	4.3	4.3		5.4	7.5	
ASC.148-2016	2.2	6.1	3.4	0.0	0.0	0.0	0.0	0.0	0.0	1.6	1.9		3.5	6.0	rancid, hay/dry
ASC.149-2016	1.4	7.1	3.2	0.8	0.6	0.0	0.0	0.0	0.0	2.5	3.6		3.3	6.5	
ASC.150-2016	6.3	1.2	2.2	0.0	0.0	0.0	0.0	0.0	0.0	1.5	1.5		2.4	6.0	rancid, fusty/sludge
RAG.151-2016	7.2	0.6	4.2	1.8	3.4	0.5	0.0	0.0	0.0	4.0	4.3		4.3	7.4	
COR.152-2016	5.5	2.2	4.0	3.5	3.0	2.8	0.0	0.0	0.0	3.1	3.4		5.0	7.5	
RAG.153-2016	5.8	2.4	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.9	1.1		2.5	6.0	fusty/sludge
MIG.154-2016	6.4	1.2	3.2	0.0	0.0	0.0	0.0	0.0	0.0	2.1	3.0		3.8	6.5	
MOG.155-2016	6.0	1.3	2.4	0.0	0.0	0.0	0.0	0.0	0.0	2.1	1.1		3.3	6.0	rancid, fusty/sludge
COR.156-2016	6.8	0.5	3.5	2.7	1.5	2.6	0.0	0.0	0.0	4.5	3.8		5.1	7.3	
MIG.157-2016	5.5	2.9	3.1	1.4	1.2	0.0	0.0	0.0	0.0	3.4	2.4		4.3	6.5	
MOG.158-2016	5.3	5.0	4.2	1.7	2.3	1.2	0.0	0.0	0.0	4.4	3.8		4.6	6.6	

RAG.159-2016	5.0	5.0	3.3	2.0	2.4	1.3	0.0	0.0	0.0	3.7	4.0	3.0	7.0	
RAG.160-2016	2.3	7.3	4.7	2.4	2.5	1.8	0.0	0.0	0.0	4.2	4.9	4.6	8.0	
RAG.161-2016	2.1	6.7	2.4	1.0	1.7	0.0	0.0	0.0	0.0	2.6	2.0	2.3	6.7	
MIG.162-2016	8.7	0.0	4.2	1.8	0.7	0.0	0.0	0.0	3.2	5.2	4.2	4.4	8.0	
RAG.163-2016	6.9	2.0	2.9	0.0	1.8	0.0	0.0	0.0	0.0	1.9	3.4	3.0	6.5	
RAG.164-2016	2.2	7.0	2.8	0.0	0.5	0.0	0.0	0.0	0.0	1.4	1.0	1.7	6.0	fusty/sludge, hay/dry
RAG.165-2016	5.7	1.1	2.6	1.0	0.0	0.0	0.0	0.0	0.0	2.0	3.1	2.2	6.5	
RAG.166-2016	8.0	2.0	3.3	1.5	2.3	1.0	0.0	0.0	0.0	3.7	3.2	4.8	7.0	
ASC.167-2016	4.9	4.1	4.7	2.9	2.0	1.8	3.4	0.0	0.0	5.0	4.6	5.6	8.0	
COR.168-2016	5.9	1.9	3.3	1.2	1.8	0.5	0.0	0.0	0.0	3.2	3.7	5.3	7.0	
RAG.169-2016	5.6	4.4	3.4	1.6	2.1	1.2	0.0	0.0	0.0	3.7	4.2	4.0	7.5	
MOG.170-2016	4.7	4.3	4.5	2.2	1.4	2.2	0.0	0.0	0.0	2.7	3.4	4.5	7.5	
MIG.171-2016	4.6	3.1	3.3	0.0	0.0	0.0	0.0	0.0	0.0	3.3	3.1	4.5	7.0	
COR.173-2016	4.3	4.1	3.6	2.2	2.2	2.2	1.2	0.0	0.0	3.3	3.9	5.4	7.6	
ASC.174-2016	5.1	2.3	4.9	2.3	1.3	1.3	2.5	0.0	0.0	3.9	3.8	4.9	7.8	
RAG.176-2016	5.6	4.0	3.9	1.2	1.8	1.7	0.0	0.0	0.0	2.5	2.7	4.8	7.0	
MIG.177-2016	3.9	5.1	3.1	0.0	0.6	1.2	0.0	0.0	2.1	3.5	4.0	4.3	7.4	
MIG.178-2016	5.7	2.0	3.1	0.5	1.0	0.7	0.0	0.0	0.0	4.1	4.0	5.0	7.3	
COR.180-2016	4.8	4.5	3.8	0.6	0.0	0.0	0.0	0.0	0.0	5.2	3.5	2.7	6.6	
MOG.181-2016	5.7	0.0	2.7	0.0	1.4	0.0	0.0	0.0	0.0	1.9	2.6	4.5	6.9	
ASC.182-2016	6.5	1.2	3.7	0.0	1.1	0.0	0.0	0.0	0.0	2.6	3.0	3.3	6.8	
MOG.186-2016	4.2	2.7	4.2	1.8	2.1	1.0	0.0	0.0	0.0	3.5	4.8	5.0	7.5	
RAG.187-2016	4.2	6.0	3.5	1.3	2.3	1.1	0.0	0.0	0.0	2.3	3.7	4.7	7.1	
COR.188-2016	4.0	4.5	4.3	1.6	1.7	1.0	0.0	0.0	0.0	4.4	4.0	4.8	7.3	
MOG.191-2016	5.5	3.4	4.6	1.7	2.3	2.5	0.0	0.0	0.0	5.2	4.3	3.2	7.6	
MOG.192-2016	5.6	1.1	3.4	1.5	2.0	1.7	0.0	0.0	0.0	3.2	3.4	4.1	6.9	
MIG.193-2016	7.3	1.6	3.4	0.4	1.0	0.0	0.0	0.0	1.6	3.7	2.8	5.1	7.2	

Table 53. Sensorial analysis results

References

[1] http://www.dietamedunesco.it

[2] M. Paz Aguilera, G. Beltràn, D. Ortega, A. Fernàndez, A.Jiménez, M. Uceda, 2005. Characterisation of virgin olive oil of Italian olive cultivars: 'Frantoio' and 'Leccino', grown in Andalusia. Food Chemistry 89, 387-391.

[3] R. Aroca-Santos, J. C. Cancilla, A. Pérez-Pérez, A. Moral,J. S. Torrecilla, 2016. Quantifying binary and ternary mixtures of monovarietal extra virginolive oils with UV–vis absorption and chemometrics. Sensors and Actuators B 234, 115-121.

[4] T. Cecchi, P. Passamonti, P. Cecchi, 2010. Study of the quality of extra virgin olive oil stored in PET bottles with or without an oxygen scavenger. Food Chemistry 120, 730-735.

[5] S. Predieri, C. Medoro, M. Magli, E. Gatti, A. Rotondi, 2013. Virgin olive oil sensory properties: Comparing trained panel evaluation and consumer preferences. Food Research International 54, 2091-2094.

[6] European Food Safety Authority Journal, 2011;9(4):2044.. Olive oil related health claims.

[7] A. Piscopo, A. De Bruno, A. Zappia, C. Ventre, M. Poiana, 2016. Characterization of monovarietal olive oils obtaines from mills of Calabria region (Southern Italy). Food Chemistry 213, 313-318.

[8] www.oleadb.it

[9] T. Cecchi, B. Alfei, 2013. Volatile profiles of Italian monovarietal extra virgin olive oils via HS-SPME–GC–MS: Newly identified compounds, flavors molecular markers, and terpenic profile. Food Chemistry 141, 2025-2035.

[10] T. Cecchi, P. Passamonti, B. Alfei, P. Cecchi, 2011. Monovarietal extra virgin olive oils from the Marche region, Italy: analytical and sensory characterization. International Journal of Food Properties 14, 483-495.

[11] M. D'Imperio, G. Dugo, M. Alfa, L. Mannina, A. L. Segre, 2007. Statistical analysis on Sicilian olive oils. Food Chemistry 102, 956-965.

[12] http://www.olimonovarietali.it/database

[13] COMMISSION REGULATION (EU) No 61/2011 of 24 January 2011 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis.

[14] R. Aparicio, M. T. Morales, D. L. Garcìa-Gonzalez, 2012. Towards new analyses of aroma and volatile to understand sensory perception of olive oil. European Journal Lipid Science and Technologies 114, 1114-1125.

[15] A. Sánchez-Ortiz, M. Aymen Bejaoui, A. Quintero-Flores, A. Jiménez, G. Beltrán, 2018. "Biosynthesis of volatile compounds by hydroperoxide lyase enzymatic activity during virgin olive oil extraction process". Food Research International 111, 220-228.

[16] C. M. Kalua, M. S. Allen, D.R. Bedgood Jr, A.G. Bishop, P.D. Prenzler, K. Robards, 2007. Olive oil volatile compounds, flavour development and quality: A critical review. Food Chemistry 100, 273-286.

[17] REGOLAMENTO (CEE) No 2568/1991 DELLA COMMISSIONE dell'11 Luglio 1991 relativo alle caratteristiche degli oli d'oliva e degli oli di sansa d'oliva nonché ai metodi ad essi attinenti.

[18] A. Venditti, C. Frezza, D. Celona, A. Bianco, M. Serafini, K. Cianfaglione, G. Celenza, 2017. Polar constituents, protection against reactive oxygen species, and nutritional value of Chinese artichoke (Stachys affinis Bunge). Food Chemistry, 221, 473-481.

[19] M. Biedermann, A. Bongartz, C. Mariani, Koni Grob, 2008. Fatty acid methyl and ethyl esters as well as wax esters for evaluating the quality of olive oils. European Food Research Technologies, 228, 65-74.

[20] M. Ricciutelli, S. Marconi, M. C. Boarelli, G. Caprioli, G. Sagratini, R. Ballini, D. Fiorini, 2017. Olive oil polyphenols: A quantitative method by high-performance liquid-chromatography-diode-array detection for their determination and the assessment of the related health claim. Journal of Chromatography A, 1481, 53-63.

[21] F. Hammer, D. A. T. Harper, P. D. Ryan, 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4, 1-9.

[22] A. Quintero-Florez, L. Sinausia Nieva, A. Sanchez-Ortíz, G. Beltran, J. S. Perona, 2015. The Fatty Acid Composition of Virgin Olive Oil from Different Cultivars Is Determinant for Foam Cell Formation by Macrophages. Journal Agricultural and Food Chemistry 63, 6731-6738.

[23] M. Cocchi, L. Tonello, J. Martínez Álvarez, G. Lercker, G. M. Caramia, 2009. Extra virgin olive oil and oleic acid. Nutricion Clinica Dietetica Hospitalaria 29, 12-24.

