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EFFECTS OF SOME PRACTICES OF CITRUS POSTHARVEST MANAGEMENT ON FRUITS QUALITY AND AROMATIC FINGERPRINT

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Preface

The citrus flavor is one of the most attractive and recognized flavors worldwide, and it is one of the major characteristics able to influence the consumer acceptance. The main objective of this research work is to investigate the relation among flavor, chemical characteristics and postharvest management.

This thesis is organized in two parts: the first one is a short state of art of citrus and the second one represents the experimental part of the work carried out during the three years of my PhD program.

More precisely, the first chapter contains the citrus world's economic situation, and an overview on the physico-chemical composition of citrus fruits and juices. A small review of the most popular varieties cultivated in Sicily, with particular regard to traditional cultivars, will be also presented. Considering the extent of these arguments, only major characteristics will be highlighted.

In the second chapter, the main theme of the thesis will be discussed. Citrus flavor will be approached from the chemical point of view, as the chemical classes that contribute to the overall aroma. Then, its relation with post harvest management will be analyzed.

Regarding the experimental part, first the motivational approach and the main objectives of the experiments conducted will be explained. Then, the methods and the techniques applied will be described.

The first experiment is focused on three lemon traditional varieties cultivated in Sicily. Different part of the experiment investigates different characteristics, mainly aromatic pattern and antioxidant properties, of lemon juices. These experiments were carried out thanks to the collaboration with the Research Center for Citrus and Mediterranean crops (CRA – ACM) and with the RoccaCoop that provided the fruits and collaborated with me making available their habits in citrus postharvest management.

The second experiment was done in Spain, in collaboration with the Polytechnic University of Valencia. The leitmotif of this experience was to evaluate the aromatic pattern of Salustiana oranges, focusing on different postharvest treatments.

Moreover, in this work an instrumental comparison between two different Electronic Nose instruments was performed. This experiment were carried out thanks to the Emilio Esteve farm, in Xeraco in the region of Valencia, that kindly provided the fruits and the methods to perform the experimental conditions.

Finally, the global results of the work and the general conclusions will be presented.

Index

Part I

1. INTRODUCTION

1.1 Citrus: Production and Consumption

Citrus are the most widely cultivated fruit crops and rank first in the world fruits production (FAOSTAT2012). Citrus are cultivated in more than 50 countries worldwide, and their production grew enormously during the last four decades of the twentieth century reaching in 2011 almost 9 million ha of growing areas and a production of about 130 million tons (FAOSTAT2012).

Due to their need of a temperate climate, the mayor producer countries are located in the tropical and subtropical regions, with the Mediterranean region ranking around 20% of the world citrus production. The main producer countries are China, Brazil, USA, and Mexico (CLAM, 2007; Liu et al., 2012; Youseif et al., 2014).

In all these countries the cultivated area has been almost constant in the last ten years, with a slight increase in the total production. China is the only country in which, compared with a slight increase in cultivated area, there has been a more than doubling of production (Calabrese, 2009).

Among the Mediterranean regions Italy and Spain are the main contributors to citrus production, with a harvested area of 168.802,00 ha and 317.605,00 ha respectively (FAOSTAT20212).

Italy is the second citrus producer in the Mediterranean region and it produces around 4% of the world orange and lemon crops (Schimmenti, 2009; Baldi, 2011). Like in the other producer countries, in the last 10 years there was a slight decrease in total citrus area, mainly due to the abandoning of orchards, the lack of organization, the increasing of input costs and the small size of the farm (Pergola et al., 2013; Baldi, 2011; Aguglia et al., 2008).

All this factors caused a loss of competitiveness on both the foreign and domestic markets with the benefit of the other Mediterranean producing countries (Baldi, 2011).

Despite the reduction of the cultivated areas, in the last years the production rate increased with a total production around 3.8 million tons in 2011.

Oranges constitute the bulk of citrus fruit production, accounting for approximately 60% of global citrus production, followed by lemons and limes group with a total production about 12%. The remaining 28% consists of grapefruit, tangerines, mandarins, clementines and satsumas (FAOSTAT2012).

The distributions of the citrus area in Italy is concentrated in the southern regions, particularly in Sicily and in Calabria that together are responsible of more than 80% of total citrus production (ISMEA, 2013).

The worldwide importance of citrus, both on the fresh and processed markets, is in constant rising mainly due to their preferred flavor and important role in human health (Ting, 1980).

In the last years in fact, the awareness of the health benefit deriving from the consumption of citrus fruits has been increasing. About two-thirds of the citrus produced worldwide is consumed as fresh fruit, the rest is processed primarily into juice (Rouseff and Perez-Cacho, 2007; Liu et al., 2012). The bulk of citrus fruits produced in Mediterranean region are used primarily for fresh market and domestic consumption, especially regarding Italian blood oranges (Calabrese, 2009; Tounsi et al., 2010; Baldi, 2011). The rest of the production is intended for industrial processing for the production of juices, essences and secondary derivatives, or for exportation. In fact, more than 30% of fruits are exported especially to Northern European markets, such as Germany, France and United Kingdom, and to Eastern European countries and Russia (Aguglia et al., 2008; ISMEA, 2013).

Italy contributes to exportation only for 3%, while the rest is made up of products of Spanish origin (Calabrese, 2009; ISMEA, 2013). Industrial processing affects approximately 20% of production for the obtaining of juices and essences. Even secondary products and by-products deriving from citrus processing are valuable on markets, like the oils derived from the flavedo that are used as source of flavor in the industries (Rouseff and Perez-Cacho, 2007).

From the point of view of the cultivated varieties, a large breeding program has been conducted on the mandarins group, including mainly tangerines and mandarins. It is

a very dynamic sector, especially due to the consumer interest in new varieties, characterized by easy peeling and seedless fruits with optimal size and shape, and early or late ripening, extending the commercialization calendar. Not the same massive work has been conducted on lemons and oranges varieties, which have remained almost the same over time (Lorente et al. 2014; Calabrese, 2009). The static nature of lemons and oranges markets resulted in an aging of the varietal panorama of these two species. For lemons, for example, the majority of selection programs were directed principally to increase the resistance to "Mal Secco" disease, caused by the fungus *Phoma tracheiphila* (Calabrese and Barone, 2009). So, the last breeding programs of this species lead to selection of the varieties mainly on the basis of their ability to survive and produce, without focusing on the quality and commercial characteristics of the fruits. An intensive study on the aromatic characteristics of old and traditional varieties, combined with an investigation on the antioxidant properties, could lead to re-assessment the importance of these cultivars.

1.2 Citrus: Varieties cultivated in Italy

The main citrus varieties cultivated in Italy are the Navel orange group ('Navelina', 'Newhall', Navelate' and 'Lane Late'), the pigmented oranges ('Tarocco', 'Moro', and 'Sanguinello' with their hybrids), the lemons ('Femminello' and hybrids, 'Monachello', 'Interdonato', and 'Lunario'), and the mandarin-like fruits (Mandarins, Clementines, hybrids and Satsumas). Concerning blond oranges, this group is very restricted in Italy due to the major interest towards pigmented ones (Pergola et al., 2013; MiPAF 2006). Briefly, a description of the major varieties cultivated in Italy is reported, as described in The Citrus Industry (Reuther et al., 1967)

Lemon (*Citrus limon* L. Burm)

Femminello Group: it is the most important lemon group in Italy, covering almost 70% of the cultivated area. In general, all the selections within the Femminello group are characterized by a good tolerance to *mal secco* disease. The trees set fruit throughout the year, and are characterized by a constant production over the years. Trees are culturally managed so as to produce four crops per year. The autumn crop

is called Primofiore, the winter to spring crop is called *Limoni Invernali*, the spring crop is called Bianchetti, and the summer crop is called Verdelli. **Femminello Comune** is the most representative variety of the group. Fruits are medium sized and elliptical to oblong. The rind is medium thick, finely pitted with sunken oil glands, and yellow at full maturity. The flesh is pale greenish-yellow, low-seeded to seedless, juicy, and very acidic. This cultivar, through several bud mutations and human hybridizations, arose a number of local cultivars and clones like F. Santa Teresa; F. Zagara Bianca; F. Sfusato.

Femminello Santa Teresa is one of the oldest varieties. It has unknown genetic origin, as said before probably originated from a mutation of 'Femminello Comune'. Its cultivation has been confined to a small area due to its low production rate and poor quality of the production. Fruits are rich in essential oils and have high juice content, which is acidic and rich in seeds. The major advantage of this cultivar is its high tolerance to *mal secco*. According to Reuther and Webber (1967) "the parent tree was an old disease-free tree discovered in a Femminello orchard that had almost been destroyed by the disease (Russo, 1955). It is said to be the variety currently most planted as a replacement in areas of Italy where the disease is severe." **Femminello Zagara Bianca** is one of the most appreciate variety because of the high quality of the fruits, the high rate of reflorescent, high tolerance to mal secco and constant production rate. The name derives from the characteristic color of the flowers that are totally white and similar to oranges.

Monachello: unknown genetic origin. With the cultivar F. Santa Teresa is the most tolerant cultivar to mal secco. For this reason this cultivar has been one of the most diffused in Italy, and currently it is planted only in areas where mal secco is very severe. However the fruits are medium-small, rich in seeds, low in juice and acidity. The rind is thin, the surface is smooth but with large sunken oil glands, very tightly adherent. Trees are slow growing, and with low production rate in comparison with Femminello and well adapted to forcing but with markedly reduced winter crop. Even from this cultivar arose several clonal selections, like the" nucellare" produced by Research Center for Citrus and Mediterranean crops (CRA – ACM) of Acireale. Certain characteristics of this variety, particularly the distinctive growth habit and cross-sectional shape of the larger branches, suggest that it is a lemon-citron hybrid.

Interdonato: It is the earliest of Italian varieties, which produces in fall and early winter. According to Burke (1962), in origin this variety has been planted because of its resistance to mal secco disease, to which its resistance is said to be intermediate between the Femminello and Monachello varieties. Respect to these varieties, however, the Interdonato produces fruits of better quality that are large and juicy with oblong-cylindrical shape. The rind is thin, very smooth and adherent, and the flavor is highly acid with slight bitterness. This cultivar is moderately productive but does not respond well to "forzatura" treatment, aimed to Verdelli production, and hence grown primarily for early fruit. Interdonato is considered a lemon-citron hybrid.

Orange (*Citrus sinensis* Osbeck)

Navel group: Almost all the varieties of this group derive from a bud mutation of 'Washington Navel' that is the forefather of the entire navel group. **Navelina** is the most diffused cultivar of the group in Italy, due to the high quality of fruits and juice. In fact, fruits of this cultivar present a little navel, big and spherical shape and seedless, the color is reddish-orange at maturity. The juice is very sweet with a high sugar content and with a sweet flavor that is less sprightly than Washington navel. Maturation is in October-November but fruits can be hold on the tree for a long time with an increase of the sugar content as the only effect. The **New Hall** variety originated as a limb sport of a Washington navel orange. It produces fruits that are seedless, elongated, big shape and weigh, and it is characterized by a big navel. With Navelina, represent the earlier orange production in Italy. The juice is sweet, with a good ratio between sugars and acids. It is used mainly for fresh consumption and only rarely for industrial processing. Regarding the late navel oranges, major varieties are **Navelate** and **Lane Late**, whose maturation starts in January to June. Fruits of both varieties are seedless and can store on the tree for several months after reaching maturity before the quality deteriorates. The juice is abundant and sweet, due to a high content in soluble solids. In general, Navelate trees have a low production rate, and for this reason the cultivar 'Lane Late' is preferred.

Oranges without navel: **Ovale Calabrese** Unknown origin. Fruits do not have navel, are ever blooming and not very productive. Fruits are well colored at maturity stage, but re-greens if held on the tree long thereafter. This cultivar is characterized by late

maturation, from March till May, fruits are hard peeling and with seeds. The juice possesses high quality, being very sweet and easy to squeeze. Nowadays in Italy this cultivar is not diffused anymore, except in the traditional growing area, Calabria and Sicily near the Tyrrhenian coast, where it is used mainly for domestic or industrial juice extraction. **Valencia** is the widespread cultivated orange variety in the world. It is characterized by a late maturation from April to June and it is almost the last orange on the market. Good for long-term storage but not for long term holding on the tree because of the re-greening of the peel, that is more accentuated than in the 'Ovale Calabrese'. It is characterized by good juice content, high sugar and vitamin C content. **Salustiana** is an ancient variety probably originated in Spain in the Valencia community, where is cultivated nowadays. It is characterize by early and extended maturation, from December until March. Fruits are seedless, juicy and sweet. Good for juice extraction.

Pigmented oranges: **Tarocco** is the most known and appreciate. Probably introduced in Italy at the beginning of '800, nowadays it's almost the leader orange in local markets reaching 45% of the total Italian production of oranges (Recupero and Russo, 2009). The characteristic red color is due to the presence of anthocyanin, a colored pigment present just in mature oranges. Anthocyanin production is very dependent on genotype and environmental factors. Among growing factors, temperatures play a key role in the synthesis of anthocyanin being low temperature during night essential for their formation in the ripening and maturity stages. The fruits are big and easy peeling, but not very resistant to storage. This is the main reason for the intense breeding programs started in '60 and focused on lengthening the harvesting season; increase and stabilization of the anthocyanin content; increase the sugar/acid ratio and the persistence of the fruit on the tree. **Moro** is the most pigmented orange in the group of blood oranges, due to an impressive content of anthocyanin. It's used mainly for industrial processing, especially by mixing with non-pigmented juices. The juice, in fact, has a very distinctive flavor, which is very sweet and rich compared to that of the navel oranges. Unlike the 'Tarocco', this variety has not been subjected to genetic improvement and the fruit does not have characteristics of particular value. **Sanguinello** is characterized by a reddish skin, few seeds and a sweet and tender flesh. It ripens in February, but fruits can remains on the tree until April.

1.3 Citrus fruit morphology and ripening

The genus Citrus belongs to the family of *Rutaceae*, which is probably originated in subtropical and tropical regions of Asia, whereas new researches suggested that some species of citrus are actually native to Australia and New Guinea (Liu et al., 2012).

Botanically, citrus fruit is a hesperidium, a specialized berry composed in general of three parts: the outer peel, a leathery aromatic rind called "flavedo", rich of oil glands and carotenoids; the inner peel, called "albedo", a spongy parenchyma tissue rich in sugar and peptic substances; and the endocarp, the edible portion composed by segments filled with multiple-fluid filled sacs. Segments are usually aligned around the soft central core of the fruit and covered by a thin membrane called septum. The cytoplasm content of sacs is the primary source of the juice (Liu et al., 2012).

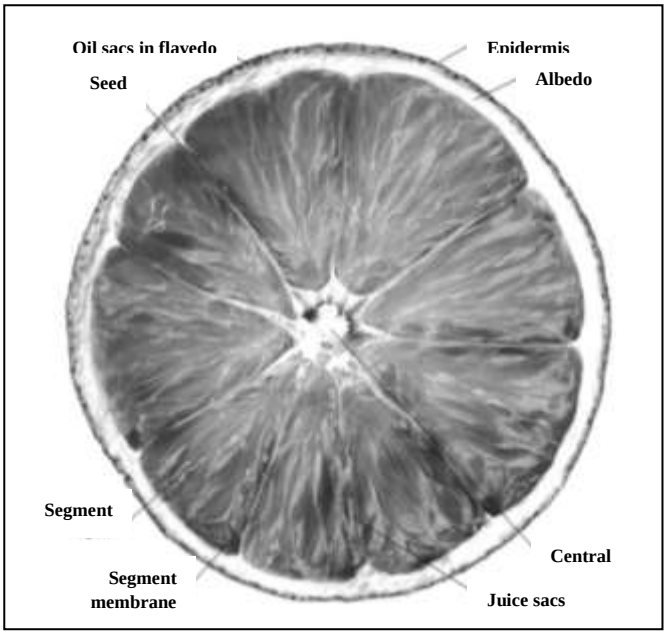

Fig. 1. Schematically representation of a citrus fruit, from Liu et al., 2012

Citrus fruits growth and development follow a characteristic sigmoid growth curve, divided into three clear-cut phases (Bain, 1958). The initial stage, or phase I, is characterized by a high division process and slow growth of cells, including the period between anthesis and June drop. Phase II is a rapid growth period, with a tremendous increase in cells sizes determined by water accumulation during four to six months. The last growth stage, phase III, is the final ripening period. Cells growth

is mostly arrested and fruits undergo a non-climacteric process (Iglesias et al., 2007). Citrus fruits, in fact, are classified as non-climacteric, based on the absence of a postharvest, ripening-associated rise in ethylene evolution and respiration (Katz et al., 2004). During ripening, fruits growth slows down, ethylene production is low, respiration is attenuated and changes in texture and composition proceed gradually (Eaks, 1970; Goldschmidt et al., 1993). Major ripening symptoms are: color break; rise of soluble solids and nitrogenous compounds contents; decrease of total acidity level. Metabolite accumulation and increasing are strictly and inversely related with temperature. After complete ripening, maturation process starts. During this process, fruits reach their complete development, assuming all the external differences, flavor, and texture that are characteristic of mature fruits. Main maturity symptoms are: accomplishment of final external color, due to complete degradation of chlorophylls and synthesis of carotenoids; weight loss; sugars enhancement; decrease of acidity. After maturity, other changes may occur that define senescence of fruits: turgidity loss; parting of tissues; overall quality loss determined above all by senescence of peel tissues (Agustí, 2009). Quality traits are acquired during phases II and III, and are related to many physical properties, such as size, shape, color, texture, and chemical components, such as sugars, acids, flavor compounds, volatiles and nutraceutical substances like vitamin C (Iglesias et al., 2007). Evolution of major quality characteristics during citrus fruits growth is reported in Figure 2.