[24] D. Fiorini, M. C. Baorelli, P. Conti, B. Alfei, G. Caprioli, M. Ricciutelli, G. Sagratini, D. Fedeli, R. Gabbianelli, D. Pacetti, 2018. Chemical and sensory differences between high price and low price extra virgin olive oils. Food Research International 105, 65-75.

[25] R. B. Gòmez-Coca, R. Cruz-Hidalgo, G. D. Fernandes, M. C. Perez-Camino, W. Moreda, 2014. Analysis of methanol and ethanol in virgin olive oil. MethodsX 1, 207-211.

[26] R. Costa, G. Bartolomeo, E. Saija, R. Rando, A. Albergamo, G. Dugo, 2017. Determination of Alkyl Esters Content in PDO Extra Virgin Olive Oils from Sicily. Journal of Food Quality.

[27] H. Jabeur, A. Zribi, R. Abdelhedi, M. Bouaziz, 2015. Effect of olive storage conditions on Chemlali olive oil quality and the effective role of fatty acids alkyl esters in checking olive oils authenticity. Food Chemistry 169, 289-296.

[28] R. B. Gomez-Coca, W. Moreda, M. C. Perez-Camino, 2012. Fatty acid alkyl esters presence in olive oil vs. organoleptic assessment. Food Chemistry 135, 1205-1209.

[29] A. Bendini, E. Valli, L. Cerretani, E. Chiavaro, G. Lercker, 2009. Study on the Effects of Heating of Virgin Olive Oil Blended with Mildy Deodorized Olive Oil: Focus on the Hydrolytic and Oxidative State. Journal of Agricultural and Food Chemistry 57, 10055-10062.

[30] A. Bendini, L. Cerretani, E. Valli, G. Lercker, C. Mazzini, 2009. Application of analytical methods to determine mildy deodorized olive oils in commercial extra virgin olive oils. Industrie Alimentari, 46-51.

[31] G. Blekas, M. Tsimidou, D. Boskou, 1995. Contribution of α-tocopherol to olive oil stability. Food Chemistry 52, 289-294.

[32] G. Singh, R. Sachdeva, B. Rai, G. S. S. Saini, 2017. Structure and vibrational spectroscopic study of alpha-tocopherol. Journal of Molecular Structure 1144, 347-354.

[33] S. B. Khedir, D. Moalla, M. Mzid, Z. Sahnoun, T. Rebai, 2018. Effects of Minor Components of Olive Oil on Health. Journal of Complementary Medicine and Alternative Healthcare 5.

[34] J. A. Cayuela, J. F. García, 2017. Sorting olive oil based on alpha-tocopherol and total tocopherol content using near-infra-red spectroscopy (NIRS) analysis. Journal of Food Engineering 202, 79-88.

[35] E. Câmara Grilo; P. Nunes Costa; C. Santos Sânzio Gurgel; A. F. de Lima Beserra; F. Niéce de Souza Almeida; R. Dimenstein; 2014. Alpha-tocopherol and gamma-tocopherol concentration in vegetable oils. Food Science and Technology 34.

[36] Y. Uchihara, T. Kidokoro, K. Tago, T. Mashino, H. Tamura, M. Funakoshi-Tago, 2018. A major component of vitamin E, α-tocopherol inhibits the anti-tumor activity of crizotinib against cells transformed by EML4-ALK. European Journal of Pharmacology 825, 1-9.

[37] V. Sicari, 2017. Antioxidant potential of extra virgin olive oils extracted from three different varieties cultivated in the Italian province of Reggio Calabria. Journal of Applied Botany and Food Quality 90, 76-82.

[38] M. Brenes, A. Garcìa, P. Garcìa, J. J. Rios, A. Garrido, 1999. Phenolic Compounds in Spanish Olive Oils. Journal of Agricultural and Food Chemistry 47, 3535-3540

[39] M. Suárez, A. Macià, M. P. Romero, M. J. Motilva, 2008. Improved liquid chromatography tandem mass spectrometry method for the determination of phenolic compounds in virgin olive oil. Journal of Chromatography A 1214, 90-99.

[40] M. I. Alarcon Flores, R. Romero-Gonzalez, A. Garrido Frenich, J. L. Martinez Vidal, 2012. Analysis of phenolic compounds in olive oil by solid-phase extraction and ultra high performance liquid chromatography-tandem mass spectrometry. Food Chemistry 134, 2465-2472.

[41] A. Bendini, L. Cerretani, A. Carrasco-Pancorbo, A. M. Gómez-Caravaca, A. Segura-Carretero, A. Fernández-Gutiérrez, G. Lercker, 2007. Phenolic Molecules in Virgin Olive Oils: a Survey of Their Sensory Properties, Health Effects, Antioxidant Activity and Analytical Methods. An Overview of the Last Decade. Molecules 12, 1679-1719.

[42] A. De Torres, F. Espinola, M. Moya, S. Alcalà, A. M. Vidal, E. Castro, 2018. Assessment of phenolic compounds in virgin olive oil by response surface methodology with particular focus on flavonoids and lignans. LWT - Food Science and Technology 90, 22-30.

[43] M. Brenes, F. J. Hidalgo, A. Garcia, J. J. Rios, P. Garcia, R. Zamora, A. Garrido, 2000. Pinoresinol and 1-Acetoxypinoresinol, Two New Phenolic Compounds Identified in Olive Oil. Journal of the American Oil Chemists' Society 77, 715-720.

[44] A. López-Biedma, C. Sánchez-Quesada, M. Delgado-Rodríguez, J. J. Gaforio, 2016. The biological activities of natural lignans from olives and virgin olive oils: A review. Journal of Functional Foods 26, 36-47.

[45] A. De Torres, F. Espínola, M. Moya, E. Castro, 2016. Composition of secoiridoid derivatives from Picual virgin olive oil using response surface methodology with regard to malaxation conditions, fruit ripening, and irrigation management. European Food Research and Technology 242, 1709-1718.

[46] T. A. Wani, F. A. Masoodi, A. Gani, W. N. Baba, N. Rahmanian, R. Akhter, I. A. Wani, M. Ahmad, 2018. Olive oil and its principal bioactive compound: Hydroxytyrosol - A review of the recent literature. Trends in Food Science & Technology 77, 77-90.

[47] REGULATION (EU) No 432/2012 of 16 May 2012, establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health.

[48] I. Romero, D. L. Garcìa-Gonzàlez, R. Aparicio-Ruiz, M. T. Morales, 2015. Validation of SPME-GCMS method for the analysis of virgin olive oil volatiles responsible for sensory defects. Talanta134, 394-401.

[49] G. Luna, M. T. Morales, R. Aparicio, 2006. Characterisation of 39 varietal virgin olive oils by their volatile compositions. Food Chemistry 98, 243-252.

[50] F. Angerosa, M. Servili, R. Selvaggini, A. Taticchi, S. Esposito, G. Montedoro, 2004. Volatile compounds in virgin olive oil: occurrence and their relationship with the quality. Journal of Chromatography A 1054, 17-31.

[51] IOOC Sensory analysis: general basic vocabulary. In C.O.I./T.20/Doc. n. 4, 1987.

[52] F. Angerosa, 2000. Sensory quality of olive oils. In Handbook of olive oil: Analysis and properties; J. Harwood, R. Aparicio. Ed.; Aspen publications, Inc, Gaithersburg, Maryland, USA.

[53] IOOC Organoleptic assessment of virgin olive oil. In C.O.I./T.20/Doc. n.15, 1996.

[54] M.T. Morales, G. Luna, R. Aparicio, 2005. Comparative study of virgin olive oil sensory defects. Food Chemistry . 91, 293-301.

Abstract

Ergosterol ((3β,22*E*)-ergosta-5,7,22-trien-3-ol) is produced by fungi and yeasts, i.e. organisms involved in the degradation of olives. In the present study, it was investigated whether ergosterol could be used as a marker to assess the quality of the olives from which the oil is produced. Ergosterol was quantified in extra virgin olive oils (EVOOs) having different level of quality developing an on-line HPLC-GC-MS method based on the on-line HPLC-GC method previously used to determine total sterol content. Oils were transmethylated to liberate ergosterol that was isolated from the far larger amounts of other sterols by HPLC on silica gel and transferred to GC by concurrent eluent evaporation. Preliminary results will be presented and discussed.

Chapter 3.

Ergosterol: a new molecule to determine olives and oils quality

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3.1 Introduction

3.1.1 Ergosterol

Ergosterol ((3β,22*E*)-ergosta-5,7,22-trien-3-ol) (Figure 1), is a natural compound. It is a biological precursor of vitamin D_2 (ergocalciferol) and a principal component that maintain the fungal cell membranes integrity [1]. Ergosterol has received much attention due to its healthy properties; in fact it has been connected to several beneficial pharmacological functions in humans, including anti-inflammatory, anti-oxidative, cardiovascular and kidney-protective functions, as well as anti-hyperlipidemic properties or its ability to reduce microbial and tumour growth [1]-[2]. For all these reasons, ergosterol is in fact a substance typically used in traditional Chinese medicine [1]. Ermakova et al. [3], instead, studied the effect of different concentration of ergosterol on the structural, mechanical, and dynamical properties of the fungal membranes using molecular dynamic simulation (MD), demonstrating that the presence of ergosterol induces the ordering of lipids leading to their denser packing, to reduce the lateral diffusion of lipids and lipid surface area, to increase the thickness of bilayer and compressibility modulus.

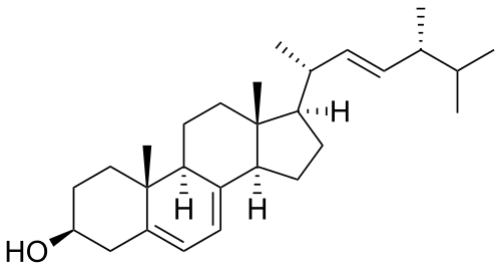


Figure 1. Ergosterol structure

Vitamin D_2 and its precursor ergosterol exist only in the kingdom Fungi, like for example in *Ganoderma lucidum* (called also *Reishi*), and the edible fungi mushrooms are the most important source for such compounds [1]-[4]. Studies have demonstrated that mushrooms ergosterol content is between 0.6 % and 0.7 % of dried weight, whereas cultivated mushrooms are deficient in vitamin D_2 content [4]. Ergosterol is present in two main forms (free and esterified) and the relative abundances should depend on the fungal species [2]-[5]. The percentage of free ergosterol and ergosteryl esters in the cell is regulated by different factors, like biosynthesis, exogenous uptake, transport and storage [2]-[5]. Free ergosterol has an important role in fluidity, permeability, and integrity of the cell membrane; moreover, this molecule could be involved in the effects of membrane-bound proteins associated with nutrient transport and chitin synthesis. On the other hand, ergosteryl esters, stored in the hydrophobic core of cytosolic lipid particles,

seem to have an important role in sterol homeostasis. Considering that ergosterol is only present in cell membranes of fungi and some bacteria and protozoa (for example the parasitic *Trypanosoma*), it is an important biochemical marker for undesirable fungal mycelia growth, thus can be used to determine the extent of fungal and microbial contaminations and as an important quality control measure for dry fungal material [1]. Therefore, for all these reasons, it becomes necessary to develop an analytical method to identify and quantify this molecule. In literature, many studies report the determination of sterols. In general, the total sterol content is determined, after alkaline saponification of extracted lipids, using gas chromatography with mass spectrometry (GC-MS). Hence, the obtained value includes ergosterol and other sterols present in free or esterified form [5]. In other cases, saponification was followed by high performance liquid chromatography with mass spectrometry (HPLC-MS) [6]. Other studies, proposed to introduce a simpler method to determine free and esterified sterols without the need for lengthy sample preparation or without any previous separation. An example is reported by Hatzakis et al. [7], that combines ^{1}H NMR for the determination of the total fraction of both free and esterified sterols and ^{31}P NMR for the quantification of free sterols; and from Flakelar et al. [8], that suggested an optimized method for the simultaneous quantification of major tocopherols, carotenoids, and, free and esterified phytosterols in canola oil using a normal phase high performance liquid chromatography coupled to sequential diode array detection and tandem mass spectrometry (NP-HPLC-DAD-MS/MS). Villares et al. [2], instead, described an isocratic reversed-phase liquid chromatography method for the separation and analysis of free ergosterol and ergosteryl derivatives present in mushrooms.