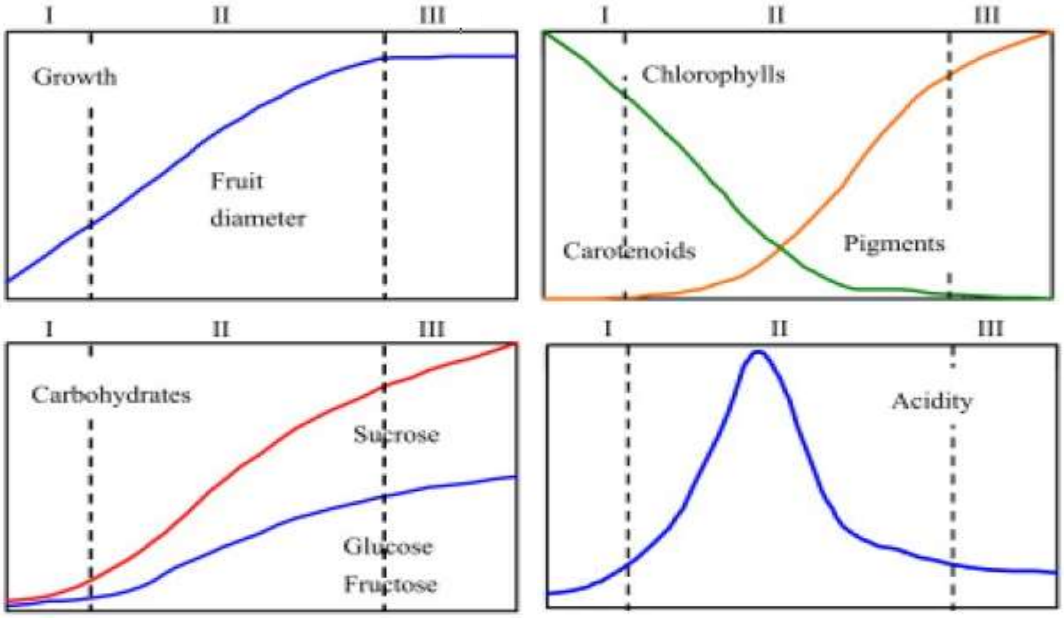

Fig. 2. Schematically representation of metabolic changes associated with maturation process, from Iglesias et al., 2007

The chemical composition is strictly dependent from environmental factors and growing conditions such as rootstocks, stage of maturity as well as genetic factors (Ranganna et al., 1986). In general, citrus fruits are rich in macronutrients, like simple sugars and dietary fiber, and contain several micronutrients including folate, thiamin, niacin, vitamin B, riboflavin, pantothenic acid, potassium, calcium, phosphorus, magnesium, and copper, which are essential for the normal growth and the correct functioning of the human physiological system (Liu et al., 2012; González-Mas et al., 2010).

Generally, the juice is an aqueous solution with high acidity level and cloudy appearance, caused by colloidal and dissolved pectin. The levels of different compounds vary according to species, cultivars, maturity stage, and growing factors (Lorente et al., 2014).

The main components in citrus juice are:

- Sugars:

  Sucrose, glucose and fructose, generally in the ratio of 2:1:1, are the major components of this fraction, and are responsible for the sweetness of the juice (Kefford, 1966; Ting and Attaway, 1971). The total sugar content could range from lower than 1%, in some limes fruits, to as high as 15% in some oranges, depending on the specific fruit and variety (Sass-Kiss et al., 2004). Other sugars, like mannose, maltose and galactose, are present in extremely low amount. Maturity and variety are the main factor affecting the sugar content in citrus juice. Actually during ripeness the content of the different sugars can vary tending to an increase with maturity (Ting and Attaway, 1971; Izquierdo and Sendra, 1993).

- Polysaccharides:

  Represent the main component of the insoluble portion. The biggest parts of this fraction are pectic substances, cellulose, lignin and hemicelluloses that are contained in peel, pulp, juice and membrane (Ting 1980). They contribute to the body of the juice and to a desirable juice quality (Nagy and Shaw, 1990; Hirsch et al., 2012). In general, the polysaccharides of citrus fruits, particularly in the peel and pulp, are considered a source of dietary fibers, and play an important role in human health (Liu et al., 2010). In literature the role

of the dietary fiber as antioxidant factor is well claimed. Several studies showed that dietary supplementation of pectin determines a decrease in levels of blood cholesterol and serum glucose (Fernandez et al 1994; Larrauri et al., 1996) and it has been shown to have potential beneficial effects in human health (Kertesz, 1951; Baker et al., 1994; Yamada et al., 1996)

- Organic acids:

  The most representative in citrus fruits are citric, malic and succinic acid (Kefford, 1966). These are carboxylic acids that can be found in the free form or in the form of salts, such as citrates and malates (Clements, 1964). The contemporary presence of free acids and cations, like potassium, calcium and magnesium, origins a buffer system that regulates internal pH. The ratio between sugars and acidity content is called maturity index, which plays a key role in the consumer and commercial acceptability providing the delightful and typical taste of citrus fruits. As well as sugars, also the acid content depends on the maturity, storage, climate and temperatures. In general, during maturity the gradual decrease of citric acid leads to declined acidity, whereas acid malic content remains relatively constant (Rasmussen, 1963).

- Nitrogenous compounds:

  This small fraction consist of compounds present in rather small concentration, but essential in assessing juice purity (Reid et al., 2006). Free amminoacids are the most important compounds, representing about 70% of total nitrogen fraction in the juice (Zamorani et al., 1973; Ranganna et al., 1986). The most abundant are asparagine, arginine, alanine and proline, which are considered non-essential amino acids (Block and Bolling, 1944). Also, citrus fruits contain a small amount of proteins which are basically enzymes, like oxidoreductases, transferases, hydrolases and lysases (Vandercook, 1977).

- Lipids:

  Lipids can be divided in three classes: non polar, nonionic polar and ionic polar. Free fatty acids form an essential part of the non-polar group, with linolenic, oleic, palmitic, and linoleic acids as the major components. The nonionic polar lipids consist of a sugar containing lipids that includes glycosyl glycerides and sterol glucosides. The ionic polar consist essentially

of phospholipids, that represent almost 50% of the total juice lipid content (Nagy et al., 1978). They are primarily found in seeds and rinds, although they can also be found in the flesh in small quantities (Nordby and Nagy, 1971). The significant difference in the lipid content of the various citrus fruits can allow distinguishing among different species, and the fatty acid profile can even be used to identify the cultivar (Tounsi et al., 2011; Nicolosi-Asmundo et al., 1987; Nordby and Nagy, 1971). In general, the most representative fatty acids are linolenic, 21-39% of total content of fatty acids, oleic, palmitic, linolenic and palmitoleic (Nordby and Nagy, 1973; Nordby and Nagy, 1971). Despite their low content, about 0.1% in orange juice, lipids play a key role in the development of off-flavors during juices storage because of their breakdown caused by oxidative stress (Moufida and Marzouk, 2003).

- Inorganic elements:

  Citrus fruits are a good source of potassium that could constitute up 40% of the total ash, although they are generally low in sodium (Guthrie et al., 1995). Other main inorganic elements are calcium, magnesium, phosphorous. Even traces of copper, zinc, iron and manganese, which are essential in sever enzymatic reactions, can be found (Rouseff and Nagy, 1994; Liu et al., 2012). The percentage of ash and the relative concentrations of inorganic constituents are dependent upon growing conditions, like fertilization, soil type, and climate; cultivars, stage of maturity, season of harvest and geographic origin. Likewise, the percentage distribution of inorganic elements in processed products is dependent on several processing parameters like pressure used to juice fruit, pulp control, finishing and pulp washing. It was shown that some trace elements, like iron, copper and manganese, are effective in prevention and treatment of atherosclerosis (Gey et al., 1993).

1.4 Citrus Bioactive Compounds

Bioactive compounds deserve to be treated in a part, because of the growing consumers demand of high nutritional and health quality fruits. Nowadays citrus fruits are recognizing as an important aid in human health, as they possess an high

level of bioactive and natural antioxidant compounds (Lorente et al., 2014; González-Molina et al., 2010; Finley, 2005; Gorinstein et al., 2001; Craig, 1997). So citrus fruits represent a very important part of a balanced diet, particularly for their role in prevention of disease, such as obesity, diabetes, cardiovascular disease and certain types of cancer (Lin, 1994; Larrauri et al., 1996; González-Molina et al., 2010).

The antioxidant and antiradicals activities are mainly provided by the hydro soluble fraction, that contains vitamin C, flavonoids and polyphenols, and by the a polar fraction, that includes carotenoids (Gorinstein et al 2001; Tripoli et al 2007).

- Vitamins:

  Vitamins can be divided in two groups: fat-soluble vitamins and water-soluble vitamins. Vitamin A is the only fat-soluble vitamin present in citrus juice in considerable amount (Liu et al., 2012). It exists in the form of provitamin A carotenoid, with the carotenes and β-cryptoxanthin as the major vitamin A precursors (Ting, 1977; Stewart, 1977; Agocs et al., 2007). Total provitamin A carotenoids vary widely among different citrus fruits: mandarins, tangerines and pink grapefruits are the major sources (Holden et al., 1999), while red grapefruits and oranges contain lower concentrations (Lime et al., 1954; Ting and Deszyck, 1958; Holden et al., 1999). The most representative water-soluble vitamin contained in citrus juice is ascorbic acid, also known as vitamin C (Kays and Paull, 2004; Gadjeva et al., 2005). This term commonly indicates both ascorbic (AA) and dehydroascorbic (DHAA) acid. The first one is the reduced, dominant and active form (Zumreoglu-Karan, 2006). Ascorbic acid is very labile and can be oxidized into the DHAA form very easily (Halliwell, 1996; Davey et al., 2000). It is an essential water-soluble vitamin, plays a key role in human health, like the formation of collagen, a primary component of much of the connective tissue in the body, and the absorption of inorganic iron (Rowe et al., 1999). It is also a very important aid in prevent oxidative stress (Gorinstein et al., 2001). The antioxidant function of vitamin C is based on its ability as hydrogen donor that lets it inactivate free radicals preventing proteins, lipid and DNA damages (Gardner et al., 2000; González-Molina et al., 2010).

The edible portion of the fruits contains about one-fourth of the total vitamin C content, the rest in contained mainly in the peels that possess the higher concentration than the other components of the whole fruit (Gorinstein et al., 2001) Total content depends in the species and the cultivar and its level vary with ripening time, storage, processing, and climate and agronomic factors (Mozafar, 1993; Lee and Kader, 2000; Wang et al., 2007; Huang et al., 2008; Rapisarda et al., 2008). In addition to Vitamin C, citrus fruits are a source of vitamin B complex (Liu et al., 2012). In particular, vitamin B_1 (thiamin); vitamin B_6 (pyridoxal phosphate); folate, the natural occurring form of folic acid; niacin; riboflavin, and pantothenic acid (Hill et al., 1971; Rampersaud, 2007).

- Carotenoids:

  Carotenoids are the only non-polar compounds that possess antioxidant activity in citrus fruits. It is proved that carotenoids exert a potential action against certain types of cancer, cardiovascular disease and cataracts (Narisawa et al., 1999; Voutilainen et al., 2006; Trumbo and Ellwood, 2006). These compounds are also responsible for the color of the fruits, and are contained in the plastids of the flavedo and of the internal juice vesicles (Rodrigo and Zacarias, 2007). The color can range from light yellows in lemon to deep red in oranges and tangerines. Moreover, they could also contribute to the flavor developing in some citrus species, like tangerine, being precursors of potent aroma-active volatiles (Winterhalter and Rouseff, 2002). Citrus fruits contain a large number of complex carotenoids. In literature approximately 115 different carotenoids are reported, and their composition can vary depending on the location in peel or in the pulp (Goodner et al., 2001). This variation is more accentuated in orange, clementine and lemon (Agocs et al., 2007). However, almost all citrus, except lime, have similar carotenoid profile even differences can be found in the proportion of various compound. Among carotenoids present in citrus, the most representative are: α- and β-carotene, lycopene, β-cryptoxanthin, and lutein. Their content fluctuate with maturation, being higher in the last maturity stage, growing conditions and postharvest treatments, and it is very dependent on cultivars (Rodrigo and Zacarias, 2007; Kato et al., 2004; Navarro et al., 2010; Alós et al., 2006).

- Phenolic compounds:

  The phenolic substances in citrus can be classified into two groups: phenolic acids and related compounds, and flavonoids.

  The most important **phenolic acids** in citrus juices are benzoic and hydroxycinnamic acids. Gallic acid is the most representative of the hydroxybenzoic acid in citrus, even it was shown that its presence and quantity is strictly dependent by the growing conditions and by the variety diversity (Tounsi et al., 2011). In addition, gallic acid has been proven to possess strong free-radical scavenging activity (Rangkadilok et al., 2007). Citrus fruits also contain hydroxycinnamic acid and its derivatives: ferulic, p-coumaric, sinapic, caffeic and chlorogenic acids (Robards and Antolovich, 1997). Their antioxidant potential is associated with their effectiveness as hydrogen donors, which is dependent on the number and arrangement of the hydroxyl groups and on the extent of structural conjugation, as well as the presence of electron-donating and electron-withdrawing substituents in the aromatic ring (Rice-Evans et al., 2000; Clegg and Morton, 1968). In addition to their antioxidant capacity, fruit phenolics have been the subject of increased interest in the last few years because their presence can contribute to the sensory quality of the fruit and juice through their effect on color, bitterness, astringency and flavor (Sousa et al., 2004).

  Flavonoids are aromatic secondary plant metabolites that possess physiological and pharmacological activities (Del Rio et al., 2004; Tusa et al., 2007). Epidemiological studies have shown that the intake level of flavonoids is associated with a reduced risk of certain chronic disease (Sun et al., 2002; Manach et al., 2004; Burdock et al., 2006). The most important flavonoids in citrus can be classified into different groups, on the basis of their carbon skeleton: flavanones, flavones, flavanols and anthocyanins (Tusa et al., 2007). Flavonoids can exist in the glycoside or aglycone forms, but most of them commonly occur as *C*- or *O*-glycosides (Gattuso et al., 2007).

  Fresh fruits and juices contain mostly flavanones and flavones in their glycoside forms (Robards and Antolovich, 1997). Normally the glycosilation of flavanones occurs at the 7-position by two disaccharides: rutinose or neohesperidose. The most important difference between these two kinds of glycolsilations is that the flavanone neohesperidosides are strongly bitter,

whereas the corresponding rutinosides are tasteless (Tusa et al., 2007). The major flavanone glycoside that can be found in citrus are: didimin, eriocitrin, hesperidin, narirutin, naringin and neohesperidin. Anyway, each species of citrus contain a characteristic flavanone glycoside pattern that makes the flavonoids profile suitable as chemotaxonomic marker (Ortuño et al., 1997; Abad-García et al., 2012). Another group of compounds that belong to this class are the polymethoxyflavones (PMFs). These are usually found as components of the essential oils fraction of citrus peels, and their composition varies among citrus species (Gattuso et al., 2007; Peterson et al., 2006). They can also be found in the flesh as glycosides, with the same mechanism described above. Anthocyanins are water-soluble glycosides that belong to the flavonoid compounds. The red color characteristic of the rind and flesh of blood oranges is due to water soluble anthocyanins, which are reduced from the yellow flavonoids due to loss of oxygen (Merken and Beecher, 2000). Anthocyanin content has been considered as an important quality attribute in both fresh fruit market and processing industry due to its biological activity (Barbagallo et al., 2007). The major anthocyanin identified in blood oranges are cyaniding-3-glucoside and cyaniding-3-(6^{II}-malonyl)-glucoside.

- Limonoids:

  Limonoids are a group of structurally similar triterpene derivatives, that can be find only in plant family of Rutaceae and Meliaceae. In citrus fruits and juices, limonoids appear in large amount as water soluble limonoid glucosides or in seeds as water insoluble limonoid aglycones. The aglycone form is responsible for the development of delayed bitterness in citrus, and is converted to the non-bitter limonoid glucosides during fruit maturation (Jacob et al., 2000). The persistence of the extremely bitter taste can cause problems in consumer acceptance. Limonin and nomilin are the most abundant aglycone limonoids in citrus. A number of studies were conducted on these compounds, showing that both limonin and nomilin could inhibit the development of carcinogen-induced cancers in a variety of different animal models, including models for stomach, lung, and skin cancer (Miller et al., 2004).

2. CITRUS FLAVOR

Citrus flavor is one of the most appreciate flavor worldwide and it is one of the main characteristics of citrus fruits influencing consumer choice, beginning with the visual selection and leading to the consumption of the fruit. As reported by the Dictionary of Flavors, flavor is the combination of the total sensory experience (De Rovira, 2008). Although flavor is perceived by receptors in the eye, tongue, nose and mouth lining, the brain interprets the overall sensation as occurring in the mouth, localizing all the sensory information into the mouth (Taylor and Hort, 2004).

The flavor composition is influenced both by genetic and environmental factors, so it is specific to species and variety, and strictly dependent on pre and postharvest handling of fruits (El Hadi et al., 2013; Sanz et al., 1997). It derives from a complex combination of soluble compounds, principally sugars, acids, flavonoids and volatile compounds (VOCs). The overall combination of the volatile compounds that represent the odoriferous portion of the flavor profile is defined as aroma (De Rovira, 2008). Although a large number of chemical compounds have been detected in citrus fruits, only a fraction of compounds have been identified as impact components of flavor and aroma, based on their quantitative abundance and olfactory thresholds (Willye et al., 1995). For example, *linalool*, *limonene*, *valencene*, and *β-pinene* are the key aroma of many citrus species (El Hadi et al., 2013; González-Más et al., 2011). In general, the big number of compounds that compose the aromatic pattern can be divided in two classes: impact compounds, that are the key compounds responsible of the characteristic aroma, and compounds that contribute to the overall aroma. Another important characteristic is the odor threshold, which indicates the minimum concentration producing an olfactory response and permitting to be detected by the human nose/human sense of smell. The threshold values are frequently determined by smelling (orthonasal value) and by tasting the sample (retronasal value). The threshold value for an aroma compound is dependent on temperature, medium, and interaction with other odor-producing substances that can result in a strong increase in the odor threshold (Belitz et al., 2009). The ratio between the concentration of an individual substance and its odor threshold is defined as Odor Activity Value (OAV). Generally compounds that are present in

concentration higher than their odor thresholds are considered key contributors to the aroma, while the others had no or minimal effect. In orange juice, 12 compounds were demonstrated to be prominent based on the odor activity values, being *nootkatone*, *ethyl butanoate*, linalool and limonene the higher contributors (Kelebek and Selli, 2011).

Citrus VOCs are comprised of diverse classes of chemicals, predominantly terpenes and terpenoids, alcohols, esters, aldehydes, and ketones. The difference between terpenes and terpenoids is that terpenes are hydrocarbons, whereas terpenoids contain additional functional groups. In general, aromatic compounds are characterized by low molecular weight, ranged between 30 and 300 Da, and by their chemical structure and functional groups. In fact, depending on polarity, number and type of bonds, volatility and functional groups, and enantiomeric properties, the overall shape of the molecules can lead to a particular aroma and flavor sensation (Gardner and Bartlett, 1999). Aroma compounds are often released upon cell disruption, when previously compartmentalized enzymes and substrate interact (Buettry, 1993). Moreover, sometimes VOCs are bound to sugars as glycosides or glucosinolates. The odorous aglycones can be released from the sugar moiety during maturation, processing and storage, or by the effect of enzymes, acids or heat.