3.1.2 Aim of the work

Extra virgin olive oils (EVOOs) of high quality are produced from olives of best quality. The superiority of the EVOOs over other oils is also associated to the content and composition of the several health promoting substances present in the unsaponifiable fraction of the oil, like phenolic compounds, tocopherols, chlorophylls, caratenoids and sterols [9]. Even if they represent only 1-2% of the olive oils composition, these minor components are considered as important quality indicators. In particular, total sterols content and their various forms in olive oils are largely dependent of many factors such as the cultivar, olive ripening degree, environmental conditions (for example agronomic and climatic conditions), harvesting techniques, oil extraction method and storage conditions, as well as a possible refining process [10]-[11]. More in details, ergosterol (ES), produced by fungi and yeast fermentation, could be used for detection of spoiled olives. It could have several advantages for the detection of spoiled olives, since it is completely transferred to the oil owing to its lipophilicity and it cannot be removed by deodoration. This research work was developed at food chemistry control laboratory of Kantonales Labor Zurich (Zurich, Switzerland). This study aims to assess the relation between the olive health state and the ergosterol content, as well as to report a new analytical method to determine and quantify ergosterol content in EVOOs having different level of quality, by means of an on-line HPLC-GC-MS based on the previously developed on-line HPLC-GC method to determine total sterol content [12]. For this kind of study, Swiss market extra virgin olive oils (SEVOOs) and Italian monovarietal extra virgin olive oils (IMEVOOs) were investigated. Spoiled olives were obtained from a private producer in Turkey. They were collected in November 2017, brought to Switzerland and deep-frozen after a few days. Oils (0.5 mg) were transmethylated (using sodium methylate 10%/MTBE 40.60, v/v) to liberate ergosterol that was isolated from the far larger amounts of other sterols by HPLC on silica gel and transferred to GC by concurrent eluent evaporation. Cholesterol (CHS) and 7-dehydrocholesterol (DEHC) was used respectively as verification standards (VS) for GC-MS detector and internal standard (IS). The method was validated by determining linearity (R^2), repeatability, recovery at two fortification levels, limit of detection (LOD) and limit of quantification (LOQ). The final purpose of this work is to investigate and propose ergosterol as a direct parameter to assess the quality of the olives used in the production of high quality olive oils, thus correlations between the ergosterol concentrations and conventional parameters (like peroxide value, alkyl esters and sensorial analysis) are also investigated. The preliminary results of this work, still in progress, are presented and discussed.

3.2 Materials and Methods

3.2.1 Reagents and standards

The analytical standards of DEHC, ES and $VitD_2$ were purchased from Sigma-Aldrich (St. Louis, MO). CHS was obtained from Fluka (Buchs, Switzerland). $VitD_2$ and CHS were used as verification standards (VSs) respectively for LC-UV and GC-MS analysis; DEHC was used as internal standard (IS). Standard stock solutions of each compound were prepared by dissolving 10 mg of pure analytical standard in 20 mL of methyl-tert-butylether (MTBE). Standard working solutions, at various concentrations, were prepared when needed by appropriate hexane dilution of stock solution aliquots. Sodium methylate at 30% and citric acid reagents were purchased respectively from Sigma-Aldrich (St. Louis, MO) and VWR-Merck (Germany).

3.2.2 Samples

Swiss market extra virgin olive oils (SEVOOs) and Italian monovarietal extra virgin olive oils (IMEVOOs) are investigated. Spoiled olives were obtained from a private source in Turkey. They were collected in November 2017, brought to Switzerland and deep-frozen after a few days. Their analysis was performed in early February 2018.

3.2.3 Sample preparation

Two different types of methods were used:

1. **Transesterification treatment**

   A solution of IS (50 µL of 20 ng/µL of DEHC in MTBE) was added to 0.5 g of oil followed by addition of 10 mL of sodium methylate at 10%/MTBE (40:60, v/v). The solution was vortexed and after 20 min, 10 mL water and 14 mL hexane were added. The supernatant was washed twice with 10 mL 0.1% aqueous citric acid. The final solution, after addition of 20 µL VS1 (500 ng/µL $VitD_2$ in MTBE), was ready for the instrumental analysis.

2. **Sample as such**

   0.5 g oil, added with IS (50 µL of DEHC in MTBE at 20 ng/µL), were dissolved into 20 mL of hexane. The solution, added with 20 µL VS1 ($VitD_2$ in MTBE at 500 ng/µL), was ready for the instrumental analysis.

3.2.4 LC pre-separation

The LC-GC instrument (Brechbühler, Schlieren, Switzerland) was composed of a Phoenix 9000 pump, Trace GC Ultra (Thermo, Milano, Italy), a PAL COMBI-xt autosampler (CTC Zwingen, Switzerland) and a microUV-VIS20 detector (Carlo Erba Instruments, Milano, Italy). The transesterified oil (100 µL) was pre-separated on a 250 mm x 2 mm column packed with silica gel (Hypersil Si, 5 µm, Stagroma®) kept at 35 °C, using a gradient of hexane (A) and MTBE (B) at a flow rate of 0.3 mL min^{-1}: 0-20 min, 20% B; 20-30 min, 100% B; then reconditioned with 20% B for 5 min. ES, determined at 282 nm, was eluted between 12.0 and 13.7 min.

3.2.5 GC-MS analysis

GC-MS analysis was performed using a GC (Thermo, Milano, Italy) equipped with a PAL RTc autosampler (CTC Zwingen, Switzerland) and a DSQ II quadrupole mass detector (Thermo, Milano, Italy). The fractions collected from the LC, added with VS2 (25 µL of 1 ng/µL of CHS in hexane), were injected (100 µL) into a 15 m x 0.25 mm column coated with trifluoropropylmethyl polysiloxane (RTX200-MS, 0.25 µm, Restek®) with a 1 m x 0.53 mm pre-column coated with dimethyl polysilicon (PS-225, 0.7 µm). The oven temperature was initially set at 40 °C for 3.50 min, then programmed at a rate of 15.0 °C min^{-1} to 320 °C and held for 2.00 min. The injection speed was set at 5 µL s^{-1}; a programmed temperature vaporizing (PTV) injector in on-column mode was used. The closure of the valve was set at 1.0 min after the beginning of injection. Helium was used as carrier gas at a flow rate of 2 mL min^{-1}. The temperature of the transfer line and the ion source (EI) were set to 300 °C and 200 °C, respectively. The mass spectrometer was operated in SIM mode. The following masses were monitored: *m/z* = 386 relevant to CHS, *m/z* = 351 and *m/z* = 384 relevant to DEHC, *m/z* = 337, *m/z* = 363 and *m/z* = 396 relevant to ES.

3.2.6 Validation method

The method was validated by determining linearity (R^2), repeatability, recovery at two fortification levels, limit of detection (LOD) and limit of quantification (LOQ).

3.2.7 Extraction from olives

Olives were divided into four different groups on their appearance under UV at 366 nm: intact olives, degraded olives with reddish color and wizened olives with greenish color and moldy olives. The oil extraction was made weighting 10 g of homogenized sample, deriving from 55 g of olives, in a flask and adding 50 mL of ethanol. After 1 hour, the ethanol phase was separated into a new clean flask and 40 mL of hexane were added to the sample. The sample was homogenized and then left at room temperature overnight. Then the hexane phase added to the ethanol phase and washed with 80 mL of water. At the end, the organic phase was separated and all the solvent evaporated. The reproducibility of extraction method was evaluated, analyzing three different aliquots of the same sample.

3.2.8 Microbiological analysis

Yeast and molds were enumerated using Dichloran 18% glycerol agar (DG18; LAB M). Olive-homogenates (10 g) were placed into stomacher bags. The initial suspension was made by adding 90 mL of dilution buffer (8.5 g/L sodium chloride (J. T. Baker) and 1 g/L Tryptone (Oxoid)) and was homogenized with a BagMixer (Interscience) for 1 min. Then, 10-fold serial dilutions were prepared using Maximal Recovery Diluent (MRD; LabRobot). The initial suspension (0.1 mL) and the three subsequent decimal dilutions were transferred and spread over the surface of the agar plates using a sterile spreader and a new sterile pipette for each decimal dilution. The samples were analyzed in duplicates. The inoculated agar plates were incubated at 25°C under aerobic conditions. After 4 days of incubation, dishes containing less than 150 colonies, propagules or germs were enumerated. The average of the two duplicate results was calculated. Yeast colonies were confirmed by microscopy.

3.3 Results and Discussion

3.3.1 Ergosterol quantification

The final objective of this research work was to determine and quantify ergosterol in different oils samples. One of the important points of this study was to evaluate the need to apply the transesterification procedure. As already said, in the oil, ergosterol can be found in the free or tied form. Therefore, we were interested to study the effect of transesterification. In order to do this, three different oil samples were used. The samples were treated with and without transesterification (according to the procedure described in the paragraph 3.2.3) and then analyzed by GC-MS. The analyses were repeated three times. Once decided the most appropriate sample preparation method (samples treated with transesterification to liberate ergosterol from ergosteryl esters), the SEVOOs and IMEVOOs were prepared and analyzed. In the figure below (Figure 2), an example of the chromatogram obtained from the ergosterol analysis is reported.