- Terpenes:

  This is by far the most representative class of aroma compounds presents in fruits. Terpenes basic structure is formed by isoprene units, $(C_5)_n$, that build up the carbon skeleton (Breitmaier, 2007) following the *isoprene rule*. The isoprene units can be linked together "head and tail" forming linear chains or can be arranged forming rings structures (Fig. 3). Depending on the number of isoprene units terpenes are classified sequentially by size as *hemi-* (C_5), *mono-* (C_{10}), *sesqui-* (C_{15}), until *polyterpenes* $(C_5)_n$ with $n>8$. In nature, terpenes and terpenoids derived from the universal C_5 precursor isopentenyl diphosphate and its isomer dimethylallyl diphosphate. In citrus terpenes occur mainly as hydrocarbons, aldehydes, ketones, esters, and alcohols and their glycosides. Characterized by high volatility, terpenes are the mainly compounds found in citrus essential oils.

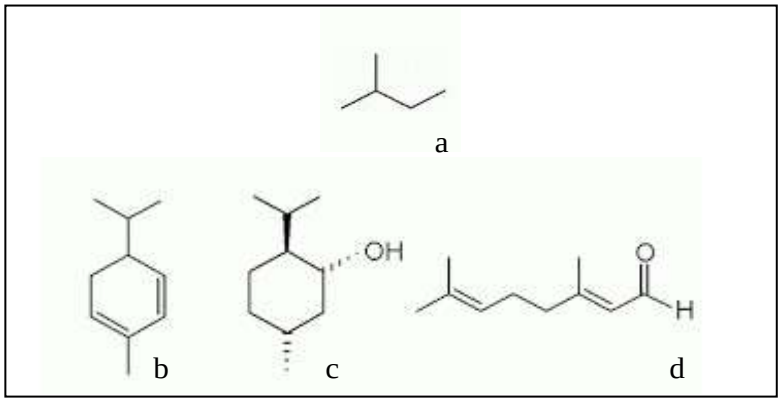

Fig.3. Chemical structure of isoprene and example of possible rearrangement.
a) Isoprene unit; b) α-phellandrene; c) menthol; d) citral

- Aldehydes:

  This is by far the largest group of aroma-active compounds in citrus and particularly in oranges. Aldehydes are formed from the oxidative cleavage of linoleic and linolenic acids. Frequently these compounds appear soon after the disintegration of tissue in the presence of oxygen, and a part of them is enzymatically reduced to the corresponding alcohol (Belitz et al., 2009). Together with alcohols they serve as precursors of esters synthesis, so their composition reflects the esters present in fruits. Alcohol dehydrogenase can reduce the aldehydes into the corresponding alcohols. The short chain aldehydes and alcohols are produced by plants in response to wounding and play an important role in the plant defense strategies (Matsui, 2006; Stumpe and Feussner, 2006).

- Alcohols:

  Chemically, an alcohol is formed by a hydroxyl functional group bounded to a carbon atom. Due to their relation with esters, that are present mostly as ethyl ester of C_3 to C_4 organic acid, linalool is by far the most important alcohol As said before, often alcohols are the simply versions of the more potent aldehydes forms. The reduction of aldehydes to the corresponding alcohol can be very slow, thus with the high enzyme specificity can result in an alcohol/aldehyde ratio in which aldehydes are predominant. Moreover, alcohols can be oxidized to the corresponding ketone.

- Esters:

This class of compounds derives from a reaction between a carboxylic acid and an alcohol. Due to their massive diffusion in almost all the plants, esters constitute one of the most important aromatic families. In general, they are responsible for the fruity flavor character (Berger, 2007). They are synthesized only by intact cells, so when the fruits are homogenized, such as in the processing of juice, esters are rapidly hydrolyzed by the hydrolase enzymes present, and the fruit aroma flattens (Beliz et al., 2009).

- Ketones:

  Chemically, this class of compounds is characterized by a carbonyl group bounded to two carbon containing substituents. Sometimes ketones possess an odor threshold lower than the alcohol's, so they can contribute greatly to the overall aroma. Due to this characteristic, they are often considered as off-flavors. For example, in oranges, they are oxidation products or products of microbial contamination. Their presence above threshold levels severely degrades the quality of the juice and is an indication of thermal abuse and/or storage abuse (Rouseff and Perez-Cacho, 2007).

2.1 Factors Affecting Citrus Flavor

There are several factors that can affect citrus fruits flavor, and can be divided in two general classes: pre- and post- harvest factors. The post harvest development of citrus fruits can alter significantly their commercial properties (Marcilla et al., 2006)

During the commercial packing of citrus, fruits are subjected to a number of processes on the packing line which include: washing, rinsing, waxing, drying, sizing and placement into boxes. Almost all the steps in the packing line have the potential to induce physiological changes in the fruit that can results in flavor changes. It is well known that all this process, combined with subsequent storage of the fruit, acts to reduce eating quality of the fruit (Obenland et al., 2008).

2.1.1 Pre harvest handling, genotype and harvest time

As said before, the aromatic pattern derives from a complex combination of numerous factors. So different species of citrus are characterized by different aromatic patterns (Moufida et al., 2002; González-Mas et al., 2011; Allegrone et al., 2006; Dharmawan, 2008), showing that there is a genetic control in the expression of the aromatic profile (Sanz et al., 1997; Schwab et al., 2008; El Hadi et al., 2013).
Even often the differences are mainly quantitative, and only a few compounds are variety-specific (Gonzales-Mas et al., 2011).
The harvest time is strictly related to the content and the composition of fruits. So it is able to affect internal characteristics of the fruits (Bruckner et al., 2008; Rekha et al., 2012).
Five standards has been usually used to define mature citrus fruits, color break, minimum juice content, minimum percentage of total soluble solids, minimum acid content, and total soluble solids/acid ratio (Nagi et al., 1978).

2.1.2 Post harvest handling

- Washing and Packing line: When arrived in the packing house, fruits are usually washed using mechanical brushes (Obenland et al., 2008). This process, if too strong, can enhance water loss from the peel and lead to changes in the internal atmosphere of the fruits (Hagenmaier and Baker, 1993). In fact an aggressive wash, causing the water loss from the peel, can result in an increase of the resistance to gas exchange (Ben-Yehoshua, 1969). Moreover, even passing through the packing line fruits are dropped and squeezed. Both these process can induce a wounding effect and lead to an increasing of the respiratory rate and of the accumulation of ethylene (Petracek et al., 1998). Production of ethanol is a very efficient indicator of wound injury, because its production is stimulated by all those factors which are capable of damaging the fruits (Cohen et al., 1990). It is proved that ethanol production is enhanced by various steps of the packing line like washing, packing and waxing, demonstrating that the metabolism of the fruit is altered by passage through all the portions of the packing line,

presumably due to mechanical injury of the fruit (Obenland et al., 2008). Important factors in washing step are: temperature and pressure of the applied water, velocity of brushes, and chemical product applied. Due to the high susceptibility of citrus fruit to green and blue molds during post harvest storage, some modification to the washing step has been proposed, like the addiction of fungicide or other chemical products to the washing water (Rodov et al., 1995; Brown and Chambers, 1996; Smilanick et al., 1999; Smilanick et al., 2003). Moreover, positive effect of heat treatments, between 50 ° and 60 °C depending on citrus fruit, on the storability of citrus fruits is well proved (Ben-Yehoshua et al., 1987; Del Rio et al., 1992; Rodov et al., 1995; Porat et al., 2000). Heat can be applied as hot water dips, vapor heat, hot dry air or by hot water rising and brushing (Schirra et al., 2000; Fallik, 2004). It is proved that the main effect of this practice is to reduce chilling injuries and mould infections, without altering fruit quality (Schirra et al., 2011; Ozdemir and Dundar, 2006). Hot water dip is one of the most easily applied and environmentally safe fruit treatments to reduce the incidence of mould injuries (Rodov et al., 1995). For example it has been applied effectively on Kumquats fruits (Schirra et al., 2004; Rodov et al., 1995); on red grapefruit, Satsuma, blood oranges, and fortune mandarins without increasing the respiration rate during storage, and without exerting negative effects on the overall quality of the fruits (Schirra and D'Hallewin, 1997; Porat et al., 2000; Hong, 2007) Despite this, it was proved that eat treatments deteriorate taste and flavor of blood oranges, and mandarins, mainly enhancing the production of ethanol and others off-flavors (Schirra et al., 2002; Schirra et al., 2004; Hagenmaier and Shaw, 2002; Moshonas et al., 1992). Different results were obtained on another study conducted on mandarin that showed no detrimental effect on fruit flavor, and no overproduction of off-flavor related compounds (Perez et al., 2005).

- Waxing and coating: Application of external waxes on the fruits surface is usually used to replace the natural waxes that have been removed by washing and brushing procedures (Marcilla et al., 2009). It is also used to improve consumers visual attraction to the fruits. But coating does not have the only attracting effect. Application of wax or non-wax based coatings can alter internal atmosphere of the fruits, leading to the production of anaerobic metabolites such as ethanol and acetaldehyde (Hagenmaier and Goodner, 2002, Tietel et al.,

2011). As said before, over production and accumulation of these compound is associated to poor flavor and production of off-flavor related compounds in waxed citrus (Cohen et al., 1990; Hagenmaier, 2002), and with an overall loss of quality of citrus fruit (Shaw et al., 1991; Del Rio et al., 1999). Among citrus fruits, mandarins are prone to the accumulation of ethanol and off-flavors following waxing (Hagenmaier, 2002). These two compounds are not the only flavor-related volatiles that are altered in citrus fruits. Coated fruits have increased levels of several volatile components, some of them being potentially beneficial to the flavor of the fruit (Nisperos-Carriedo et al., 1990; Baldwin et al., 1995; Obenland et al., 2009). The patterns of change varied depending on the compound, some increasing and others decreasing during storage with waxing and type of wax being key factors in determining the amounts present (Baldwin et al., 1995). Moreover, it was shown that the development and perception of ethanol as an off-flavor, is strictly dependent from the absolute content and odor threshold of a particular compound (Martínez-Javéca, 1991; Hagenmaier, 2000; Hagenmaier, 2002). Several studies tried to relate postharvest treatments, like coating, with fruit sensory quality (Hagenmaier and Baker, 1994; Manheim and Soffer, 1996; Bioatto et al., 2005; Shi et al., 2005; Marcilla et al., 2009). Of course, type wax used is an extremely important factor. The most commonly coating formulations are composed of either synthetic or natural waxes dispersed in water or resin solutions (Marcilla et al., 2009). In general, polyethylene waxes do not promote modification of internal atmosphere of citrus fruit (Hagenmaier and Baker, 1993), whereas waxes with high shellac content are those that affect more internal quality, due to their low permeability to gases (Baldwin et al., 1995; Fallik et al., 2004; Marcilla et., 2009). The oxygen permeability of coatings can be used for predicting flavor changes (Hagenmaier, 2002).

- Ethylene degreening:

  According to their respiration rates, citrus fruits are classified as non-climacteric, since the fruits show no or slight variation in the production of carbon dioxide and ethylene during maturation (reviewed in Iglesias et al., 2007). Ethylene is a plant growth hormone, also known as stress hormone, that has numerous effects on the growth, development, storage life of many fruits and vegetables (Saltveit, 1999). Its production in albedo and flavedo tissues of citrus fruits is stimulated in response to a variety of stress, like wounding, low temperature, and pathogen

infections (Eveson et al., 1991; Eaks, 1980; McCollum and McDonald, 1991; Achilea et al., 1984; Mullins et al., 2000). Exposure of harvested fruits to exogenous ethylene induced several physiological changes, mainly destruction of chlorophyll, and synthesis and development of carotenoids (Rodrigo and Zacarias, 2007; Iglesias, 2007). The major effect of these processes is the color change of the fruit flavedo, from green to the characteristic color of each species. However, prolonged exposure of fruits at high concentrations of ethylene can produce unpleasant effects related to the senescence of fruits (Saltveit, 1999), causing important fruit quality loss and reduction in shelf-life (Wills and Warton, 2000; Wills et al., 1999; Wills et al., 2001). There are different effects of quality loss mainly enhanced of respiration and ethylene production rates, both indicators of biochemical changes in citrus flesh, such as breakdown of sugars and acids that serve as respiratory substrates. Moreover, different studies proved that ethylene affects various metabolic pathways like: decrease acidity in 'Mosambi' oranges (Ladaniya and Singh, 2001); increase production of aroma volatiles in green lemons (Norman and Craft, 1968); increase susceptibility to stem-end rots, enhances weight loss; and accelerate rind and calyx senescence (Barmore and Brown, 1985; Carvalho et al., 2008; Porat, 2008). Notwithstanding, more recent works showed that ethylene had only minor effects on content and composition of juice aroma volatiles of several citrus fruits, such as 'Navel' oranges, 'Star Ruby' grapefruits, and 'Satsuma' mandarins; and on the overall antioxidant activity (Chaudhary et al., 2008; Mayuoni et al., 2011). Concluding that, maintaining adequate temperature and duration, ethylene is probably not involved in regulation of internal ripening process in citrus flesh and did not affect fruit quality attributes, including perceived flavor and nutritional quality (Mayuoni et al., 2011).

- Storage:

  It was shown in the older literature (Biale, 1961), that the packing process combined with the subsequent storage determines a reduced eating quality of the fruits (Obenland et al., 2008). A lot of investigations have been conducted to better understand the physiological changes that occur during this process and what are the factors that affect it majorly. It is proved that most of the storage effects are determined by temperature (Marcilla et al., 2006; Rapisarda et al., 2001; Obenland et al., 2011), being citrus fruit native from tropical and

subtropical regions and sensitive to low temperature. The consequence of the exposure of fruits to low but not freezing temperature, typically below 10 °C is the chilling injury (Schirra et al., 1998; Kader and Arpaia, 2002). It is a physiological disorder that often appears on the surface of fruits, probably due to the rupture of the oil glands with consequent water loss. Gravity of symptoms depends on citrus varieties and on maturity stage of the fruit (Chalutz et al., 1985; Underhill et al., 1995; Bajwa and Anjum, 2007).

Injury symptoms increase as temperature decrease and storage period is extended (Henriod et al., 2005). Instead storage at temperature higher than 20 °C caused degradation of anthocyanins and ascorbic acid in blood oranges (Rapisarda et al., 2001) and flavor loss in mandarins (Obenland et al., 2011). Changes are rapid if fruits held under hot and dry ambient conditions, while under optimum refrigerated conditions with high relative humidity, changes are gradual and at times may be insignificant.

Several studies report that the main effects on fruit quality are a general reduction in flavor quality, and a small increase in the volatile compound content, weight loss, and maturity index due to an increase in soluble solid content and a decrease of acidity level (Baldwin et al., 1995; Obenland et al., 2011; Tietel et al., 2012; Marcilla et al., 2009). Nevertheless panel test showed that in some cases panelist revealed differences that were not instrumentally determined (Harker et al., 2002; Marcilla et al., 2006). In a study conducted on ‘Navel’ orange, sensory panel evaluation indicates that the freshness of the orange flavor decrease progressively as a result of storage, and hedonic ratings indicates that stored fruits are liked less by the panelist (Obenland et al., 2008).

3. FLAVOR ANALYSIS TECHNIQUES

In order to investigate the changes occurring during the citrus packing line and the subsequent storage of the fruits, it is essential to establish the chemical nature of the VOCs and the overall aromatic pattern that characterize the fruits aroma. To achieve these objectives it is necessary to isolate, and sometimes to concentrate, the volatile fraction from the non-volatile bulk of the fruit matrix. Afterward, a wide range of techniques can be applied to obtain different qualitative and quantitative information on all compounds of possible sensory importance.

3.1 Extraction Techniques

Although in flavor research the direct analysis of the sample is a common practice, pre concentration of the samples is often required to obtain the maximum of information from the sample matrix (Bazemore, 2011). The most widely used sample preparation techniques are rapid and precise, and are based on products that incorporate polydimethylsiloxane (PDMS). It is a hydrophobic polymeric material that extracts the volatile components present in a sample matrix by absorption into the polymer liquid phase, without binding water appreciably. Moreover, it does not require the use of solvents (Lötters et al., 1997).

Headspace sampling with SPME

The term *headspace* is referred to the gas phase located above the surface of a liquid or solid sample present in a sealed vessel (Bazemore, 2011). In headspace sampling techniques, the atmosphere adjacent the sample, that contains the volatiles, is analyzed leaving the actual sample material behind (Wampler, 2002).

The partition of VOCs into the gas phase is strictly dependent on a big number of factors, all related to each other. The main variables that regulate this process are: solubility in water, polarity, molecular weight, ionic nature of analyte and solvent, and temperature (Bazemore, 2011).

For this thesis **SPME** technique was used to sampling the static headspace of samples. SPME is one of the most widely solvent free techniques used to extract

VOCs from a complex matrix. It consists of a microfiber sorbent coated on a fused silica fiber, that adsorbs the analytes until equilibrium is reached in the system. The first were developed by Arthur and Pawliszyn in 1990 and were made only with PDMS, nowadays different coatings are available depending on the matrix and the type of compounds analyzed. The most common coatings are PDMS, Carboxen, divinyl benzene (DVB), polyacrylate, and polyethylene glycol (Carbowax). Fibers can be made of one or a combination of different coatings. The amount of analyte extracted is determined by the partition coefficient of the analyte between the sample matrix and coating material (Pawliszyn, 1997). So, the choice/selection of different coatings and film thickness is a fundamental factor, and it is mainly based on the molecular weight and polarity of components (Bazemore, 2011). In general, thicker film, higher analyte loading into the polymer, and higher analyte detection. For high polarity are recommended fibers made by DVB/Carboxen or PEG (Shirey, 1999). The main advantages of this technique are that it is rapid and simple, requires no solvent addiction, can be applied for liquid, solid and gas, and can be performed without heating the samples (Harmon, 2001). The commercial product that utilizes this technology is commercialized by Supelco Corp.

For the experiments presented in this thesis, a biphasic fiber made of CAR/PDMS was used.

Briefly, other major extraction techniques will be described:

- **Static headspace:** in this kind of extraction the sample is placed in a vial crimped with an inert material, like Teflon (in order to avoid volatiles from sticking to the surface via adsorption, or being absorbed into the septum material), and allowed to reach equilibrium between the sample and the gas phase. Then, an aliquot of the headspace is removed with a gas-tight syringe and usually directly injected in a GC system to be analyzed. This technique provides a good representation of the volatile compounds responsible of the aroma, because it reflects natural headspace concentration. But, it can be difficult to detect potentially important components due to the non-concentration of the samples that can lead to the detection only of the compounds present in higher concentrations (Reineccius, 2006).
- **Dynamic Headspace:** in this technique that is also known as purge and trap VOCs are continuously swept from the headspace into a trap by a flow of

inert gas, like nitrogen or helium. Traps can contain one or a combination of substances, included activated carbon, Tenax (2,6-diphenylene-oxide polymer), or PDMS foam. Once trapped, volatiles are usually released for chromatographic analysis. The major advantages of this technique are the lower detection limits, due to the possibility of concentration of the samples, and the possible application to solid samples (Goodner and Rouseff, 2011).

3.2 Gas Chromatography – Mass Spectrometry

Many different techniques are available in flavor research. The choice among them depend primarily on the kind of desired information, and on the type of samples analyzed (pure or mixtures, their volatility, physical state or solubility) (Rouseff and Goodner, 2011). However, the most widespread techniques are Gas Chromatography (GC) coupled with Mass Spectrometer (MS) detector (Reineccius, 2006). GC-MS is an instrumental combination of the separation power of capillary gas chromatography with the identification power of the mass spectrometer. It is the most ubiquitous analytical technique for the identification and quantification of volatile organic substances in complex matrix.

This technique allow the separation of the volatile compound contained in the volatile fraction of the sample using GC separation, and their classification based on the mass spectra of the detected compounds using MS identification.

3.2.1 Principles of Gas Chromatography

GC is a high resolution technique that enables the separation of gaseous substances on the basis of physical-chemical properties such as boiling point, polarity and size of the gas molecules. During a GC analysis, samples are volatilized and transported by the carrier gas (mobile phase), through the column, where separation occurs (stationary phase). Usually the carrier gas is inert, like Helium, Nitrogen or Hydrogen. Stationary phase is usually a high molecular weight liquid that is deposited either on the surface of finely divided particles or on the walls of a long capillary tubing (Karasec and Clement, 1988). It may also consist of molecules

chemically bonded to the wall of the column; or it may be an adsorptive or inert porous solid (Jonsson, 1987). The time taken for a specific volatile to elute from the end of the column after injection is a characteristic of the volatile molecule and its interaction with the column stationary phase (Rouseff and Goodner, 2011). The separation of the individual components of a mixture involves the partitioning of a compound between the two different phases, mobile and stationary. The compounds with greater solubility in the stationary phase take longer to emerge from the column than those with lesser solubility (Karasec and Clement, 2005). So the relative affinity of the substances for the stationary or mobile phase determines the difference in migration velocity and ultimately leads to physical separation of the components in a sample (Jonsson, 1987). The relative affinity is strictly dependent from the partition coefficient that is specific for each molecule and is defined as the ratio of concentrations of a compound in the two phases of a mixture of two immiscible liquids at equilibrium (Leo et al., 1971). Subtle differences in a compound partition coefficient result in differential retention on the stationary phase and thus changing the separation. So it is possible to discriminate molecules according to their rate of elution, or through the measurement of the volumes eluted or, more commonly, through the detection of the times elapsed between the introduction of the sample and the time at which the analyte reaches the detector. This time interval is defined as the "retention time".

Key features of gas chromatograph are: separate ovens that heat the individual injectors, the column, the transfer line and the detector. Column and injector oven allow the temperature to be increased at a regular rate during the separation of the compounds in the sample (Sparkman et al., 2011).

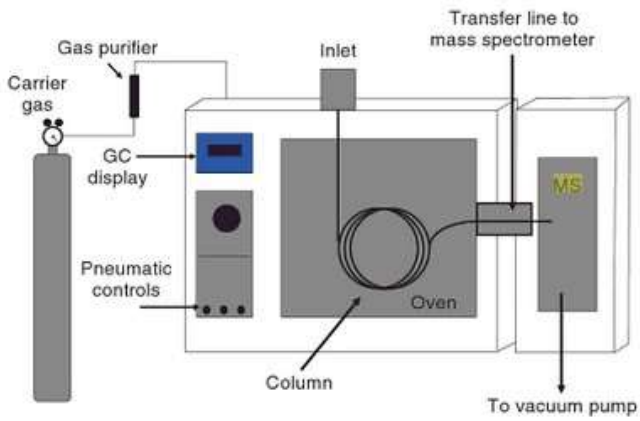

Fig.4. Schematic of a typical simple GC-MS, from Sparkman et al., 2011

3.2.2 Principles of Mass Spectrometry

Mass spectrometry is one of the analytical techniques of major application since it allows performing quantitative-qualitative analysis of any chemical species, from metal ions to organic macromolecules, with extremely low detection limits, and in samples of any type. The sample, pure or in mixture, is brought to the state of gas or vapor at low pressure and then ionized by bombardment by a beam of particles that disintegrates into fragments of different mass and charge ratio (*m/z*). It works on the principle that volatiles are fragmented into ions of predictable size and frequency (Rouseff and Goodner, 2011). The weakest chemical bonds holding the molecule together will be the place at which the molecule is most frequently fragmented and ions form. The degree of fragmentation depends on the energy of the particles that bombard the sample.

The ions that are formed, accelerated by an electric field within a magnetic field, run through different trajectories according to their respective mass/charge (*m/z*) ratio and therefore separated. The most common ionization system uses electron impact (EI), in which the sample is bombarded with a high energy stream of electrons, to approximately 70 eV, that fragments the volatiles as they elute from the end of the capillary column of GC. The ions formed are focused and then sent to a mass analyzer, such as a Quadrupole Mass Analyzer, that sorts the ions in terms of their *m/z* ratio. The resulting fragmentation pattern is characteristic for each molecule, and is called mass spectra. The peak in the mass spectrum with the greatest intensity is called the base peak (Cozzi et al., 1998).

Key features of mass spectrometer are: the ion source; the mass analyzer; and the detector. The ion source is the core of the spectrometer and, because ions are very reactive and short lived; their formation and manipulation must be conducted under vacuum.

A GCMS-QP2010 (Shimadzu) was used during the PhD study. This instrument uses a single quadrupole mass analyzer that is responsible for filtering sample ions, based on their m/z ratio and the stability of their trajectories in the oscillating electric fields that are applied to the rods of the quadrupole. This kind of analyzer permits selection

of an ion with a particular m/z, or allows scanning for a range of m/z-values by continuously varying the applied voltage.

The instrument was equipped with a SLB5-ms column (Supelco). It is a capillary, non-polar column, made of silphenylene, a polymer virtually equivalent in polarity to poly(5% diphenyl/95% dimethyl siloxane) phase. The low phenyl content provides a boiling point elution order with a slight increase in selectivity, especially for aromatic compounds.

3.3 Electronic Nose

The **Electronic Nose (E.N.)** instruments are able to simulate the human nose, replicating the four fundamental functions of the sense of smell: detection, recording, memory search and identification. The first two functions are simulated by the use of chemical sensors; the other can be simulated by Artificial Intelligence software (Win, 2005). The most common E.N. are based on the use of an array of electronic chemical sensors, with partial or no specificity, coupled with an appropriate system of pattern recognition. The statistical treatments of the data use complex algorithms to extract all the information that can be useful for the different applications (del Cueto Belchi et al., 2013). Contrarily to the classical techniques used in aroma analysis, like chromatography, E.N. does not identify the composition of volatiles compounds but provides a fast comparative measure of patterns of odors, representative of compounds disengaged by a substratum (Steine et al., 2001). The E.N. offers a fast non-destructive alternative to sense aroma, and in the last decade there have been several reports on electronic sensing in environmental control, medical diagnostics and food industry (Olarte et al., 2013; Reinhard et al., 2008; Tang et al., 2010; Lebrun et al., 2008; Saraolglu and Kocan, 2010; Horvath et al., 2010). Some authors reported positive applications of the E.N. technology to the discrimination of fruit of different quality, but as yet few literatures refer to control the fruit maturity in the shelf life state (Hernandez-Gomez A. et al., 2007).

3.3.1 Sensors description

Different type of sensors can be used for this objective: Metal Oxide Semiconductors (MOS); Gas sensitive Field Effect Transistor (GasFET or MOSFET); Conducting Polymers; acoustic wave devices such as the Buck Acoustic Waves (BAW) or Quartz Crystal Microbalance (QCM); Surface Acoustic Wave (SAW) (Strike and Koudelka-Hep, 1999). In case of MOS sensors, the interaction with the volatile compounds induces mechanisms of adsorption and desorption, taking place on the surface of sensors and provoking the modification of its electrically measurable properties by a variation of resistance versus time (Aishima, 1991). The most common sensors used in an electronic nose system are metal oxide semiconductor. MOS sensors are able to detect gases through a decrease in resistance values when reducing gases are adsorbed on the sensor surface. The general operating principle of a MOS gas sensor is based on the changes that occur in the sensing material when it is heated (Fig. 4). In fact when a metal oxide crystal, such as SnO_2, is heated, the oxygen is adsorbed on the crystal surface causing a negative charge, and the donor electrons are transferred to the adsorbed oxygen. As a result of this process, a positive charge is formed in a space charge layer forming a surface potential that serve as a potential barrier against electron flow. Inside the sensor, at the level of SnO_2 micro crystals, an electric current flows and at grain boundaries, the adsorbed oxygen forms a potential barrier that prevents carriers from moving freely. This potential barrier determines the electrical resistance of the sensor. In this way when a deoxidizing gas arrives on the sensor, the surface density of the negatively charged oxygen decreases, so the barrier height in the grain boundary is reduced. The reduced barrier height decreases sensor resistance and increases the electrical conductivity. For a target gas, the relationship between the sensor resistance and the gas concentration is:

$$Rs= A(C)-\alpha$$

Where Rs is the electrical resistance of the sensor; A is a constant, and α the slope of the curve Rs.

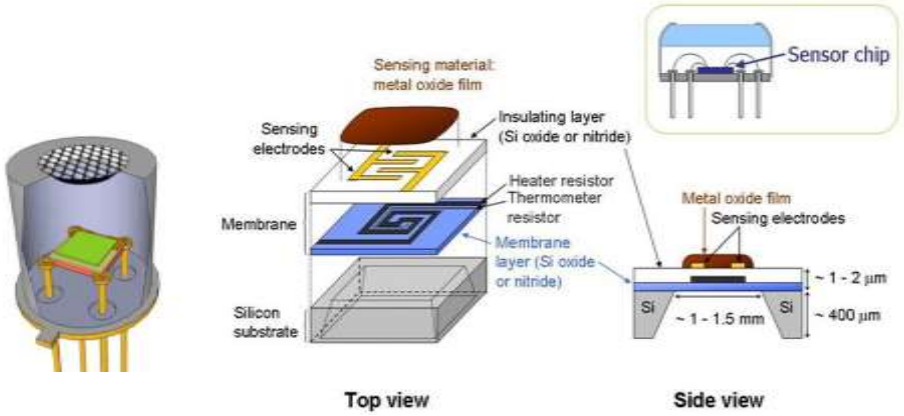

Fig.5. Schematic representation of a MOS sensor, from Simon et al., 2011

3.3.2 Instruments description

Due to their wide range of sensitivity to different gas types and the wide range of application of E.N. devices, a lot of instruments were developed both by industries and research group. Basically, an E.N. is formed by three fundamental parts: sampling system; sensor chamber; data analysis system.

Selected electronic nose instrument examples and their application in food analysis:

- AlphaMOS, Toulouse, France → instruments Fox3000; Fox4000. Used for the authentication, classification and characterization of Citrus spp. (Steine et al., 2001; Reinhard et al., 2008; Goodner and Manthey, 2005); for the discrimination of different mango varieties (Lebrun et al., 2008); or for the evaluation of Chinese tea (Qin et al., 2013);
- AromaScan, Aroma Analysis Specialist → instrument AromaScan A32 Multisample used for identification and characterization of sausages aroma (Win, 2000); and other dairy products (Visser and Taylor, 2007); and for the detection pesticide residues in crop production (Wilson, 2012)
- WMA Airsense Analysentechnik GmbH → instrument PEN2 used for several investigation of food quality monitoring, like mandarins and tomatoes (Hernandez Gomez et al., 2007; Hernandez Gomez et al., 2006), and fish (Cheli et al., 2009; Campagnoli et al., 2009)

- Neotronics Scientific → instrument Neotronic Olfactory Sensory Equipment (NOSE) Model 4000. Used for the classification of processed orange juice (Shaw et al., 2000)
- Sensigent → instrument Cyranose 320, used for assess maturity stage of tropical fruits, like mango or avocado, or for classification of honey origin (Zakaria et al., 2012; Pereira et al., 2009)

For this PhD research two different electronic noses were used:

- EOS835
- Multisensory Odor Olfactory System – MOOSY32

3.3.2.1 EOS835

It is a commercial device developed by Italian Sacmi industry (Fig. 6). The instrument employs an array of 6 MOS sensors installed inside a patented measuring cell, the sensor chamber (Tab. 1). Different sensors that can react differently to the same odor molecules generating a set of signals that is characteristics of the analyzed sample, and represent the aromatic fingerprint.

Tab. 1. MOS sensor array configuration of the EOS835. Specificity from Sacmi

Model	Sensing layer	Operating Temperature
CJ1316	SnO_2 cat SiO_2	450°C
SB0225	SnO_2 cat Ag	400°C
SD0515	SnO_2 cat Mo	400°C
SH0612	WO_3	375°C
SJ0717	SnO_2	450°C
WHT19	WO_3	400°C

The instrument consists of several parts:

- an auto sampler with a forty positions try and six positions oven that permit the conditioning of samples;
- a pneumatic section, designed to aspirate and regulate the flow of the sample being analyzed;
- a patented measuring cell, the sensor chamber;
- an electronic section which controls and samples measurement data;
- a system-resident software application which controls all the measuring experiment settings and then processes the data using specific algorithms.

The reference conditions are obtained by fluxing neutral air. The resistance variation toward the reference produces a response curve from which significant features can be extracted for numerical elaboration and classification. The instrument has two air inlets, one for reference air, whereas the other, the sample air line, is connected to a valve regulating the sample air flow directed to sensor chamber. During the reference phase, the neutral air flows over the sensors, while during measurements the inlet is switched to the sample air. Thus lead to changes in the composition of the analyzed mixture and the sensors resistance changes correspondingly generating a response curve for each sensor. At the end of the analysis the collected data must be processed in order to extract significant features from the sensors response curve to be used in odor recognition (Fig. 7).

Fig.6. EOS835 (Sacmi, Imola, Italy)

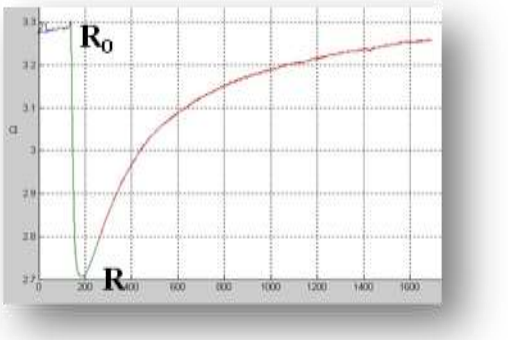

Fig.7. Typical sensor response

3.3.2.2 Multisensory Odor Olfactory System – MOOSY32

Is a homemade instrument developed by the research group of Electronic Engineering Department of the Polytechnic University of Valencia. The instrument utilized 32 commercial MOS sensors of 5 different types, all produced by Figaro Engineering Inc. (Tab. 2). So, the use of different types of sensors combined with the selected operating temperature leads to a wide range of different responses toward volatile organic compounds with a wide variety of applications.

Model	Target Gas	Typical detection Range
TGS2600	General Air Contaminants	1 – 30 ppm
TGS2610 – C00	LP gas	500 – 10.000 ppm
TGS2610 – D00	LP gas	500 – 10.000 ppm
TGS2611	Methane	500 – 10.000 ppm
TGS2620	Alcohol, solvents vapor	500 – 5.000 ppm

Tab. 2. MOS sensor array configuration of the MOOSY32. Specificity from Figaro

The system is composed mainly by two parts: the electrical part and the processing of the sample part (prechamber) (Fig. 8). The prechamber is linked with a clean air pump which air flow is splitted in two streams, one that goes directly to the sensors

and is used as a reference line, while the other passes through the sample chamber to carry the volatile molecules to the sensors chamber.

Flow velocity can be manually adjusted with a small valve. So, neutral air passes through the system to clean it during a predetermined time and then the airflow passes through the sample chamber during another fixed time. The electrical part consists in an electrical circuit that can convert the change in conductivity to an output signal that corresponds to the gas concentration. The sensor chamber consists of a piece of steel composed with eight identical electronic boards each one with four sensors and a voltage regulator, which supplies the heater for each of the four sensors (Fig. 9).

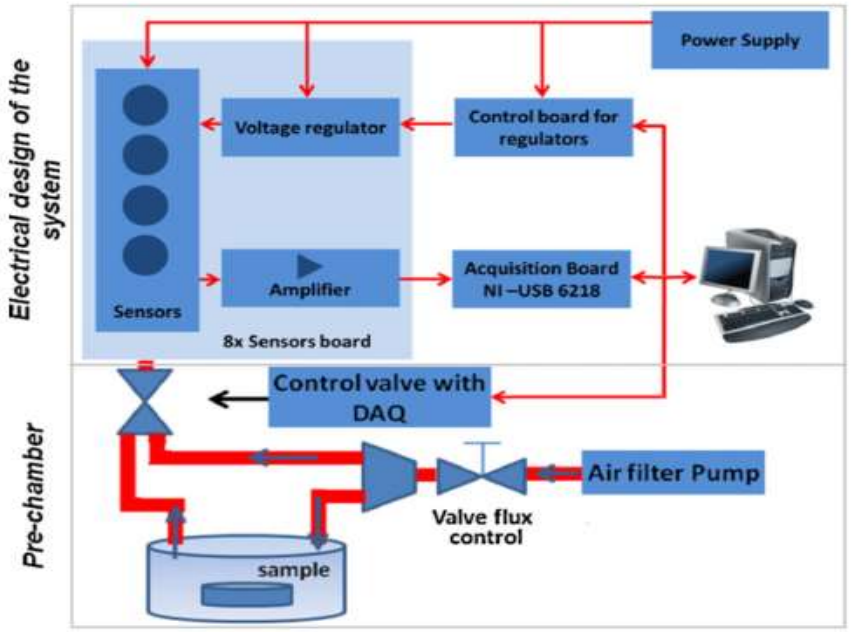

Fig.8. MOOSY32, operation of the whole system. From del Cueto Belchi et al., 2012

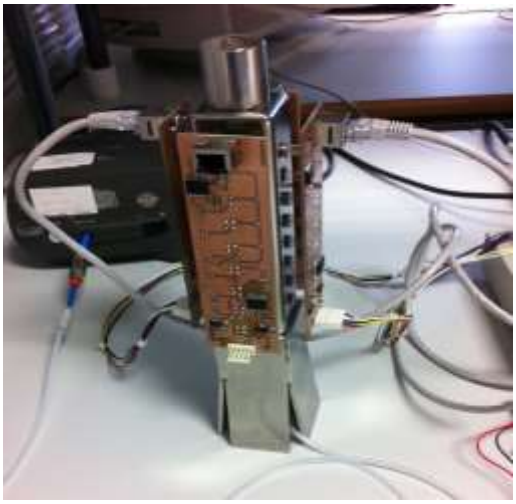

Fig.9. MOOSY32

Each part of the instrument is controlled by user interfaces that permit the setup of the measurement parameters. This kind of configuration allows to maintain every sensing element at a specific temperature, which is optimal for the sensing process, and to have different sensitivity properties by selecting the most appropriate combinations of the sensor temperature, as described in the reference of del Cueto Belchi et al. (del Cueto Belchi et al., 2012).