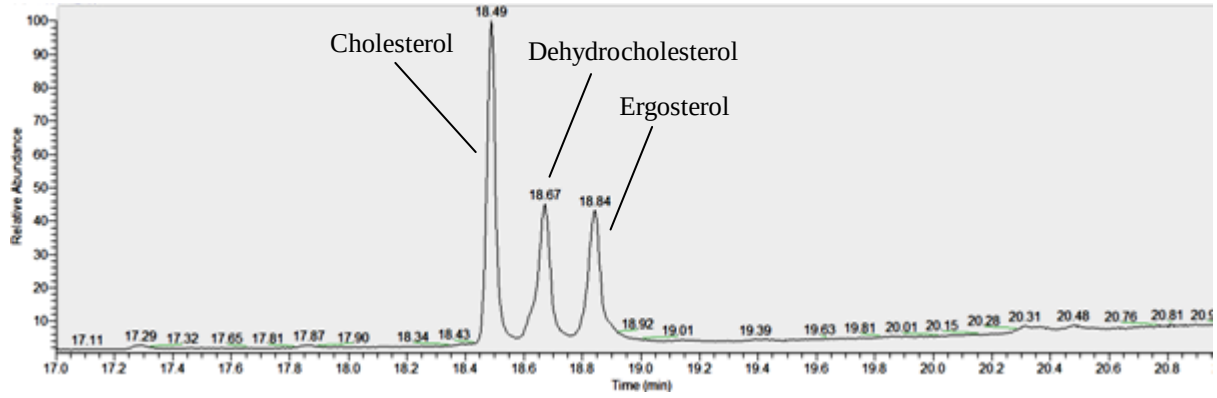


Figure 2. GC-MS chromatogram obtained from the ergosterol analysis

The chromatograms showed peaks well defined and contiguous. Once all samples were analyzed with the LC-GC-MS instrument, the identification of the different peaks, present in the resulting chromatograms, was performed. The recognition was carried out using extract ion chromatogram (EIC) from total ion chromatogram (TIC), monitoring specific fragments for CHS, DEHC and ES, chosen from their mass spectra. In particular *m/z* = 386 is selected for CHS (Figure 3), *m/z* = 351 and *m/z* = 384 are monitored for DEHC (Figure 4) and as regards ES (Figure 5) were chosen *m/z* = 337, *m/z* = 363 and *m/z* = 396.

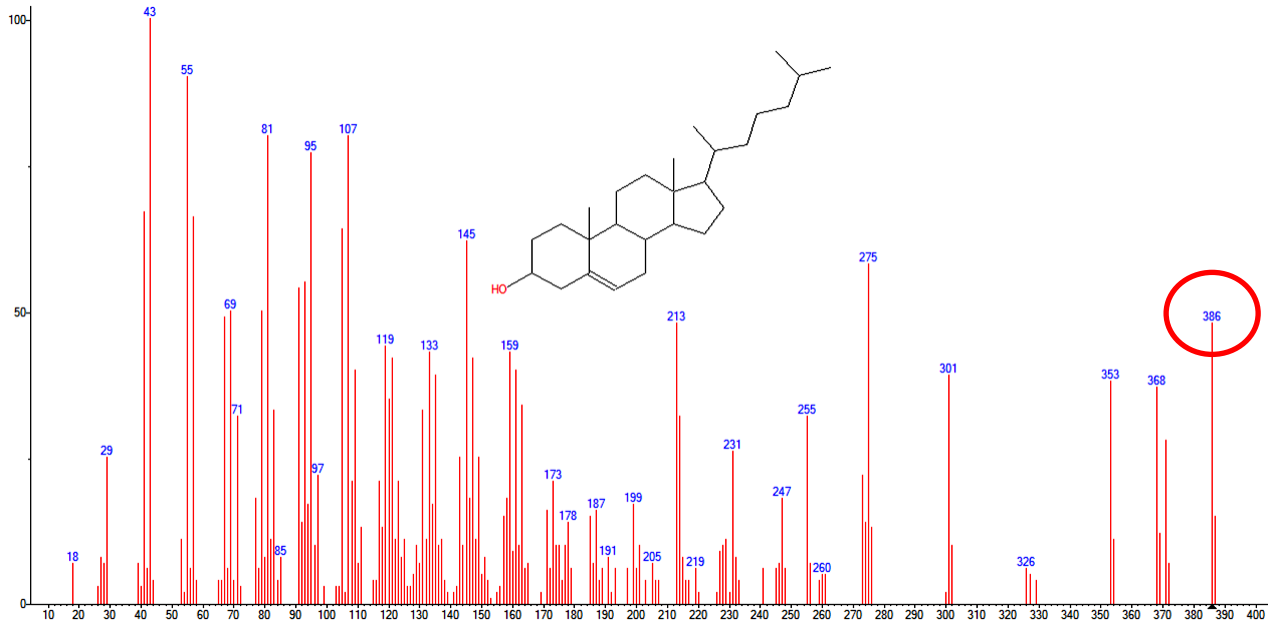


Figure 3. Cholesterol mass spectra

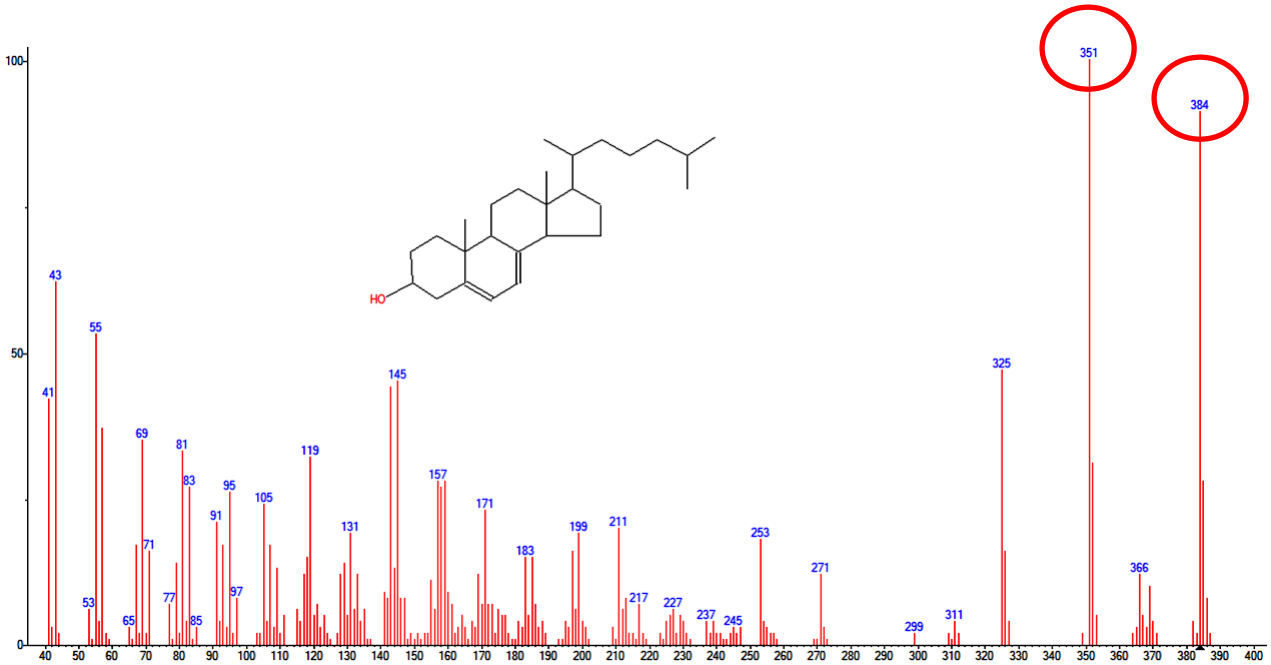


Figure 4. 7-Dehydrocholesterol mass spectra

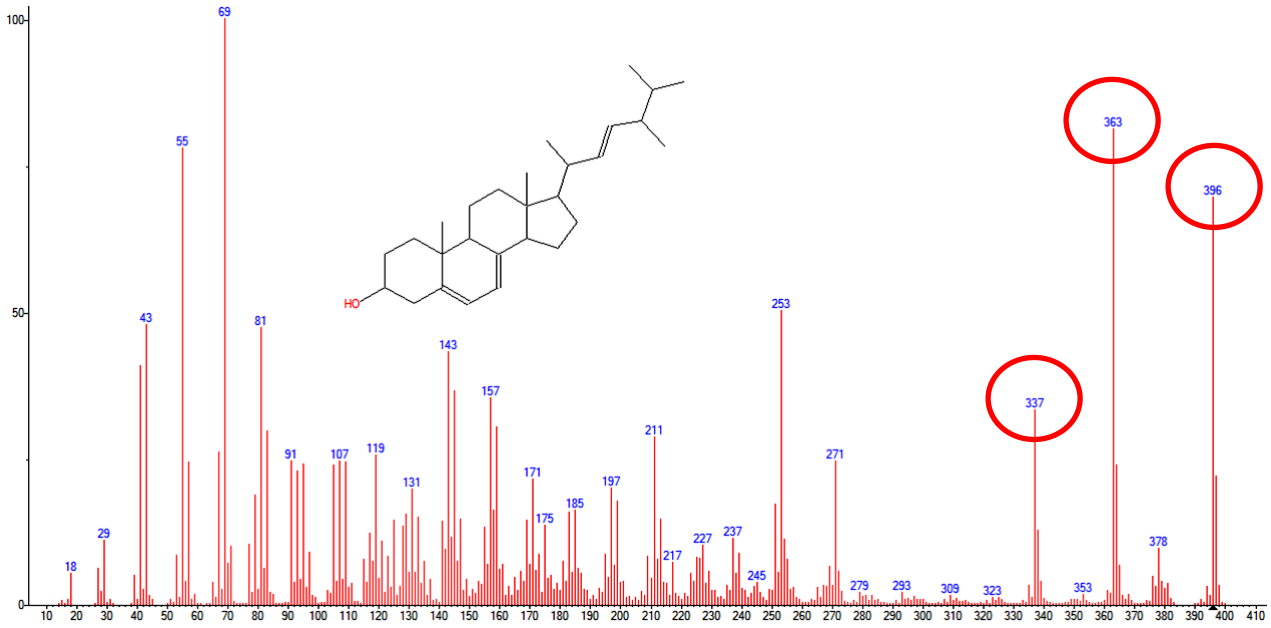


Figure 5. Ergosterol mass spectra

To calculate the ergosterol content present in all samples, calibration curves (Figure 6) were used. To create the calibration curves, an oil sample was spiked with different quantities of ergosterol solution at known concentration. The absolute areas obtained for each fragments of ergosterol were divided for the fragments relative to the internal standard in order to have the normalized values. In the table below (Table 1), the ergosterol concentrations (in ppm) and the normalized areas for each ergosterol fragments are reported.

ppm	ERGOSTEROL NORMALIZED AREAS		
	***m/z* = 337.5**	***m/z* = 363.5**	***m/z* = 396.5**
0	0.00000	0.00000	0.14149
0.5	0.04577	0.15610	0.24109
1	0.09829	0.31952	0.42327
2	0.18401	0.60083	0.72772
5	0.57450	1.53671	1.80787