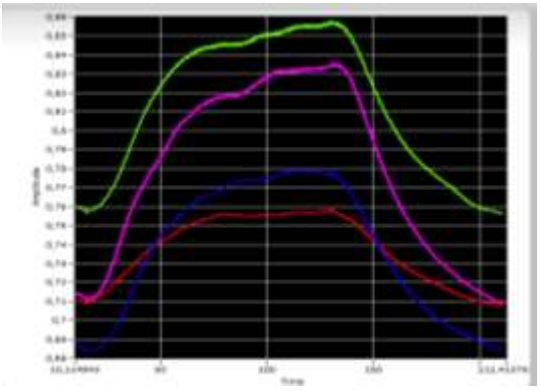

Fig.10. Example of response of sensor TGS 2600 to different samples air

The sensor signal are recorded continuously until the signal of each sensor reaches a steady state, and acquired by a board of National Instruments. Thereafter, the output signals from the sensors are digitized and stored (Fig. 10). It is possible to analyze this data in different ways, using different algorithms for the extraction of different features.

The classification features utilized for this goal are: late saturation; saturation slope; early saturation; transient slope and time to threshold.

Part II

1. GENERAL OBJECTIVES

To evaluate the effect of some practices of the postharvest management on the quality characteristics of different citrus fruits is the main objective of this thesis. In order to achieve this objective, the work was structured in two different parts, each one investigating different aspects of the main theme.

Organoleptic quality is one of the main factors that are able to influence consumer choice. A lot of investigations have been conducted on the effect of postharvest and storage on this quality trait of several fruits of Citrus spp. As said before, a great number of practices are able to modify in some way the internal atmosphere of the fruits and the subsequent storage performance.

The first experiment was conducted in Sicily, Italy, with the collaboration of the RoccaCoop, a cooperative located in the province of Messina which includes about 70 growers. The experiment was designed in order to verify if the harvest and the postharvest handling adopted by the cooperative is able to guarantee the maintenance of citrus fruits quality. Actually, harvest time is often decided taking in accounts the requirements of the market and not the real maturity stage of fruits, and also the adopted storage conditions are not always suitable to preserve organoleptic quality. Obviously, this way of behave is motivated by economical requisites, which include the need of organizing and optimizing all the corporate resources. But sometimes with just small adjustments, it is possible to significantly improve the quality of the product. In this experiment, lemon fruit samples were submitted to the same storage and handling conditions applied in the farm. The objective was to evaluate the changes that occur during fruits storage, and to evaluate the feasibility of a non-refrigerated storage. Moreover, special attention has been paid to the characterization of the aromatic pattern and of the bioactive compounds of three cultivars traditionally cultivated in Sicily.

It seems that the lack of communication among scientific community and growers and processors caused that the improvement of the knowledge regarding citrus acquired from researchers did not correspond to an improvement of the producers knowledge and habits.

The second experiment was conducted in the campus of Gandia of the Polytechnic University of Valencia, Spain. In this case, the experiment was designed and supported by the participation of the company Emilio Esteve, located in the region of Valencia, which is specialized in orange fruits commercialization. To investigate the effect of the packing line, postharvest treatments and the subsequent storage on the aromatic pattern of orange fruits was the objective of this work. In order to evaluate the effect of each treatment, samples were picked at different steps of the fruits packing line. Moreover, thanks to the collaboration of the research group of Electronic Engineering Department of the Polytechnic University of Valencia, coordinated by Professor José Pelegrí-Sebastiá, a comparison between the ability of two different electronic noses to monitor the changes during storage was performed.

2. EXPERIMENT 1:

Postharvest life and aroma quality of three lemon cultivars grown in Sicily

Abstract

The present work is focused on the study of the characteristics of the fruits of three main lemon (Citrus limon L. Burm.) varieties cultivated in Sicily: 'Femminello Comune', 'Femminello Zagara Bianca', and 'Femminello Santa Teresa'.

Physical and chemical properties as well as aroma compounds were analysed as quality discrimination factors. The effect of the storage conditions was verified. Also, to assess the antioxidant potential, vitamin C content and total polyphenols content were analysed. Standard experimental techniques were used to determine: weight, longitudinal and transverse diameters, titratable acidity, total soluble solids, and juice percentage. The volatile component was analysed by i) a gas chromatographer with a mass spectrometer detector (GCMS QP2010, Shimadzu), and ii) an electronic olfactory system equipped with an array of six MOS sensors (EOS835, Sacmi). Vitamin C content was determined with a HPLC instrument with a UV/VIS detector (Waters Alliance 2695- PDA).

Most of the physical and chemical parameters analysed allowed a statistically significant discrimination among the factors "cultivar" and "storage". The aromatic pattern was similar for the cultivars 'Femminello Comune' and 'Femminello Zagara Bianca', while 'Femminello Santa Teresa' showed different volatile composition. Differences were observed after the storage at 18°C.

2.1 Introduction

Qualitative characteristics are the main focus of many studies regarding citrus. However, to provide a specific definition of "quality" may be very difficult because it can assume different meanings depending on the step of the supply chain and on the final destination market. In fact, different quality attributes are required for the fresh market, like the carpometric parameters, or for the industrial use, like the

chemical characteristics. Moreover, since in 2010 Mediterranean diet was recognized as intangible cultural heritage by UNESCO, particular attention is paid to fruits nutraceutical characteristics. Citrus fruits are a fundamental source of antioxidant and bioactive compounds, and their consumption has been related to the prevention of several diseases such as obesity, diabetes, cardiovascular diseases, and certain types of cancer (Benavente-García and Castillo, 2008; Del Rio et al., 2004; Lin et al., 2007; Schroeder, 2007; Vanamala et al., 2006). So, chemical, aromatic and nutraceutical properties are able to directly determine customer satisfaction. All these characteristics are influenced by several factors, such as cultivar, maturity stage, growing region, cultural practices, and storage conditions of fruits (Gorinstein et al., 2001; Rapisarda et al., 2001; Lorente et al., 2014).

Particularly regarding citrus aroma, most of the knowledge gained so far is focused on oranges, grapefruits and mandarins (Buttner and Schieberle, 1999; Perez-Cacho and Rouseff, 2008 a, b; Zipora et al., 2011). With regards to lemon fruits, there are many studies in literature that analyzed the variation of physical and chemical characteristics of the fruits during the storage (Martinez-Romero et al., 1999; Undurraga et al., 2007), others that describe the volatile composition of the juices (Allegrone et al., 2006). On the contrary, papers concerning the time of storage and its effect on the aromatic pattern and volatile composition of lemon fruits are limited.

The aim of this work is to characterize some aspects of three Sicilian cultivars of lemon, namely: 'Femminello Comune'; 'Femminello Zagara Bianca'; and 'Femminello Santa Teresa'. Particularly, the fruits and the juices were analyzed for chemical characteristics, antioxidant compounds, and aromatic patterns. Moreover, their response to two different storage conditions was analyzed.

2.2 Materials and Methods

Plant Material

This study was carried out using fruits of three lemon cultivars: 'Femminello Comune', 'Femminello Zagara Bianca' and 'Femminello Santa Teresa'. The fruits were harvested on March of two consecutive years, 2012 and 2013, in a farm located in Torrenova (Messina, Sicily, Italy). All trees were of the same age, approximately 15 years, grafted on sour orange (*Citrus aurantium*), and grown in the same orchard

under conventional farming system. To evaluate the different response to the storage, 180 fruits were collected for each cultivar: 36 fruits for each plant at the cardinal points, from 5 trees and divided in 3 theses. For the storage treatments the fruits were analyzed the day after harvesting (ST0); the other fruits were stored for 4 weeks at 4 °C (ST28 4 °C) or at 18 °C (ST28 18 °C).

Cultivar ‘Femminello Comune’

Cultivar ‘Femminello Zagara Bianca’

Cultivar ‘Femminello Santa Teresa’

Physical and Chemical Parameters

Sampled fruits were individually weighted with a precision balance. Longitudinal (DL) and transverse (DT) diameters were measured with a digital caliper. For each thesis, percentage of juice (Juice %) was calculated as the weight ratio between the fresh fruits and the juice squeezed with an electric juicer. Total soluble solids (TSS) were determined by a digital refractometer (Atago) and titratable acidity (TA) was determined by potentiometric titration with 0.1 N NaOH, using 2 ml of pure juice and expressed in g/L of citric acid. Maturity Index (MI) was also calculated as the ratio between TSS, expressed in °Brix, and TA, expressed in percentage (%).

Total Polyphenols Content

Total polyphenols content (TPC) was determined by Folin–Ciocalteu method measuring the absorbance at 750 nm with a UV–VIS spectrophotometer (UV-2401 PC, Shimadzu), as described by Tounsi et al. (2010). The total polyphenols content of the samples was expressed as mg/mL of gallic acid equivalent (GAE). All the analyses were performed in triplicate.

Vitamin C

Vitamin C concentration was determined by liquid chromatography using a Waters Alliance 2695 HPLC instrument equipped with a Waters 996 photodiode array detector (PDA), and Waters Empower software. The column was a C18 Hypersil ODS (150 mm x 4.6 mm i.d., 5 µm; Phenomenex, Torrence, CA) maintained at 35 °C. The elution was performed with a buffer solution of 0.1 M KH_2PO_4/H_3PO_4 at pH 2.3, at a flow rate 1 mL/min, and the wavelength was set at 260 nm (Rapisarda and Intelisiano, 1996). The vitamin C content of the samples was expressed as mg/100 mL of ascorbic acid. All the analyses were performed in triplicate.

Gas Chromatography – Mass Spectrometry

The volatile compounds (VOCs) of the fresh squeezed lemon juices were sampled with the headspace solid phase micro extraction (HS-SPME) technique using a Car/PDMS fiber (1 cm, Supelco). An aliquot of 10 mL of juice was added with 1 g of NaCl, stirred and extracted for 30 minutes at 60 °C. The measurements were carried out with a gas chromatographer coupled with a mass spectrometer detector (GC-MS) (GCMS-QP2010, Shimadzu), equipped with a SLB5-ms column (30 m x 0,25 mm x 0,25 µm, Supelco), with the method described by Costa et al. (2010) with some modifications. The GC-MS instrument parameters were: injection temperature 270 °C, injection mode splitless, sampling time 1 min, split ratio 50:1, carrier gas Helium, pressure 33.7 KPa, linear velocity 32.4 cm/s, ion source temperature 200 °C, interface temperature 250 °C, scan interval 0.25 s, mass range 40–400 m/z. The peak identification was performed through comparison of the experimental mass spectra with those reported in the National Institute of Standards and Technology (NIST) libraries incorporated in the instrument software (GCMS solution Library, Shimadzu) and by comparison with previous studies on citrus fruits

(Yo and Lin, 2004; Allegrone et al., 2006; Dharmawan et al., 2007; Tounsi et al., 2010; Gonzalez-Mas et al., 2011; Saura et al., 2012). Libraries used were: NIST 21, NIST 107, and NIST 147. Only the molecules recognized with a percentage of similarity greater than 90% were used for this study. All the analyses were performed for each sample in triplicate.

Electronic Nose

The aroma fingerprints of fresh squeezed juices were performed by an electronic nose (EOS835, Sacmi) equipped with an array of six metal oxide semiconductor (MOS) sensors (see paragraph 3.3.2.1). Two milliliters of pure juice were placed into a 20 mL glass vial, sealed and incubated for 5 min at 50 °C under stirring. The automatic sampler draws a volume of 4 mL from the headspace by a gas syringe and a chromatographic airflow of 10 mL/min carried the sample air to the sensors chamber. The measurement duration was 27 minutes. The responses of the sensors were processed by Principal Component Analysis (PCA), a multivariate statistic method.

Statistical Analysis

Physical and chemical parameters data were submitted to two-way Analysis of Variance (ANOVA) using the software SYSTAT 13 (Systat Software Inc.) and analyzed for the effects of cultivar and storage. Means are separated using Tuckey Honestly Significant Difference Test.

For the e-nose sensors responses, a Principal Component Analysis (PCA) was used. The PCA is an unsupervised multivariate statistical analysis, which provides a transformation of many variables into a linear combination of variables, into two or three dimensions. This technique extracts features projecting the high-dimensional data set into a dimensionally reduced space formed by the uncorrelated and orthogonal eigenvectors of the correlation matrix calculated from the sensor response, called principal component. The magnitude of the single eigenvector or percentage of information is expressed by the eigenvalue, which gives a measure of the variance related to the principal component. The first principal component (PC1) accounts for the maximum of the total variance, the second (PC2) is uncorrelated with the first and accounts the maximum of the residual variance (Berrueta et al., 2007), and so on for the other components. The feature calculated for

each sensor and used in the statistical analysis is the ratio (R/R_0), between the electrical resistance of the sensor in the presence of volatile substances (R) and the resistance of the same sensor measured in the absence of volatile substances (R_0). The PCA was performed with Nose Pattern Editor (Sacmi) and with the software S-PLUS 2000 (MathSoft Inc.) using a correlation matrix.

2.3 Results and Discussion

Physical and Chemical Parameters of Lemon Fruits

As shown by the results reported in Table 1, regarding the Maturity Index and the juice percentage, in the two analyzed years the fruits were characterized by different maturation stage. It led to differences in the starting values of the fruits. Also, the interaction between the cultivar and the storage time was not statistically significant, meaning that although the physical characteristics were different among the cultivars and the storage times, the differences remained stable within the storage time. Table 1 summarizes the data obtained for each cultivar, and each treatment, in the two years.

The physical and carpometric parameters were significantly affected by the storage time, and showed the same behavior in response to the storage conditions. In fact, storage caused a significant reduction of weight and longitudinal and transverse diameters of the fruits, more accentuated with the storage at 18 °C. The juice percentage increased whit storage, probably as a consequence of the loss of total weight of the fruits caused by water loss from the peel. Regarding chemical parameters, total soluble solids content and titratable acidity remained unchanged during the storage, with a slight decrease of titratable acidity with the refrigerated storage.

Titratable Acidity: higher values were registered in 2012, and in the juice of the cultivar Femminello Santa Teresa. In both years storage time did not influence significantly TA levels, that was subjected to just a slight decrease with the refrigerated storage, as reported in literature (Del Caro et al., 2004; Marcilla et al., 2006)

Total Soluble Solids: higher values were detected in 2013. Among the cultivars, Femminello Santa Teresa showed the highest content of sugars. Storage

treatments did not affect TSS content in both years, as already described by Del Caro (Del Caro et al., 2004).

Maturity Index: this parameter showed variation between years. In 2012 the cultivars were characterized by the same maturity stage, while in 2013 the cultivars were at different maturity stage, having Femminello Santa Teresa a lower maturity index. In both years, MI was not affected by the applied storage treatments. Previous works pointed that for citrus fruits Maturity Index has no correlation with storage time, storage temperature, flavor or chilling injury (Hagenmaier and Goodner, 2002).

Tab. 1. Quality parameters of the analyzed fruits

2012								
CVS	**ST**	**Weight (g)**	**DL (mm)**	**DT (mm)**	**Juice% (w/w)**	**TSS (°Brix)**	**TA (g/L citric acid)**	**MI**
F.C.	**ST0**	134,16±11,14	77,56±3,47	61,58±1,91	34,9±4,12	7,66±0,21	60,82±3,59	1,26±0,07
	ST28 4°C	119,67±20,28	76,04±3,87	57,90±4,29	39,1±3,97	7,74±0,25	58,00±0,22	1,33±0,05
	ST28 18°C	109,89±16,14	74,28±4,77	56,27±3,78	41,5±2,86	7,68±0,50	57,84±2,59	1,33±0,12
Z.B.	**ST0**	109,90±13,00	72,24±3,83	58,86±2,43	35,7±1,26	7,48±0,13	60,51±2,65	1,24±0,06
	ST28 4°C	96,86±7,75	70,00±3,25	55,37±1,50	40,9±4,28	7,44±0,11	58,33±1,57	1,28±0,02
	ST28 18°C	87,41±8,25	68,89±4,36	52,60±1,69	43,2±1,07	7,44±0,15	58,48±4,81	1,27±0,09
S.T.	**ST0**	117,89±10,84	71,51±3,95	57,93±2,98	35,3±2,53	8,00±0,51	60,61±3,26	1,32±0,08
	ST28 4°C	106,06±10,95	67,62±2,09	55,17±1,65	43,7±2,74	8,12±0,31	60,16±5,06	1,34±0,07
	ST28 18°C	94,86±6,33	66,64±2,47	52,24±1,08	45,3±2,89	8,26±0,51	63,02±0,77	1,31±0,06
	CVS	**	**	**	***NS***	**	*	***NS***
	ST	**	*	**	**	***NS***	***NS***	***NS***

2013								
CVS	**ST**	**Weight (g)**	**DL (mm)**	**DT (mm)**	**Juice% (w/w)**	**TSS (°Brix)**	**TA (g/L citric acid)**	**MI**
F.C.	**ST0**	171,99±27,49	88,02±4,68	66,88±3,45	38,84±5,05	8,23±0,33	50,11±3,00	1,65±0,13
	ST28 4°C	165,54±13,28	85,09±2,04	65,99±1,91	39,32±4,47	8,15±0,57	49,72±3,52	1,64±0,10
	ST28 18°C	152,11±16,30	83,52±3,24	63,42±2,61	43,54±4,97	8,17±0,58	52,42±3,30	1,56±0,10
Z.B.	**ST0**	185,92±30,58	91,20±6,58	68,06±3,76	36,23±3,79	8,53±0,21	49,51±0,62	1,72±0,05
	ST28 4°C	176,01±12,65	90,82±2,77	67,51±2,61	36,21±5,33	8,43±0,48	49,44±4,18	1,71±0,15
	ST28 18°C	160,20±7,62	88,80±2,89	64,36±0,46	46,48±3,57	8,55±0,16	51,18±1,87	1,67±0,06
S.T.	**ST0**	184,77±21,55	84,92±4,46	68,40±2,83	42,33±2,00	8,63±0,56	58,47±1,99	1,47±0,07
	ST28 4°C	180,82±11,99	87,38±3,69	67,93±1,59	42,49±1,64	8,83±0,18	54,73±2,78	1,61±0,08
	ST28 18°C	159,12±14,74	81,88±3,00	63,85±2,39	50,01±0,58	8,91±0,36	56,22±2,41	1,59±0,10
	CVS	***NS***	**	***NS***	**	**	**	**
	ST	**	*	**	**	***NS***	***NS***	***NS***

F.C.) cv. 'Femminello Comune'; Z.B.) cv. ' Femminello Zagara Bianca'; S.T.) cv. 'Femminello Santa Teresa' *p-value* is determined by ANOVA. For each parameter, $p < 0,05$ indicates differences among a) CVS, Cultivars; and b) ST, Storage Time.

Bioactive Compounds of Lemon Fruits

Total Polyphenols Content (TPC): data shows variations between years, higher in 2012, and among cultivars, having Femminello Zagara Bianca a higher content. Storage time affected significantly TPC in both years with a general trend of decrease in ST28 18 °C treatment. It is interesting to notice that between the two years there is a difference in the response to storage treatments (Fig.1). In 2012 a reduction in TPC was observed in both of the applied treatments, while in 2013 TPC decreased only with ST28 18°C, and it increased with ST28 4 °C. This difference is probably due to different concentration at the harvest moment. In 2013, cv. 'Femminello Santa Teresa' behaved differently with no detectable changes in total polyphenols content during storage. Being TPC dependent on the maturity stage (Bermejo et al 2012; Kumari et al 2013; Rekha et al 2012), it is possible to suppose that the changes detected in 2012 were not only due to the storage treatments applied but also to the developing of maturation process, that could have caused the reduction of TPC. In 2013, when fruits were already more mature, this kind of reduction was not detected, while an increase in TPC with ST28 4°C could be observed.

Ascorbic Acid: data shows variation between years, higher in 2012, and among cultivars, having Santa Teresa a higher content. Storage time affected significantly vitamin C content that decreases with storage. Even for vitamin C in 2012 there was a significant reduction in ascorbic acid content with both storage treatments, while in 2013 there was a decrease with ST28 18 °C and an increase with ST28 4 °C (Fig.2).

Previous studies (Rekha et al., 2012; Kumari et al., 2013) reported that TPC and vitamin C content are higher in several unripe citrus fruits, including lemons and oranges. In 2013, fruits had a higher maturity index and juice content, so the TPC and Vitamin C content were lower. The reduced polyphenols and vitamin C content could be due to the possible decrease of both compounds during ripening. Furthermore, organic acids may provide carbon skeletons for the synthesis of phenolics, including anthocyanin and non-anthocyanin phenolics (Rapisarda et al., 2001; Kalt et al., 1999), causing the slight decrease of TA. The results obtained in this study are in agreement with previous findings: with refrigerated storage TA content decreases while TPC and Vitamin C increase. Also, the refrigerated storage induced an accumulation of TPC probably as a response to chilling adaptation. It was

demonstrated in other species that low temperature can induce an enhancement of phenolic compounds as defense mechanism for scavenging reactive species of oxygen (ROS) to mediate this stress (Mohammadian et al., 2011; Pennycooke et al., 2004; Christie et al., 1994). Stresses can induce the activation of the antioxidant system in the cell plant (Avsian-Kretchmer et al., 1999).

Tab. 2. Total Polyphenol Content, express in mg GAE/mL

CVS	ST	2012 TPC (mg GAE/mL)			2013 TPC (mg GAE/ mL)		
F.C.	**ST0**	938,16	±	52,36	618,91	±	46,34
F.C.	**ST28 4°C**	917,37	±	66,41	737,34	±	52,55
F.C.	**ST28 18°C**	851,18	±	48,02	539,04	±	48,01
Z.B.	**ST0**	939,62	±	44,96	680,44	±	46,30
Z.B.	**ST28 4°C**	914,55	±	57,20	773,82	±	52,54
Z.B.	**ST28 18°C**	879,33	±	52,14	658,61	±	13,23
S.T.	**ST0**	919,60	±	46,56	726,30	±	41,31
S.T.	**ST28 4°C**	772,20	±	35,35	711,24	±	31,84
S.T.	**ST28 18°C**	726,15	±	4,08	710,20	±	25,35
	CV		****			****	
	ST		****			****	
	CV*ST		*****			****	

Tab. 3. Ascorbic acid content, express in ascorbic acid mg/100 mL

CVS	ST	2012 Vit C (mg/100 mL)			2013 Vit C (mg/100 mL)		
F.C.	**ST0**	50,19	±	0,39	40,34	±	0,25
F.C.	**ST28 4°C**	47,74	±	4,91	42,98	±	5,48
F.C.	**ST28 18°C**	36,69	±	3,24	39,75	±	0,03
Z.B.	**ST0**	51,81	±	3,17	46,97	±	1,66
Z.B.	**ST28 4°C**	44,46	±	4,00	47,67	±	0,69
Z.B.	**ST28 18°C**	43,95	±	2,73	44,52	±	1,52
S.T.	**ST0**	55,41	±	2,34	50,56	±	0,61
S.T.	**ST28 4°C**	54,42	±	2,38	53,84	±	0,91
S.T.	**ST28 18°C**	46,96	±	1,12	41,89	±	2,54
	CV		****			****	
	ST		****			****	
	CV*ST		****			****	

F.C.) cv. 'Femminello Comune'; Z.B.) cv. ' Femminello Zagara Bianca'; S.T.) cv. 'Femminello Santa Teresa'. *p-value* is determined by ANOVA. For each parameter, $p < 0,05$ indicates differences among a) CVS, Cultivars; and b) ST, Storage Time. Main effects are indicated as non-significant (NS) or significant at either the * $p<0,05$ or ** $p<0,01$

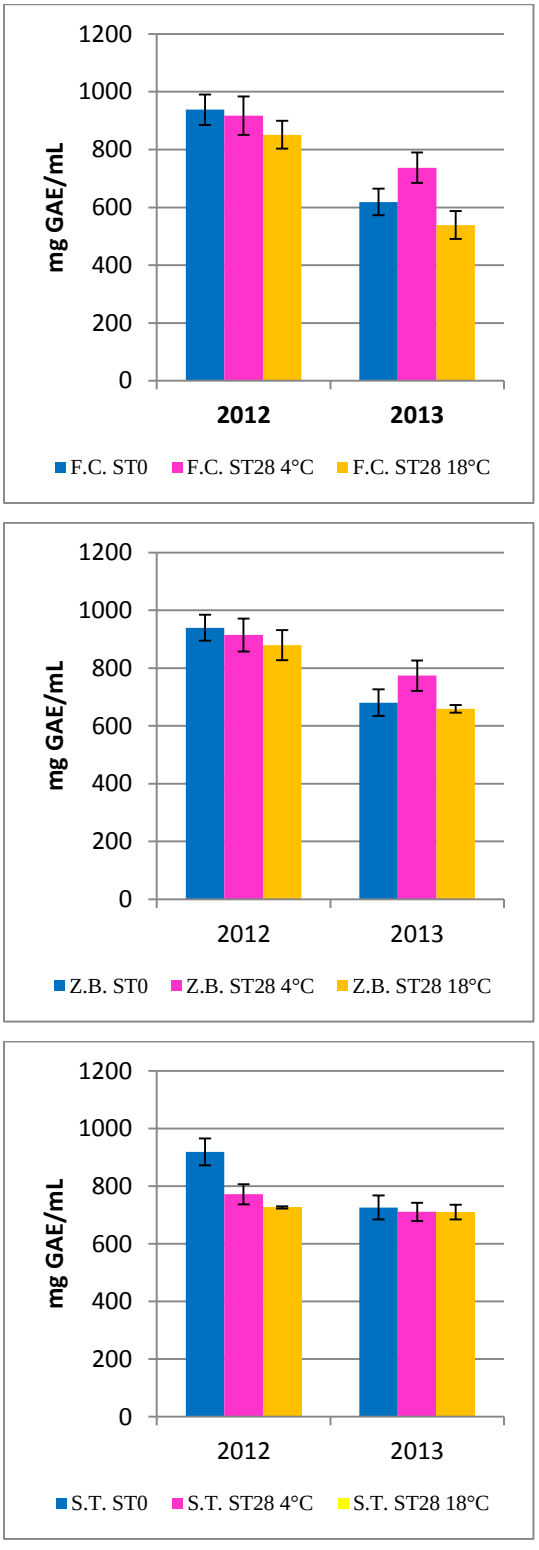

Fig.1. **Total Polyphenols Content**, express in mg GAE/mL [F.C.) cv. 'Femminello Comune'; Z.B.) cv. 'Femminello Zagara Bianca'; S.T.) cv. 'Femminello Santa Teresa']

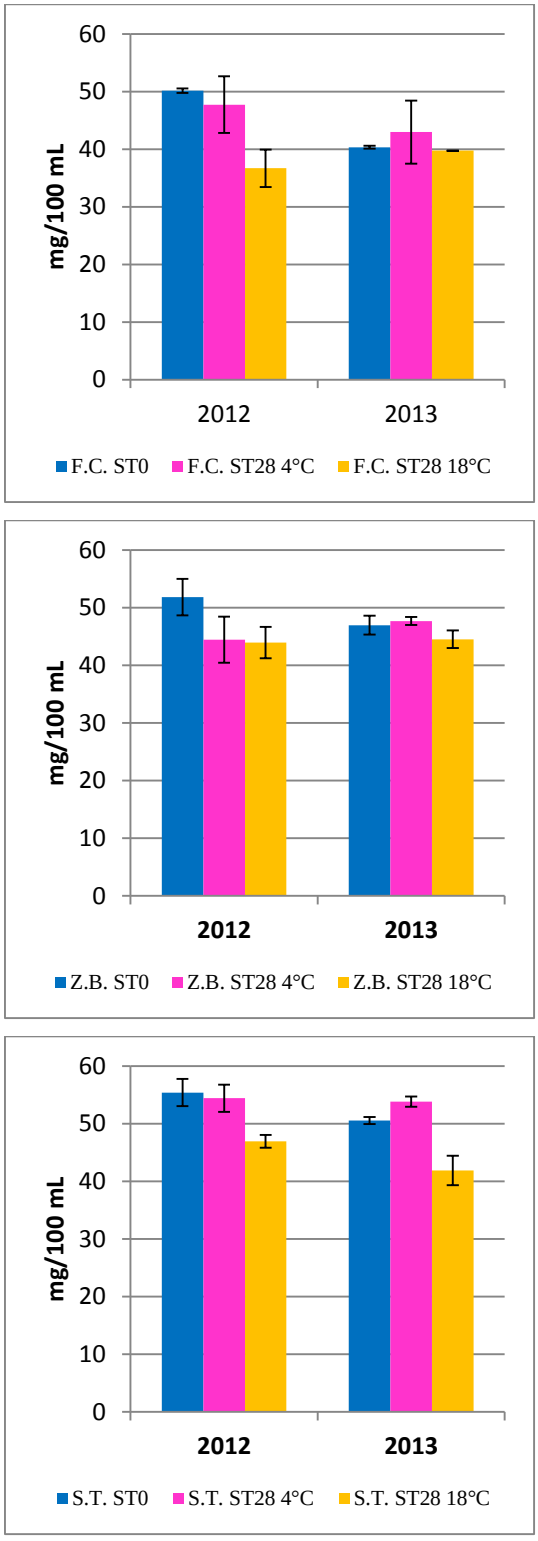

Fig.2. **Ascorbic acid content**, express in ascorbic acid mg/100 mL [F.C.) cv. 'Femminello Comune'; Z.B.) cv. 'Femminello Zagara Bianca'; S.T.) cv. 'Femminello Santa Teresa']

VOCs Analysis of Lemon Juices by GC-MS

The aroma characterization led to the detection and identification of 76 volatile molecules that can be divided in 6 principal chemical classes, as reported in Appendix 1 (Tables 1, 2, and 3).

The radar plot in Figure 3 shows qualitative analyses and comparison of the gas chromatographic peaks of the three cultivars in the two years analyzed, divided into chemical classes and expressed as relative percentage of area. To better highlight the small changes in the chemical classes, the plot scale used is logarithmic.

The overall aroma was richer in 2012, as shown by the total absolute areas analysis (data not shown), especially due to the higher content of aldehydes, esters and sesquiterpenes. Nevertheless, a larger number of molecules have been identified in 2013, and the volatile fraction of the fruits collected in this year is characterized by a higher content in monoterpenes.

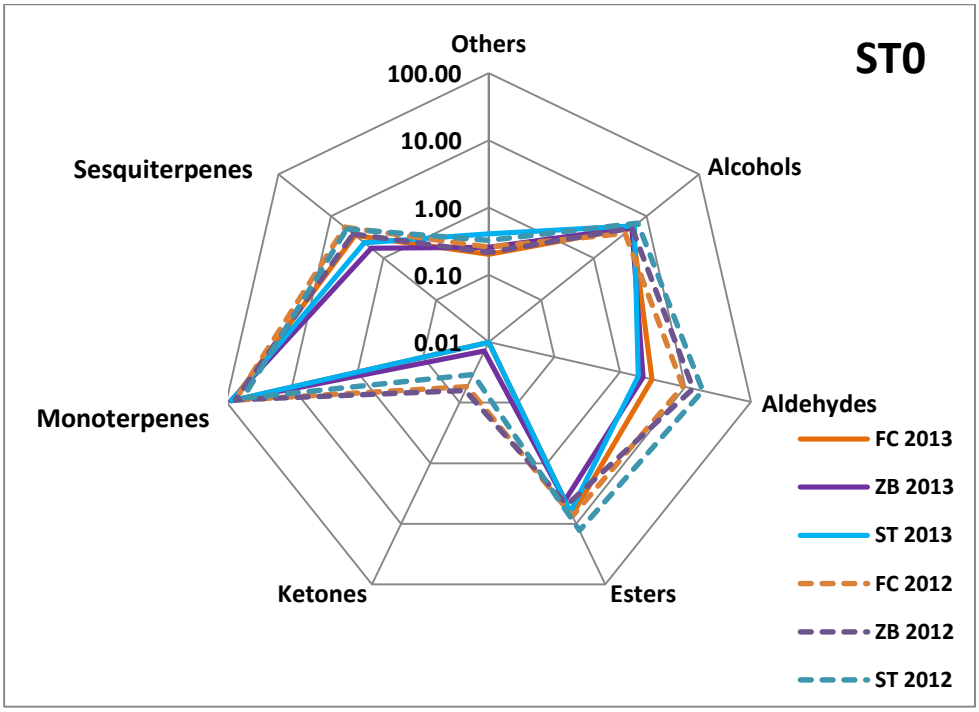

Fig.3. Chemical composition of the volatile fraction of the juices of the three cvs at Storage Time 0. F.C.) cv. 'Femminello Comune'; Z.B.) cv. 'Femminello Zagara Bianca'; S.T.) cv. 'Femminello Santa Teresa'

The analysis of the volatile fractions of the juices extracted from the different cultivars just after harvesting showed a different composition of VOCs according to the varieties. Particularly, cultivar 'Femminello Santa Teresa' is characterized by a

higher content of alcohols (mainly *4-terpineol* and *α.-terpineol*), aldehydes (especially *β-citral*), and esters (as *geraniol acetate*), and a lower content of monoterpenes, especially due to a lower content of *limonene*. Aldehydes and esters are characteristics of freshly squeezed juice and, with a small number of alcohols, contribute to citrus fresh juices green and floral odor notes (Perez-Cacho and Rouseff, 2008). These molecules contribute to characterize the flavor of the juice of this cultivar. In fact monoterpenes, aldehydes and esters contents were already proved to be discrimination factors of juices from different lemon cultivars (Allegrone et al., 2006). Particularly *β-citral* content was reported to be critical in the perceived quality of lemon flavor (Rouseff and Perez-Cacho, 2007).

Regarding the response of each cultivar to the storage conditions, slight differences were observed within the years and the cultivars. Particularly:

- Cv. 'Femminello Comune' (Fig. 4, Appendix 1 Tab. 1, and 2): alcohols increased during both storage treatments in 2012, while there were no appreciable differences in 2013. Aldehydes showed no changes with storage in both years. Esters increased with storage at 4 °C in both years, even this enhances was consisting only in 2012. Monoterpenes underwent to considerable reduction with refrigerated storage in 2012. Sesquiterpenes varied differently in response to storage, their content increased with refrigerated storage and decreased with storage at 18 °C. Ketone content was not affected by storage in both years.
- Cv. 'Femminello Zagara Bianca' (Fig. 5, Appendix 1 Tab. 1, and 2): alcohols content increased in 2012 in response to ST28 18 °C. Substantial reduction of aldehydes was observed with storage at 18 °C in 2013, while no changes were observed after storage in 2012. Ester content increased with both storage treatments in 2012, and in 2013 this enhancement was appreciable only in refrigerated storage. Monoterpenes considerably decreased with storage in both years. Solid increase of sesquiterpenes was observed with refrigerated storage. Ketone content did not vary in response to storage treatments.
- Cv. 'Femminello Santa Teresa' (Fig. 6, Appendix 1 Tab. 1, and 2): alcohol and ester contents were not affected by storage treatments. Aldehydes decreased with storage, and this reduction was consisting only in 2012. Monoterpenes substantially increased in 2012 in response to storage, while no

changes were observed in 2013. Sesquiterpene content did not vary in 2013, while in 2012 storage at 18 °C caused a valid reduction of this class.