Table 1. Ergosterol concentration and normalized area for each fragment

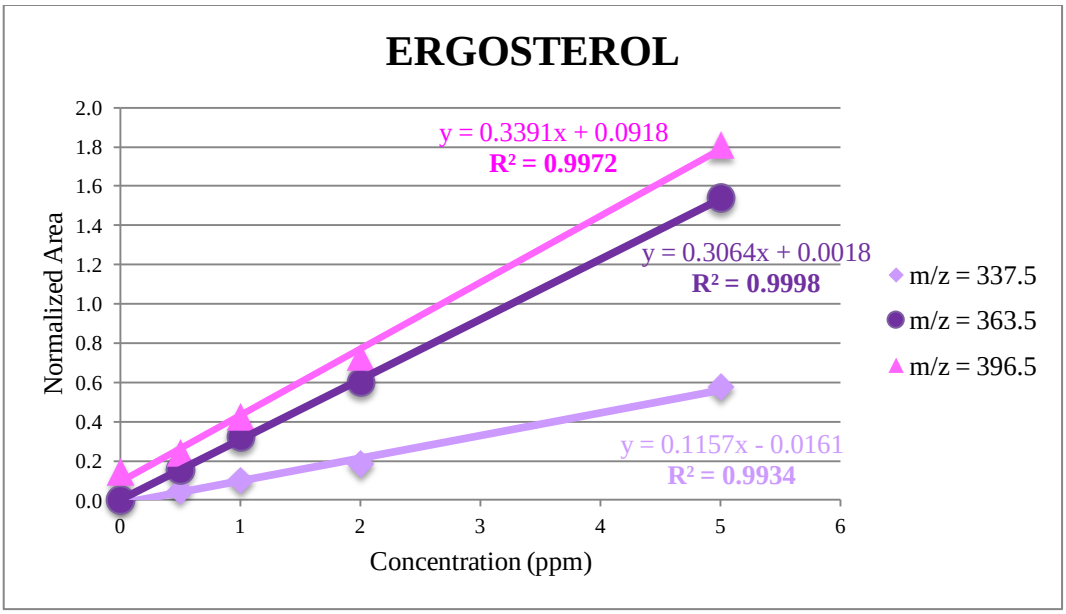
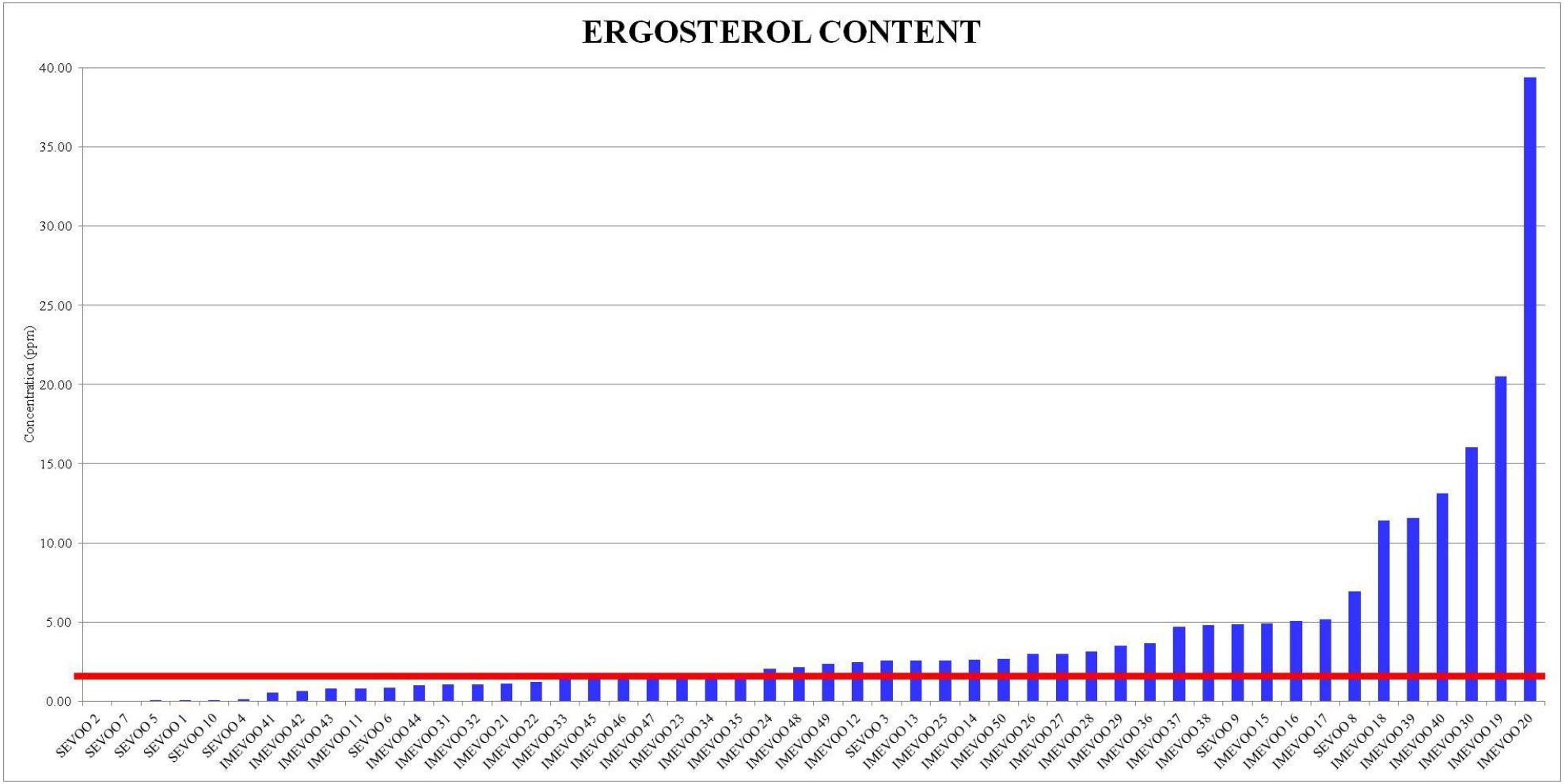


Figure 6. Ergosterol calibration curves

Interpolating the three equations with the relative ergosterol normalized areas, it is possible to obtain the concentration (ppm) of the analyte. The final concentration of ergosterol (in ppm), present in the samples analyzed, is given by the average of the values obtained from the three fragments. In the table and graph below (Table 2 and Graph 1), the results for each sample are reported. Moreover, in the table, the characteristics of these oils are shown.

	SAMPLE NAME	ERGOSTEROL CONCENTRATION (ppm)
Swiss Extra Virgin Olive Oils having different quality (Peroxide Value range: 6.6-11.8 mEq O_2/Kg and Alkyl Esters concentration range: 6-861 ppm)	SEVOO 1	0.07
	SEVOO 2	0.00
	SEVOO 3	2.55
	SEVOO 4	0.13
	SEVOO 5	0.02
	SEVOO 6	0.86
	SEVOO 7	0.00
	SEVOO 8	6.96
	SEVOO 9	4.88
	SEVOO 10	0.07
Italian Monovarietal Extra Virgin Olive Oils: Bad oils (Alkyl Esters concentration range: 18-659 ppm)	IMEVOO 11	0.82
	IMEVOO 12	2.46
	IMEVOO 13	2.56
	IMEVOO 14	2.61
	IMEVOO 15	4.93
	IMEVOO 16	5.06
	IMEVOO 17	5.16
	IMEVOO 18	11.39
	IMEVOO 19	20.52
	IMEVOO 20	39.36
Italian Monovarietal Extra Virgin Olive Oils: Bad oils (Peroxide Value range: 20.50-35.25 mEq O_2/Kg)	IMEVOO 21	1.09
	IMEVOO 22	1.21
	IMEVOO 23	1.67
	IMEVOO 24	2.06
	IMEVOO 25	2.59
	IMEVOO 26	2.97
	IMEVOO 27	3.00
	IMEVOO 28	3.15
	IMEVOO 29	3.48
	IMEVOO 30	16.03
Italian Monovarietal Extra Virgin Olive Oils: Bad oils (Sensorial Analysis Score: below 6.2)	IMEVOO 31	1.04
	IMEVOO 32	1.05
	IMEVOO 33	1.41
	IMEVOO 34	1.78
	IMEVOO 35	1.79
	IMEVOO 36	3.68
	IMEVOO 37	4.69
	IMEVOO 38	4.82
	IMEVOO 39	11.57
	IMEVOO 40	13.11
Italian Monovarietal Extra Virgin Olive Oils: Good oils (Sensorial Analysis Score: 6.6-8.0)	IMEVOO 41	0.54
	IMEVOO 42	0.66
	IMEVOO 43	0.81
	IMEVOO 44	1.02
	IMEVOO 45	1.45
	IMEVOO 46	1.51
	IMEVOO 47	1.56
	IMEVOO 48	2.16
	IMEVOO 49	2.39
	IMEVOO 50	2.69

Table 2. Ergosterol concentration (in ppm) in each sample analyzed



Graph 1. Ergosterol content

From the graph, it is possible to see that most of these samples have an ergosterol content of less than 2 ppm (see the red line). In particular, 46% of the oils contain less than 2 ppm of ergosterol, 36% contain from 2.0 ppm to 5.0 ppm, 12 % of the oils contain from 5.0 ppm to 15 ppm and 6% of the oils contain more than 15 ppm of ergosterol. Moreover, the lowest ergosterol concentrations found in SEVOOs were below 0.1 mg/kg, while concentrations in IMEVOOs, marketed as extra virgin, went up to 40 mg/kg, i.e. there is a range corresponding to a factor of at least 400. This important difference in terms of concentration between Swiss and Italian oils could depend of the cultivars or the area of production. Infact Swiss oils are commercial oils that are not produced in Switzerland but are produced in different part of the world, while the Italian oils are monovarietal oils produced only in Italy.