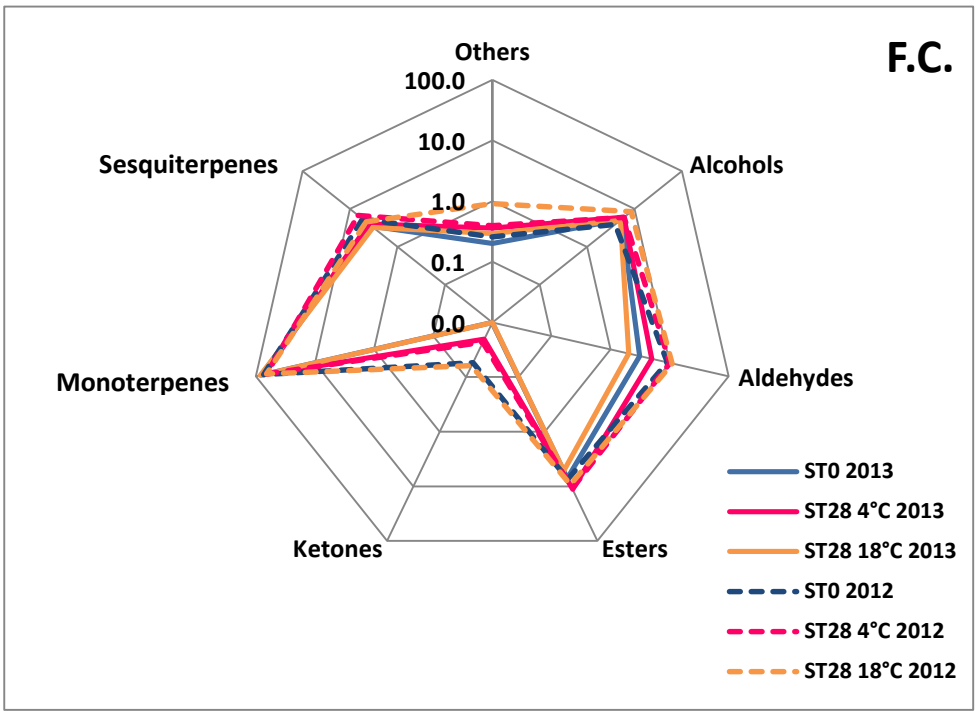

Fig.4. Chemical composition of the volatile fraction of the juices of the cv. 'Femminello Comune'

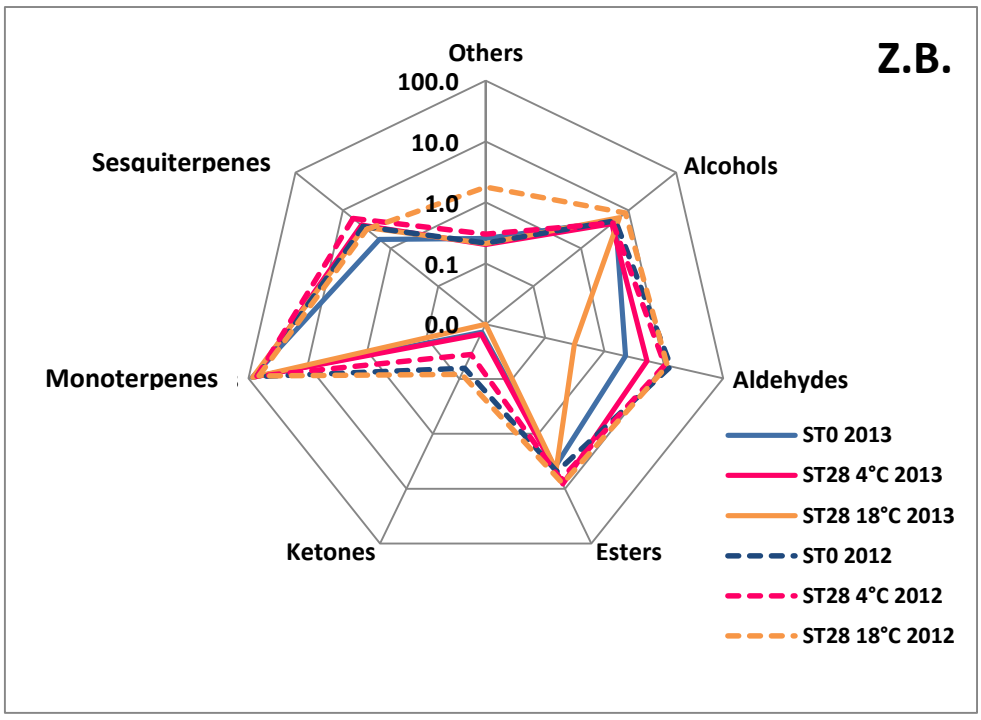

Fig.5. Chemical composition of the volatile fraction of the juices of the cv. 'Femminello Zagara Bianca'

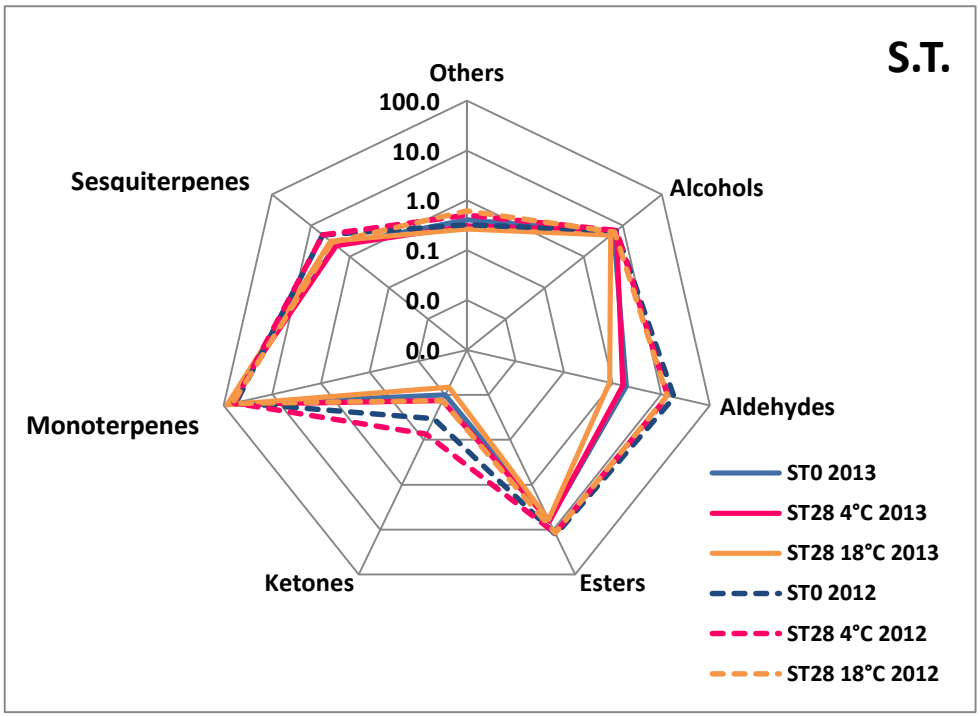

Fig.6. Chemical composition of the volatile fraction of the juices of the cv. 'Femminello Santa Teresa'

Generally, the pattern of variation depends on the cultivar and on the year. Nevertheless, it is possible to establish a general trend of variation of some chemical classes. Primarily, aldehyde, ester, monoterpene and sesquiterpene contents are influenced by storage. Aldehydes content decreases with non-refrigerated storage, while storage at 4 °C determines enhancement of esters and sesquiterpenes and decrease of monoterpenes. A previous work reported an enhancement of ester content and a decline of aldehydes in the juice during mandarins storage (Obenland et al., 2011).

Considering that the perceived aroma is the results of the complex combinations of all the molecules that constitute the volatile fraction, and is not due to the change of single molecule or chemical class, the data were submitted to PCA analysis merging all the collected data and using each molecule as a variable. This analysis confirmed that the biggest differences exist between the two years of harvest (Fig. 7). Actually, it is possible to divide the data in the PCA score plot into two big clusters: data from fruits harvested in 2012 and data from fruits harvested in 2013. As said before, the overall volatile pattern was different in the two years, and this factor probably determined the different response to the storage. The data shows that

in 2012 there was a variation in the volatile fraction caused by the storage at 18 °C, while in 2013 the biggest changes was due to the storage at 4°C. It is important to notice that in the performed PCA, the percentage of variability covered by each component was very low, about 40%, meaning that the variations in the aromatic patterns highlighted with this analysis are enlarged and the changes determined by the different treatments analyzed could not be significant.

The major components responsible of the different volatile composition of the juices and of the positioning of the data in the PCA plot are:

- *Limonene*, *trans-geraniol*, and *α-phellandrene:* the content of these compounds were higher and characteristic in 2013, and they are responsible of the shift along the right of the PC1 axis. *Trans geraniol* is characterized by odor type floral, medium odor strength, and a sweet floral and fruity odor description (Mosciano, 1997); *α-phellandrene* has a terpenic odor type, medium odor strength, and a citrusy slight green odor description (Mosciano, 1991); *Limonene* has a typical citrus odor type and a medium odor strength, its odor type is described as sweet and orange (Mosciano, 1994);
- *Nonanal*, *decanal* and *undecanal:* the content of these aldehydes was higher in 2012, and determined the shift toward the left of the PC1 axis. These three aldehydes are characterized by an odor type aldehydic, high odor strength, and a typical green citrus-lemon peel like nuance (Mosciano, 2001);
- *B-farnesene:* this sesquiterpene is the only molecule responsible of the shift upward along PC2 axis; its content was higher in 2012 and decreased in each cultivar with the non-refrigerated storage. It has a medium odor strength and woody citrusy sweet odor description (Mosciano, 1996);
- *Fenchol*, *α-terpineol*, *para-α-dimethyl styrene; 2,4,6-Octatriene, 2,6-dimethyl,* and *2,4,6-Octatriene, 2,6-dimethyl, E, Z:* the fluctuation of these molecules is the main responsible of the variation of the aromatic pattern detected in 2012 in response to the treatment ST28 18 °C. In 2012, the increase of content of these molecules with the non-refrigerated storage, determined the shift downward along PC2 axis. *Fenchol* alcohol has a balsamic odor type and medium odor strength characterized sweet lemon odor description (Luebke, 1989). *A-terpineol* has a floral, medium strength odor type, with citrusy floral odor description. *Para-α-dimethyl styrene* it has a high odor strength described as spicy and musty (Mosciano, 1996). *2,4,6-*

Octatriene, 2,6-dimethyl, and its stereoisomer *2,4,6-Octatriene, 2,6-dimethyl, E, Z* have a floral and medium strength odor type, characterized by sweet floral and tropical odor description (Luebke, 1983).

Generally, the shift along PC1, that covers the higher percentage of variance, describes the effect of the year; while the shift along PC2 describes the changes in response to storage conditions. Regarding the differences revealed between the years, is interesting to point out that in 2012, the major contributors to the overall aromatic pattern are represented only by aldehyde molecules. While in 2013 the overall volatile fraction is better represented by molecules belonging to different chemical classes, such as monoterpenes, alcohols and sesquiterpenes.

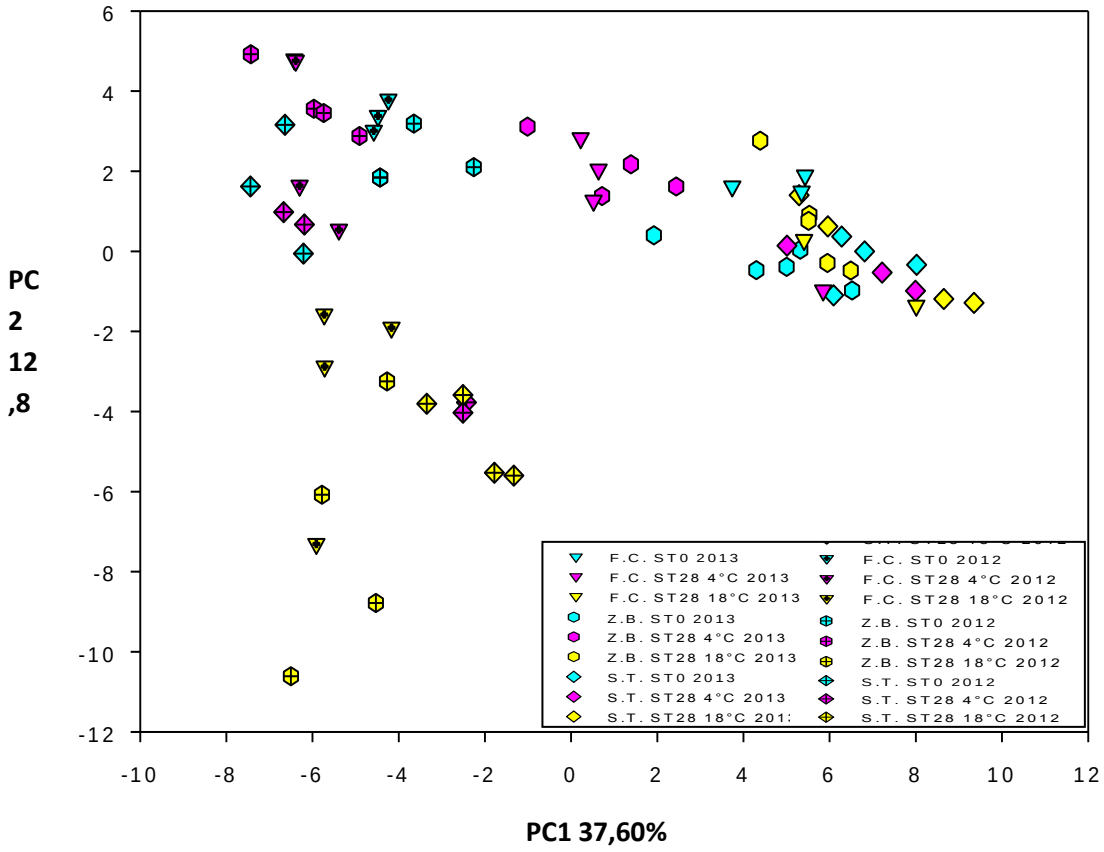

Fig.7. PCA analysis of the GC data of the years 2012 and 2013. F.C.) cv. 'Femminello Comune'; Z.B.) cv. 'Femminello Zagara Bianca'; S.T.) cv. 'Femminello Santa Teresa'

Aroma Pattern by Electronic Nose

The e-nose results are shown in Figure 8 in a PCA score plot. It is possible to note that the most appreciable differences were observed between the aromas of the juices of the fruits collected in different years, confirming the GC-MS observations. Moreover, all the cultivars showed similar aromatic patterns, and showed the same response to the storage conditions in the two years, with a slight shift of the points of the thesis ST28 18 °C towards PC1 axis. To better evaluate the response of each variety, a merge of the data of each cultivar in the two years was submitted to PCA analyses (Fig.9; Fig.10; Fig.11). All the cultivars showed the same behavior, repeatable within the years. The e-nose seems to be able to discriminate on the basis of the storage conditions: at storage time 0 and after 4 weeks at 4 °C the aroma fingerprints were very similar, while after the storage at 18 °C the aroma changed. In fact, the cluster of 18 °C measures has a shift towards the PC1 axis that represents more than 95% of the total variance. Considering also the shift that occurs along PC2 axis, in 2013 the cv. 'Femminello Zagara Bianca' showed a slight displacement due to the storage at 4 °C, as already observed in the GC data with the increase of ester and sesquiterpene classes. Anyway, considering the low variability represented by the PC2 axis, this shift is probably not significant, and the changes due to this kind of refrigerated storage are not strong enough to be well perceived by the E.N. system. Furthermore, the data of the cv. 'Femminello Comune' show no separation due the storage conditions, in all the analyzed years.

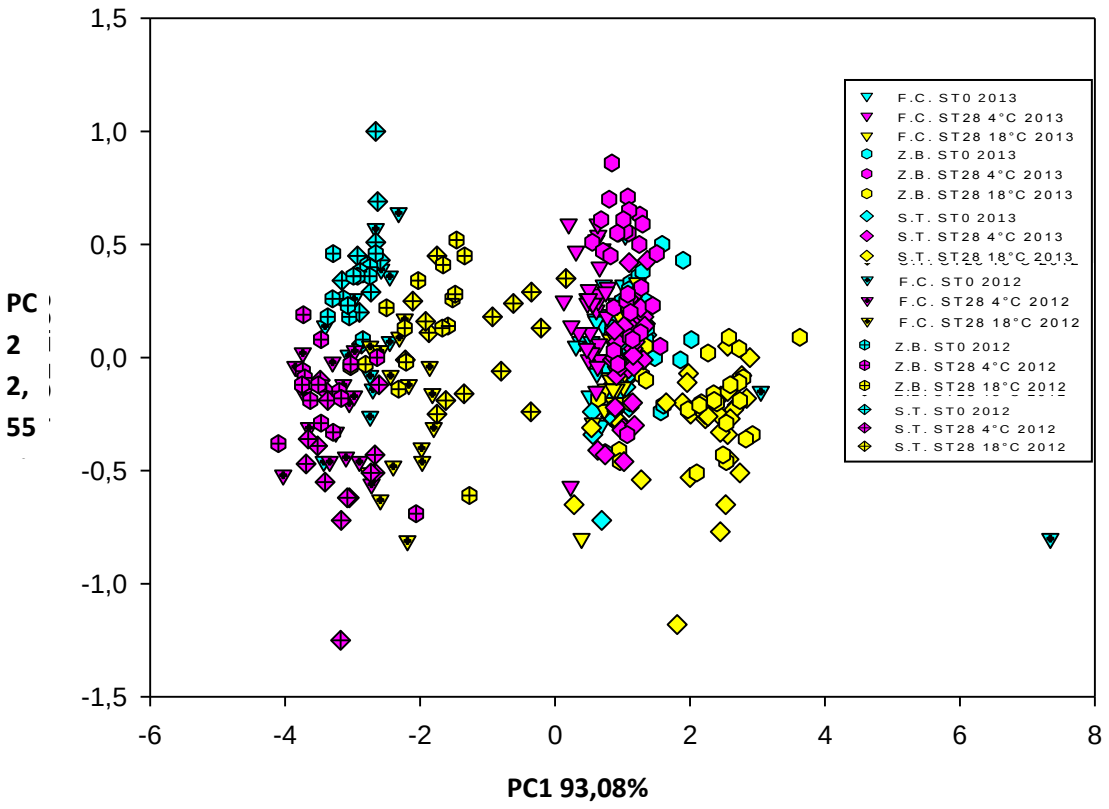

Fig. 8. PCA analysis of the E.N. data of the years 2012 and 2013

These changes are probably due to the variation in the content or in the intensity of some compound that are responsible of the aroma. To better understand which chemical classes had influenced these shifts, the e-nose results were compared with the GC-MS data. A merge of e-nose and GC-MS data was used as correlation matrix for the PCA analysis. The PCA plot shows that there are no differences between the aroma of the fresh squeezed fruits and the juices obtained from fruits stored for 4 weeks at 4 °C. Instead, the juices from fruits stored at 18 °C make a separate cluster. This kind of clusterization is very similar to the one obtained with only the e-nose data and GC data, and shows the same discrimination. So, there is a correlation between the information obtained from the e-nose analysis and from the analytical determination by the GC-MS.