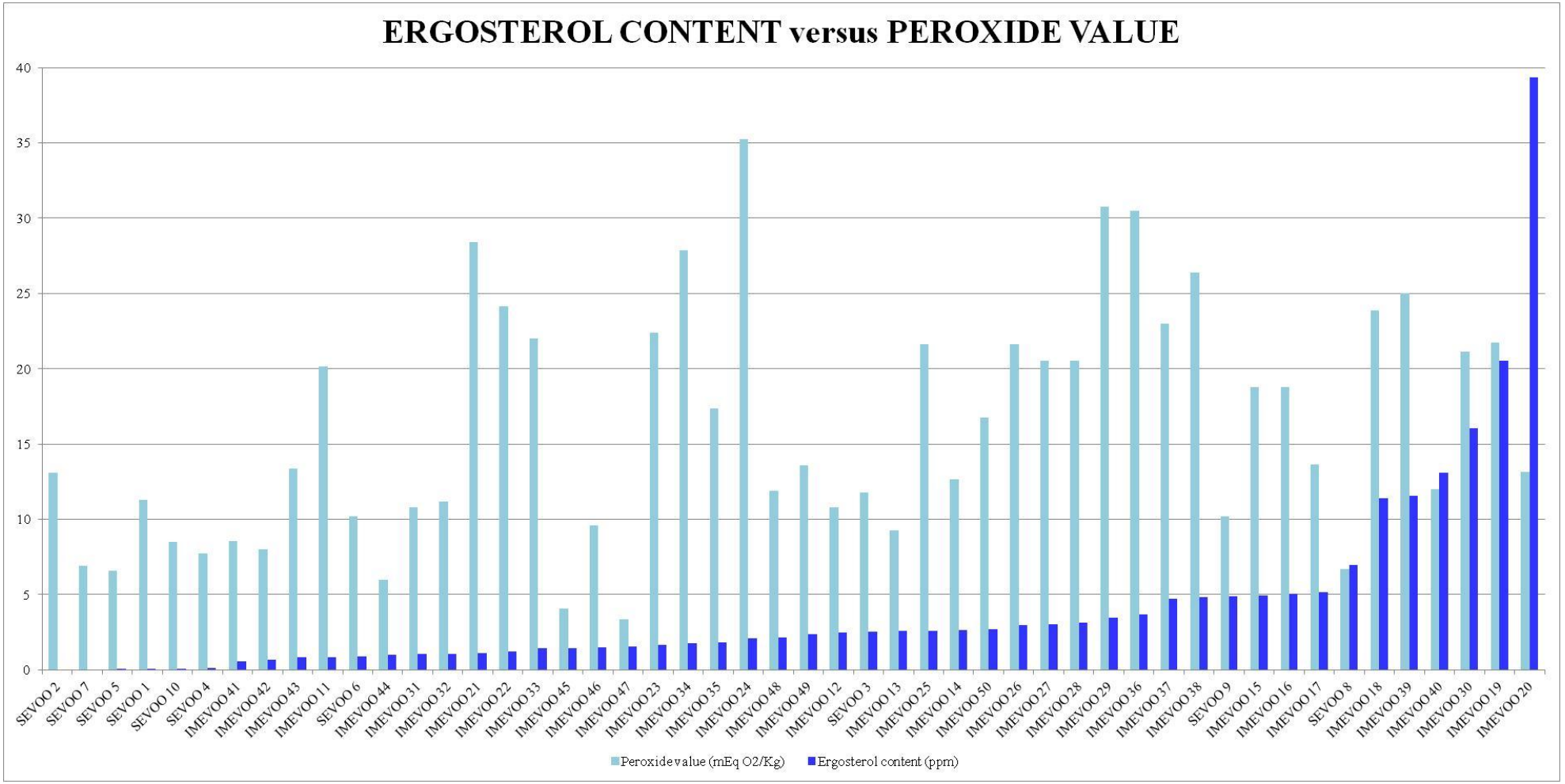
3.3.2 Correlation between ergosterol and other quality parameters

Another important point of this research work, was the comparison between the ergosterol concentration in the samples and the parameters conventionally used to determine the quality of an oil (peroxide value*, fatty acid alkyl esters* and the results of sensorial analysis*), to determine a possible correlation between them.

*see chapter 2 for the analysis procedures.

Ergosterol content versus peroxide value:

In the graph below (Graph 2), the ergosterol content and peroxide value (PV) for each sample analyzed were compared. According to the current legislation [13], for edible virgin oils (both extra virgin and virgin oils) the maximum peroxide value limit is set at 20 mEq O_2/Kg of oil, above which the oil is classified as "lampante". Observing the results, it is not found a linear correlation between these parameters. Anyway, it is possible to see that in Swiss oils, where a lower ergosterol content has already been registered compared to Italian oils (see Graph 1), there is also a low peroxide value (always below the legal limit).



Graph 2. Ergosterol content versus peroxide value

Ergosterol content versus ethyl esters content:

Ergosterol concentration was compared also with the ethyl esters content (Graph 3). In order to evaluate this possible correlation, ten oils among all those analyzed were selected. The choice of these samples was based on their high content of ethyl esters (range from 7 ppm to 596 ppm). Considering the European Commission Regulation No. 61/2011 [14], the legal limit for fatty acid alkyl esters (FAAEs) are the following:

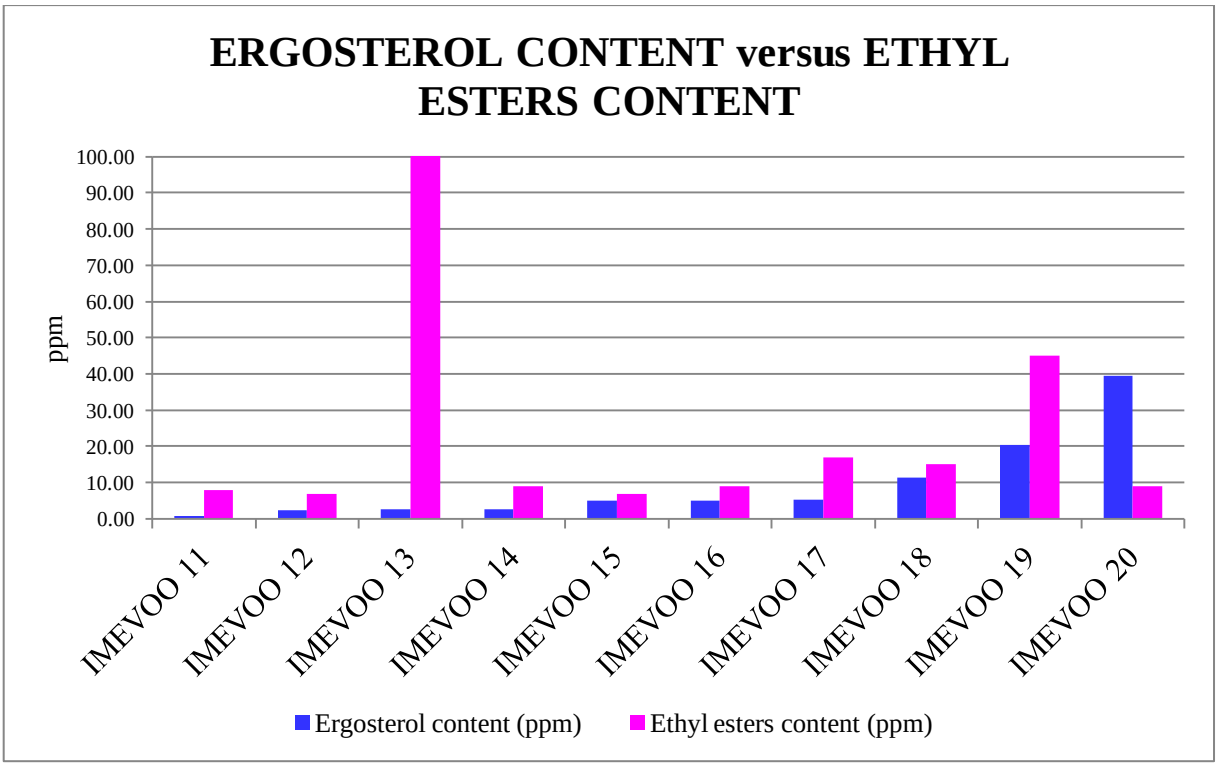
$$\Sigma\ (\text{FAME} + \text{FAEE}) \leq 75\ \text{mg/Kg}$$

or

$$75\ \text{mg/Kg} < \Sigma\ (\text{FAME} + \text{FAEE}) \leq 150\ \text{mg/Kg}$$

if $(\text{FAME/FAEE}) \leq 1.5$

FAAEs, mainly ethyl (FAEEs) and methyl esters (FAMEs), are formed by the esterification of free fatty acids (FFAs) with low molecular alcohols, such as methanol (MeOH) and ethanol (EtOH) [15]. Inappropriate practices during the olive oil extraction process and bad quality of the olive fruits promote their formation. Low amounts of MeOH and EtOH are accepted since small quantities of these alcohols may be formed during the maturation of olives. Anyway, high volumes of EtOH appear during the fermentation processes occurred mainly throughout olive oil storage [16]. It is known in fact that the alkyl esters are formed effectively as a result of degradation processes or fermentation in the olives of low quality that can be over-ripe, deteriorated or only badly stored before processing. It was also demonstrated that the methyl esters usually are formed with technological transformations of over-ripe olives, while the ethyl appear to be more related to poor raw materials or at mixing, since their formation is related to the damage of the cell structure of the olives that causes escape of aqueous fraction with consequent processes of alcoholic fermentation of the sugars with the production of ethanol. However, considering the purpose of this work, a better correlation with fatty acid ethyl esters is expected, since olives infected by any type of microorganism are likely to be infested by yeast, even if it remains to be determined, which of the two parameters is more appropriate. Anyway, ergosterol is produced not only by yeast and its transfer to the oil is straight forward.

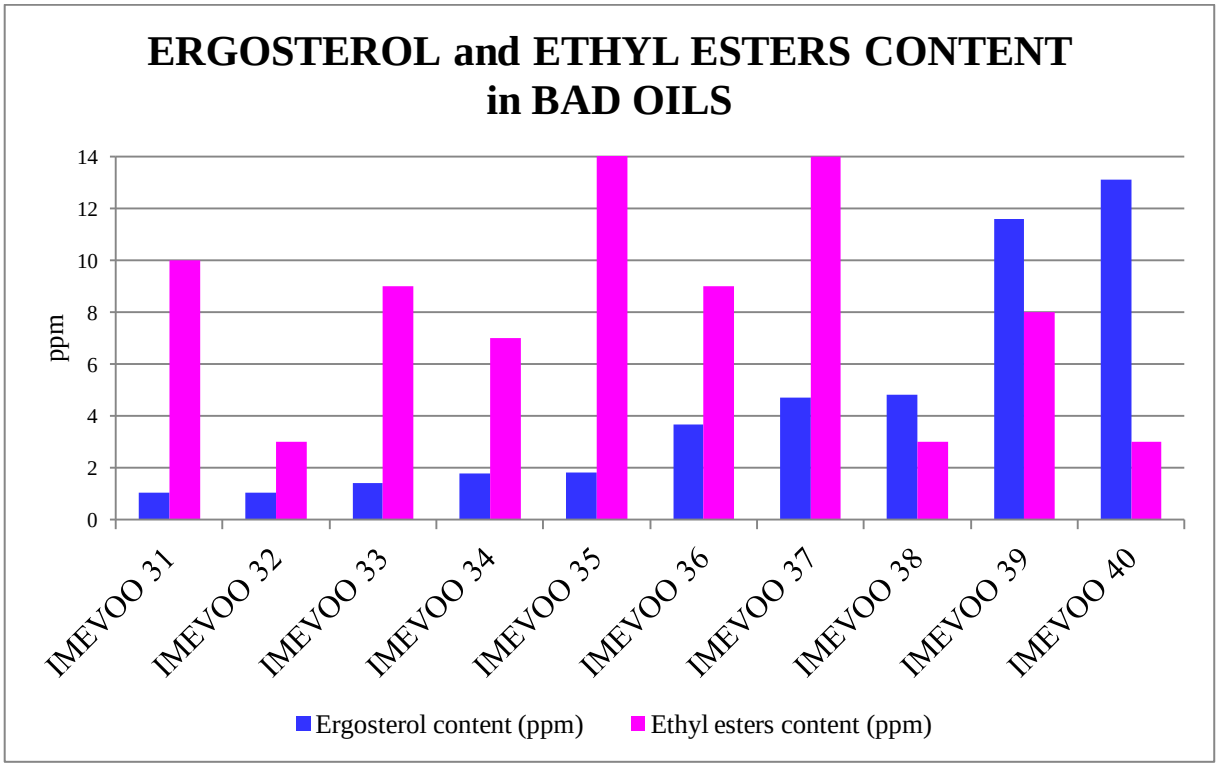


Graph 3. Ergosterol content versus ethyl esters content

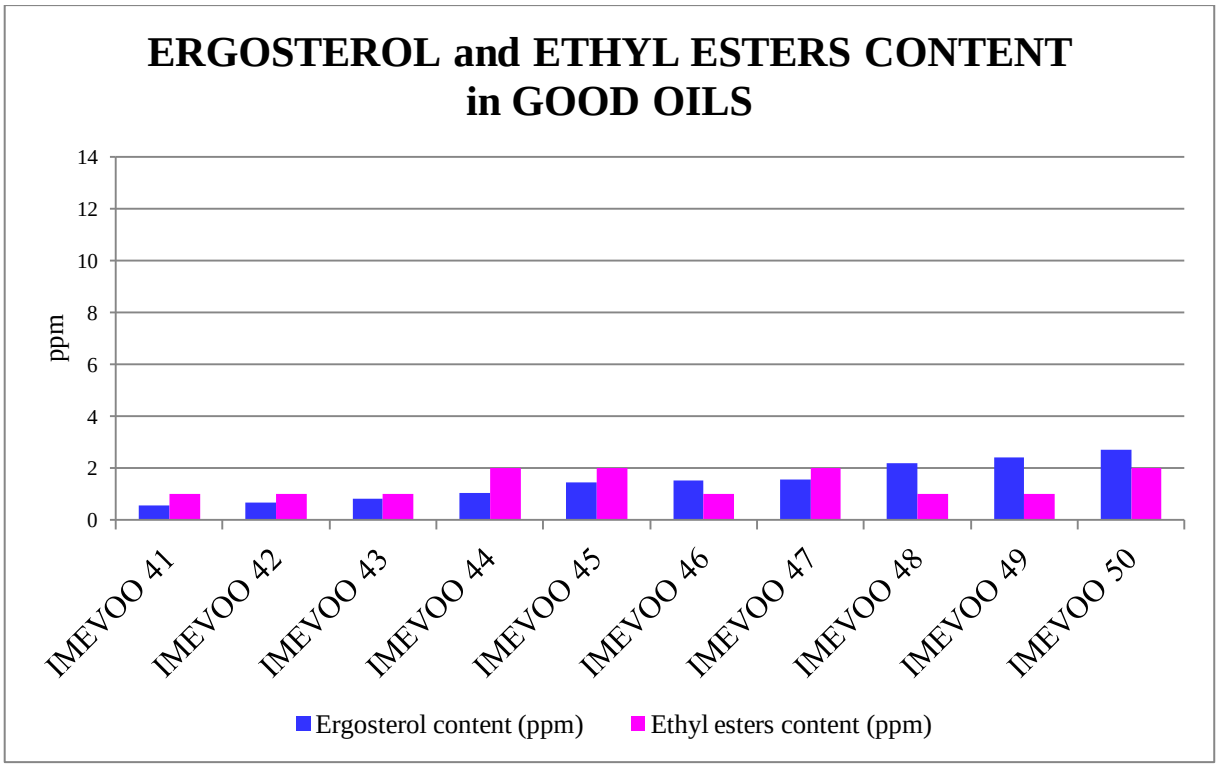
In all the samples, except for IMEVOO 20, there is higher ethyl ester content than ergosterol content. All oils with high ethyl esters also contain substantial amounts of ergosterol, it would seem that as the content of ethyl ester increases, also the content of ergosterol increases, but the ratio varies. The samples IMEVOO 13 and IMEVOO 20 instead have an anomalous behavior: IMEVOO 13 shows a high content of ethyl esters and a low content of ergosterol, at the contrary IMEVOO 20 has an high content of ergosterol and a low content of ethyl esters.

Ergosterol content versus ethyl esters content in bad and good oils:

The last comparison was made between ergosterol and ethyl esters content and the sensorial analysis results. Also in this case, among all samples analyzed, twenty samples were selected. In particular, ten of these are considered "bad" in according to the sensorial analysis (total score below 6.2); the other ten are assumed "good" according to the sensorial analysis results. More in details, in the graphs below the two different categories are reported (respectively Graph 4 for bad oils and Graph 5 for good oils).

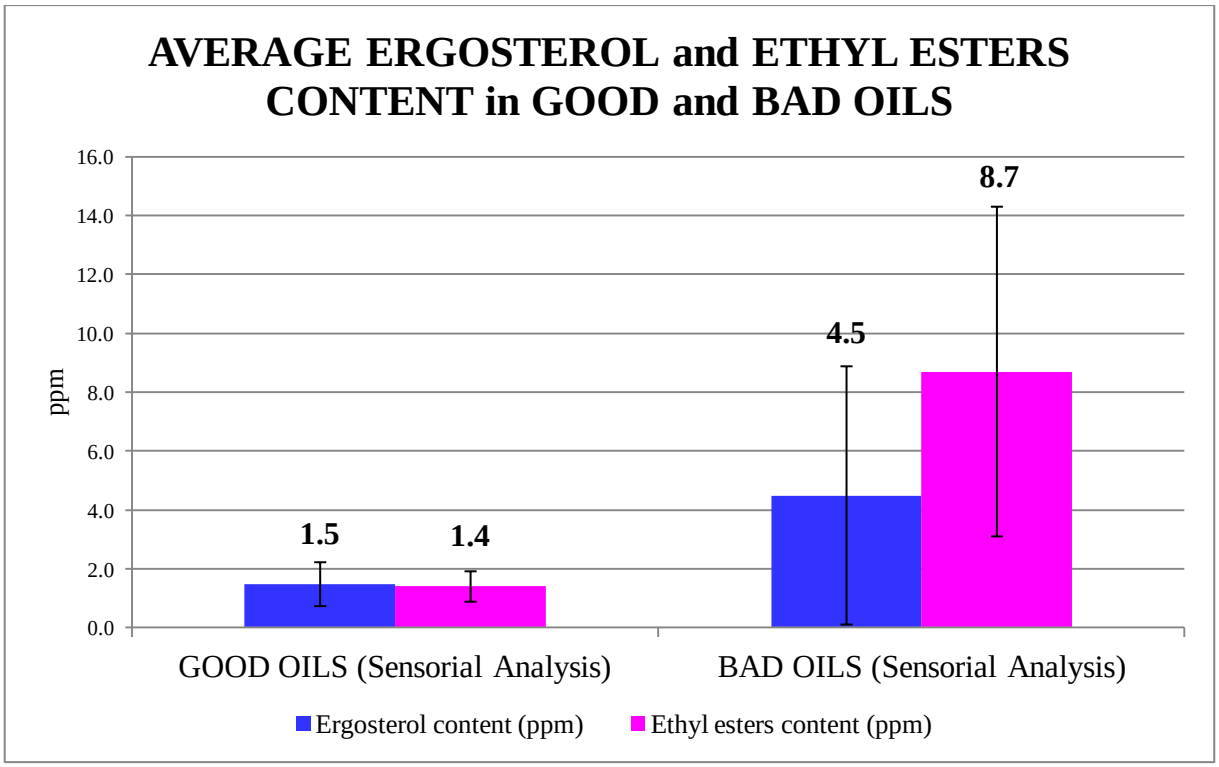


Graph 4. Ergosterol and ethyl esters content in bad oils (score from sensorial analysis below 6.2)



Graph 5. Ergosterol and ethyl esters content in good oils (sensorial analysis)

Considering only the individual oils results of both classes, it is possible to notice that most of good oils contain ergosterol below 1.5 ppm and a low content of ethyl esters (below 2 ppm); on the contrary, for bad oils only three of these (IMEVOO 31, IMEVOO 32 and IMEVOO 33) contain ergosterol below 1.5 ppm. The ethyl esters content is high in all samples of this class. Anyway, observing the graphs, it is not possible to see a linear correlation between ergosterol, ethyl esters and sensorial analysis. However, considering the average values, a clearer indication is given. In the Graph 6, the average results of ergosterol and ethyl esters content in good and bad oils are shown. In this case, moving from good to bad oils, an increase in both the average value of ergosterol (from 1.5 ppm to 4.5 ppm) and the average value of ethyl esters (from 1.4 ppm to 8.7 ppm) is found. Therefore, these results could support the hypothesis of this study.



Graph 6. Average ergosterol and ethyl esters content in good and bad oils (sensorial analysis)

3.3.3 Ergosterol in microbiologically spoiled olives

In addition to the analysis of extra virgin olive oils commercially available in Switzerland and in Italy, the present work has also paid attention to the analysis of oils obtained from the processing of spoiled olives. The olives came from Turkey and have been provided by a private source. At the beginning, using only the equipment available in the laboratory, the typical process of extracting the oil from the olives, which takes place in the common mills, was simulated. Anyway, considering the unsatisfactory results of these analyses and the operative difficulties as well as the complexity to reproduce the real conditions, the extractions were performed by means of solvents (see the applied procedure at paragraph 3.2.7).

Verified the reproducibility of the method, spoiled olives were sorted into:

- the best ones: soft, black, but not visibly damaged, called "*Good Olives*";
- somewhat wizened olives with bluish/greenish discoloration under UV at 366 nm, called "*Green Olives*";
- somewhat wizened olives with reddish discoloration under UV at 366 nm, called "*Red Olives*";
- olives with visible mold, called "*Bad Olives*".

In Figure 7, it is possible to see the different classes of spoiled olives, divided according to their coloration under the UV lamp.

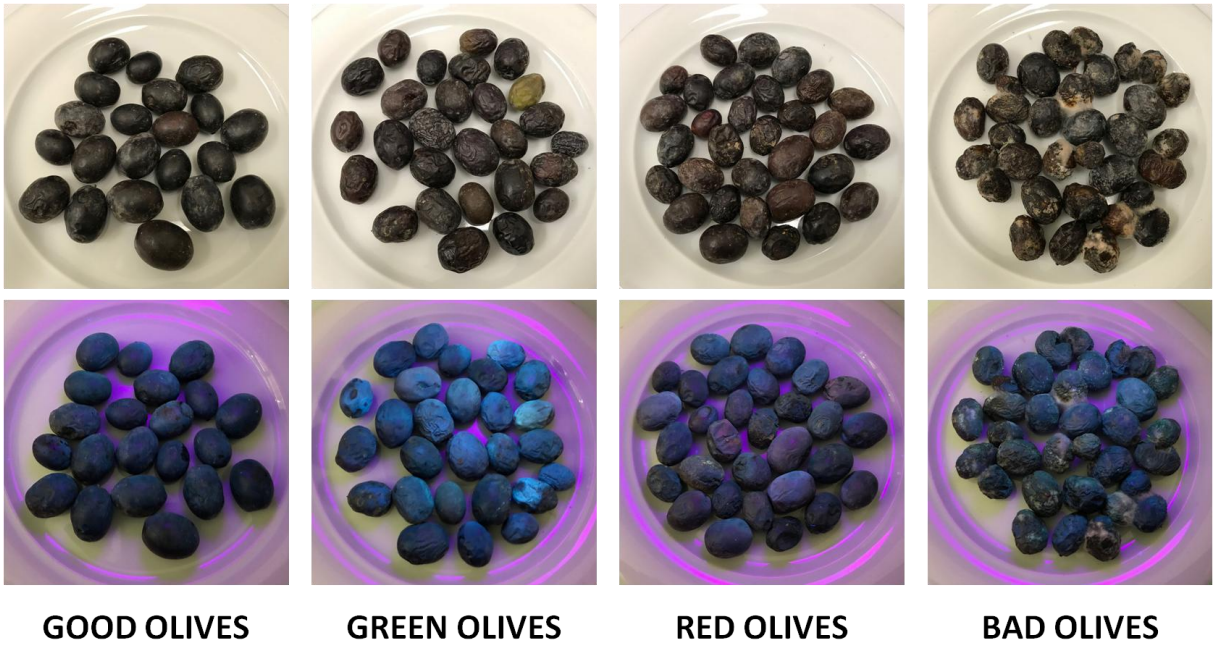
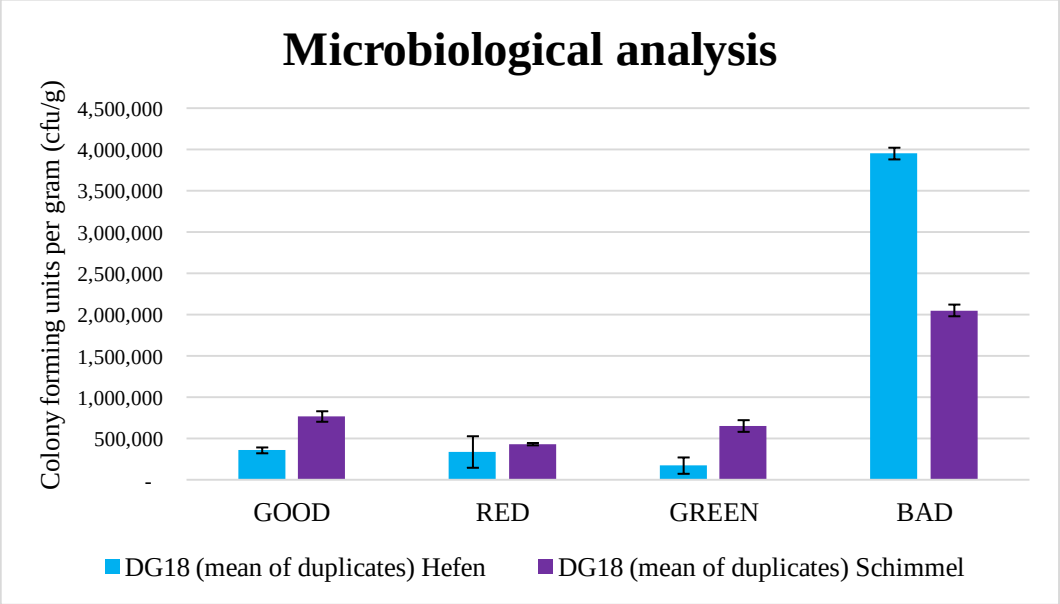


Figure 7. Olives under UV light at 366 nm

The Graph 7 and the Table 3 show respectively the microbiological analysis results and ergosterol content (in ppm). The two kinds of analyses give the same information and results. More in details, the good olives are not really good, in terms of ergosterol content, respect to green and red olives, which have a low content of ergosterol. Therefore, the visual sorting was failed, i.e. spoiled olives are not necessarily detected by visual inspection. The oil from severely spoiled olives contains roughly 10 times more ergosterol (289 ppm) than oils obtained from high quality olives (21 ppm). This means could be that a single degraded olive may cause the ergosterol concentration in oils to be strongly increased (for example, a single severely degraded olive out of 100 olives of perfect quality increases the ergosterol content above the high levels observed in the commercial oils).



Graph 7. Microbiological analysis results

SAMPLE	ERGOSTEROL (ppm)
GOOD OLIVES	197
GREEN OLIVES	21
RED OLIVES	49
BAD OLIVES	289

Table 5. Ergosterol content in olives

3.4 Conclusions

Ergosterol is a marker for the presence of yeast and also fungi in olives, but not for bacteria. Its concentration in olive oils is proportional to the abundance of degraded olives and the degree of degradation among the olives used to make the oil. It could be a more direct indicator of spoiled olives than the fatty acid ethyl esters, since the presence of the latter depends on the extent of the transesterification, such as the duration of storage of the olives, acidity and temperature.

In conclusion, considering the objective of this research work and the results obtained, we can summarize that high quality oils contained less than 0.5 mg/kg ergosterol, whereas concentrations in oils extracted from degraded olives were in the order of 200 mg/kg. The high ergosterol concentration in oils derived from degraded olives proves the validity of the concept. The correlation between this new possible marker and the parameters conventionally used to assess olive oil quality, demonstrate a certain correspondence (not always linear). Even if, for example, for the comparison with the ethyl esters it would be helpful to investigate the transesterification process and the reliability of the ester concentration for the amount of ethanol formed in the infected olives. These are only preliminary results; the study needs to be extended (for example by evaluating the stability of ergosterol during time), supported with the investigation of other samples and interpreting the results with the help of the statistical analysis.

References

[1] S. N. N. S. Hashim, L. J. Schwarz, B. Danylec, K. Mitri, Y. Yang, R. I. Boysen, M. T. W. Hearn, 2016. Recovery of ergosterol from the medicinal mushroom, *Ganodermatsugae* var. Janniae, with a molecularly imprinted polymer derived from a cleavable monomer-template. Journal of Chromatography A, 1468, 1 - 9.

[2] A. Villares, L. Mateo-Vivaracho, Ana García-Lafuente, Eva Guillamón, 2014. Storage temperature and UV-irradiation influence on the ergosterol content in edible mushrooms. Food Chemistry 147, 252 - 256.

[3] E. Ermakova, Y. Zuev, 2017. Effect of ergosterol on the fungal membrane properties. All-atom and coarsegrained molecular dynamics study. Chemistry and Physics of Lipids 209, 45 - 53.

[4] W. Guan, J. Zhang, R.Yan, S. Shao, T. Zhou, J. Lei, Z. Wang, 2016. Effects of UV-C treatment and cold storage on ergosterol and vitamin D_2 contents in different parts of white and brown mushroom (*Agaricus bisporus*). Food Chemistry 210, 129 - 134.

[5] S. Hammann, K. Lehnert, W. Vetter, 2016. Esterified sterol and their contribution to the total sterols in edible mushrooms. Journal of Food Composition and Analysis 54, 48 - 54.

[6] B. Canabate-Dıaz, A. Segura Carretero, A. Fernandez-Gutierrez, A. Belmonte Vega, A. Garrido Frenich, J. L. Martınez Vidal, J. Duran Martos, 2007. Separation and determination of sterols in olive oil by HPLC-MS. Food Chemistry 102, 593 - 598.

[7] E. Hatzakis, G. Dagounakis, A. Agiomyrgianaki, P. Dais, 2010. A facile NMR method for the quantification of total, free and esterified sterols in virgin olive oil. Food Chemistry 122, 346 - 352.

[8] C. L. Flakelar, P. D. Prenzler, D. J. Luckett, J. A. Howitt, G. Doran 2017. A rapid method for the for the simultaneous quantification of the major tocopherols, carotenoids, free and esterified sterols in canola (Brassica napus) oil using normal phase liquid chromatography. Food Chemistry 214, 147 - 155.

[9] İ. Sani Özdemir, Ç. Dağ, G. Özinanç, Ö. Suçsoran, E. Ertaş, S. Bekiroğlu, 2018. Quantification of sterols and fatty acids of extra virgin olive oils by FT-NIR spectroscopy and multivariate statistical analyses. LWT - Food Science and Technology 91, 125 - 132.

[10] R. Giacalone, S. Giuliano, E. Gulotta, M. Monfreda, G. Presti, 2015. Origin assessment of EV olive oils by esterified sterols analysis. Food Chemistry 188, 279 - 285.

[11] A. Fernández-Cuesta, L. León, L. Velasco, R. De la Rosa, 2013. Changes in squalene and sterols associated with olive maturation. Food Research International 54, 1885 - 1889.

[12] M. Biedermann, K. Grob, C. Mariani, 1993. Transesterification and On-Line LC-GC for Determining the Sum of Free and Esterified Sterols in Edible Oils and Fats. Fat Science Technology 95, 127 - 133.

[13] REGOLAMENTO (CE) N. 1989/2003 DELLA COMMISSIONE del 6 Novembre 2003 che modifica il regolamento (CEE) n. 2568/91 relativo alle caratteristiche degli oli di oliva e degli oli di sansa di oliva nonché ai metodi di analisi ad essi attinenti.

[14] REGOLAMENTO (UE) No 61/2011 DELLA COMMISSIONE del 24 Gennaio 2011 che modifica il regolamento (CEE) n. 2568/91 relativo alle caratteristiche degli oli d'oliva e degli oli di sansa d'oliva nonché ai metodi di analisi ad essi attinenti.

[15] M. C. Perez-Camino, A. Cert, A. Romero-Segura, R. Cert-Trujillo, W. Moreda, 2008. Alkyl Esters of Fatty Acids a Useful Tool to Detect Soft Deodorized Olive Oils. Journal of Agricultural and Food Chemistry 56, 6740-6744.

[16] R. B. Gòmez-Coca, R. Cruz-Hidalgo, G. D. Fernandes, M. C. Perez-Camino, W. Moreda, 2014. Analysis of methanol and ethanol in virgin olive oil. MethodsX 1, 207-211.