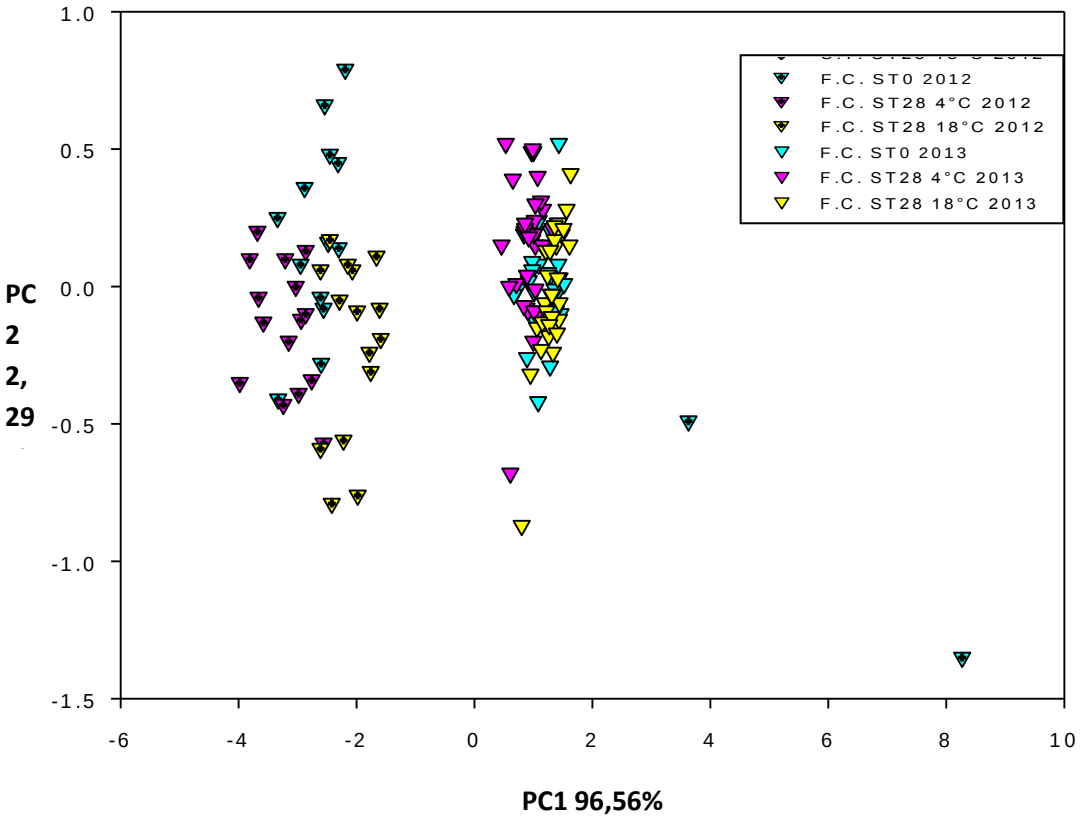

Fig. 9. PCA analysis of the E.N. data of the cv. 'Femminello Comune'. Comparison between years 2012 and 2013

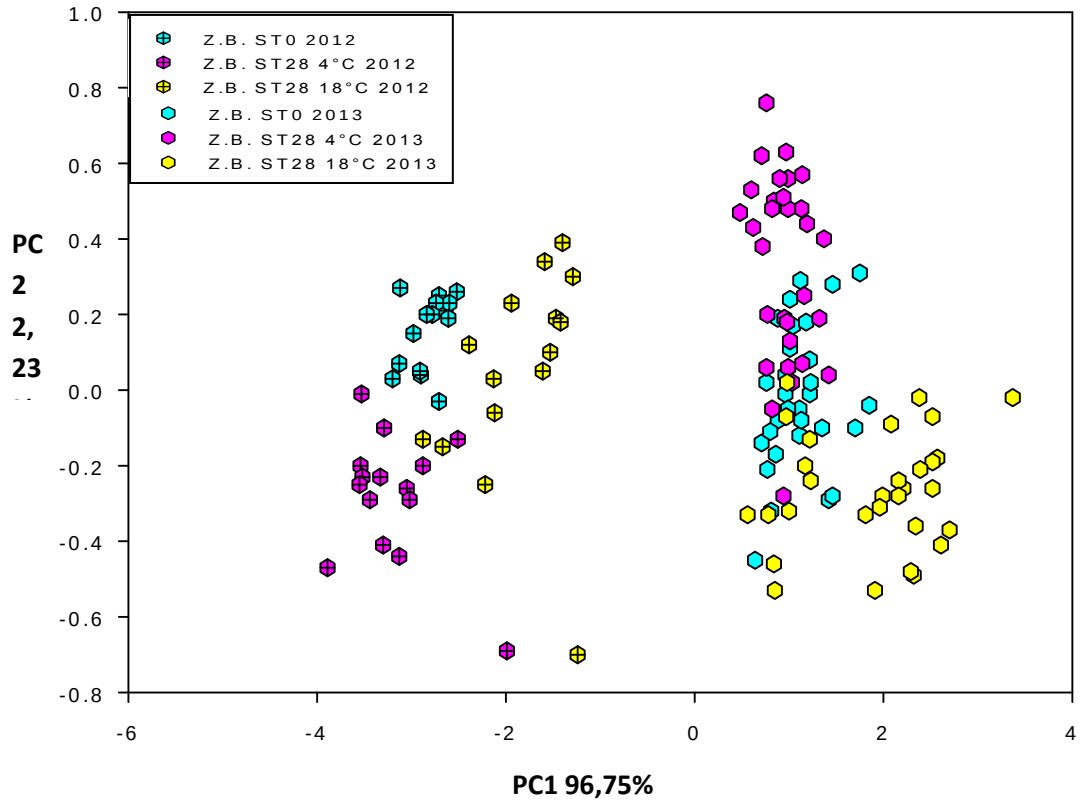

Fig. 10. PCA analysis of the E.N. data of the cv. 'Femminello Zagara Bianca'. Comparison between years 2012 and 2013

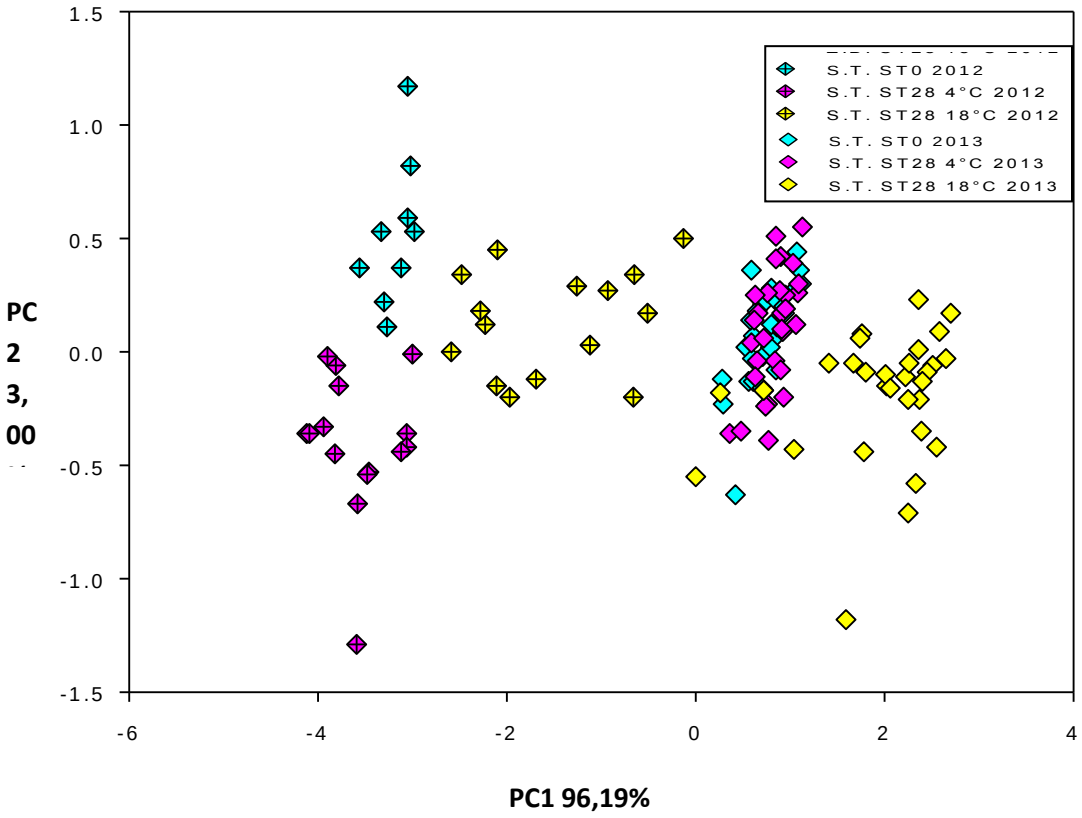

Fig. 11. PCA analysis of the E.N. data of the cv. 'Femminello Santa Teresa'. Comparison between years 2012 and 2013

2.4 Conclusions

The main effect observed in this experiment was due to the influence of the year analyzed. Probably, the high variability of the analyzed parameters could be explained according to the differences of the climate conditions between the two years.

The effect of the cultivar was statistically significant for all the physical and chemical parameters analyzed in 2012, while in 2013 this effect was reduced probably due to the environmental influence. The effect of storage was significant for the carpometric parameters, at 18 °C as well as at 4 °C in both years.

The difference in the maturity stage between the analyzed years probably determined the different responses obtained in the two years to storage treatments, especially regarding total polyphenols content and vitamin C. In 2012 storage induced a decrease of both TPC and vitamin C. On the contrary, in 2013 storage at

18 °C determined a decrease, while refrigerated storage caused an increase of bioactive compounds.

By the e–nose measurements, all the cultivars showed a variation of the aroma depending on the storage conditions. The e–nose responses were confirmed by the GC data, which show a different behavior of the chemical classes, mainly due to an increase in the percentage of alcohols, esters and sesquiterpenes, and a decrease of aldehydes and monoterpenes. The E.N. results showed no discrimination among the aromatic pattern of the three cultivars tested. GC-MS results, instead, highlighted a different composition in the volatile composition of the cultivar 'Femminello Santa Teresa'.

Regarding the changes observed in the different chemical classes, the environmental influence was stronger than the treatments imposed, providing no significant changes. Analyzing just the general content of each chemical class, it is interesting to highlights that aldehydes, esters, and sesquiterpenes were significant higher in 2012, while in 2013 there was a major content of monoterpenes. This is consistent with the different maturity stage of the fruits in the two analyzed years, determined by chemical parameters analysis. Fruits collected in 2012 were less mature and the volatile fraction was richer in aldehydes and esters that are responsible of green and fruity notes.

So, both the e-nose and the GC-MS revealed that only some modifications occurs in the volatile pattern due to the storage conditions, but the merge of data is not always easy to understand. Future work are needed do better understand the possibility of establishing a correlation between the two techniques, leading to a simple and rapid method to control the aroma quality during the storage that can be adopted by the food and storage industries.

3. EXPERIMENT 2:

Application of a new electronic nose instrument to assess the effects of some postharvest treatments on the quality of 'Salustiana' orange juice

Abstract

Salustiana oranges were picked at different steps of a commercial fruit packing line: right after harvesting, after the washing, after the ethylene degreening, and after waxing. Fruits were stored for 0, 4, or 8 weeks at 4°C followed by one week at 20°C. Fruits were individually weighted and analyzed for percentage of juice content, Total Soluble Solids, and Titratable Acidity. The aromatic patterns of orange juices were evaluated by two different electronic nose instruments, in order to taste the ability of the two instruments to monitor the changes that occur during storage. Moreover, volatile fractions were analyzed by GC-MS techniques.

3.1 Introduction

The majority of citrus fruits are consumed as fresh product, and this clearly indicates the importance to preserve the natural qualities of fresh citrus after harvesting. To achieve this objective, one of the most critical point is represented by the postharvest treatments and storage. In fact, all packing house operations until the arrival of the products to the final market play a very important role in maintaining the quality characteristics of the fruits. Storage is one of the most critical point in the commercialization of citrus fruits. In fact, all the alterations that can be induced by the postharvest treatments are amplified during the storage.

It is well known that all of the steps of the packing house have potential effects on the internal quality of citrus fruits, and for this reason in the recent years many studies has been conducted to optimize the use of all these treatments in order to reduce undesired effects and quality decay during storage (Obenland et al., 2009).

Ethylene degreening is one of the most common practice in citrus postharvest management that can stimulate various ripening related processes, as the destruction of chlorophyll pigments and the formation of carotenoids in the peel tissue (Rodrigo and Zacarias, 2007). Previous works reported different effects of the degreening on fruits and juices quality attributes, showing very slight effects (Mayuoni et al., 2011); or severe physiological and biochemical (Rodrigo and Zacaria, 2007), that can even involve alterations in the volatile composition of the aromatic pattern and the development of off-flavors (Testoni et al., 1992), related to the accumulation of ethanol (Sdiri et al., 2012).

Wax coating is usually applied with an esthetical purpose, to make fruits more attracting to consumers. But coating does not have only this effect, limiting gas exchanges between the fruit surface and the external atmosphere, and modifying the internal atmosphere of the fruits, enhancing the level of CO_2 and reducing O_2. This can lead to the production and accumulation of off-flavor volatile, such as ethanol and acetaldehyde (Tietel et al., 2011). The effect of wax on quality attributes of citrus fruits is not well clear. The modified atmosphere can lead to an alteration in the Soluble Solids Content/Titratable Acidity ratio and in the composition of aroma volatiles, some increasing, other decreasing and some of them positively correlated with taste and aroma (Obenland et al., 2008). In two different studies, Baldwin et al. (1995) and Hagenmaier and Shaw (2002) did not find any effect of waxing during the storage of oranges, grapefruit or tangerines. Also Obenland in a study conducted in 2008 on navel oranges, did not find a clear effect of waxing and storage on sugar and acidity levels. However, the effect of the coating treatment is strictly dependent of the type of wax used (Marcilla et al., 2009; Hagenmaier, 2002).

To understand the combined effect of the postharvest treatments and how they act together during the storage and the shelf life to modify the quality can be very useful.

The objectives of this research were: a) to evaluate the quality of ‘Salustiana’ juice squeezed from fruits submitted to postharvest treatments with fungicide, degreening and wax coating, by monitoring the changes in the aroma fingerprint; b) to evaluate the effect of different treatments during two different fruits storage conditions, on the aromatic pattern of juices; c) to evaluate the capacity of a new Electronic Nose instrument to monitoring the change in volatile composition of ‘Salustiana’ oranges submitted to different postharvest treatments and storage

conditions. Moreover, different methods of sample classification, such as Bayesian nets, Artificial Neuron Networks and classification tree were applied.

3.2 Materials and Methods

Plant Material

This study was carried on fruits of 'Salustiana' orange (*Citrus sinensis* L. Osbeck), purchased by Emilio Esteve (www.emilioesteve.com) farm located in Xeraco, in the region of Valencia, Spain. Fruits were harvested on 13 December 2013, at the commercial maturity stage. A total of 160 fruits were divided in four theses, 40 fruits for each sample, and four postharvest treatments were conducted, respectively. After the treatments, the fruits were transported to the laboratory of the "Departamento de Ingeniería Electrónica", Polytechnic University of Valencia for the storage tests.

Postharvest treatments

Different postharvest treatments were conducted picking up the samples in subsequent step of the packing line. Precisely:

- Control: fruits were provided and analyzed directly right after the harvest;
- Chemical treatment: fruits were drenched with a mixture of fungicide product, namely Fecundal S-7,5 6 L/1000 L, biostimulant product, namely Fortisol Ca Plus 8 L/1000 L; and oxidizing product, like Oxypure 902 DW-50 1,8 L/1000 L, for the disinfection of water;
- Ethylene degreening treatment: after the packing line fruits were stored for 72 h with the application of 1.5 ppm of ethylene and a continuous flow regulated at 22 ±1 °C, and 95% - 98% of relative humidity, and CO_2 concentration at 3.000 ppm;

- Wax treatment: to evaluate the effect of the entire packing line, fruits were analyzed at the end of the process after coating with Citrosol Imad 2 VS UE, a commercial emulsion with a polyethylene/shellac ratio of 20% (w/v) and an Imazalil content of 0.2%.

Control fruits Chemical fruits

Ethylene fruits Wax fruits

Storage conditions

The samples were analyzed in the first 24 - 48 h after harvest (T0). Treated and control fruits were stored at 5±1 °C and 85% RH for eight weeks and analyzed after four (T1), and after eight weeks (T2) to assess the changes occurring during the storage. After the storage period, all the fruit were kept at 20 °C for 7 days to simulate the shelf-life conditions (T3).

Methods

For each treatment and storage period, 10 fruits were individually weighted, and the longitudinal and transverse diameters were measured with a digital caliper. All the fruits were then squeezed and the juice was used to analysis of juice yield, soluble solids content (TSS), titratable acidity (TA), Maturity Index (MI), pH and aromatic pattern.

Total soluble solid content was evaluated with a digital refractometer with automatic temperature compensation (Atago) and expressed as °Brix.

Titratable acidity was determined by titrating to pH 8.1 using 0.1 N NaOH and expressed as g/ L of citric acid.

Maturity Index was calculated as the ratio between TSS, expressed in °Brix, and TA, expressed in percentage (%).

The **aromatic pattern** of the juice was analyzed with the two following Electronic Nose instruments:

- **Electronic Nose EOS835** (Sacmi): two milliliters of pure juice were placed into a 20 mL glass vial, sealed and incubated for 5 min at 50 °C under stirring. The measurement duration was 27 minutes. The instrument is equipped with an array of 6 MOS sensors (as described in paragraph 3.3.2.1).
- **Multisensory Odor Olfactory System – MOOSY32**: for the aromatic pattern analysis, 10 mL of juice was kept in a Petri dish maintained at 25 °C. Total acquisition time was 3,5 minutes and the air flow was settled at 0,5 m/s. After 2 minutes of equilibration time, in which the sample was placed in the acquisition chamber, for each analysis were made 5 repetitions with a delay between repetitions of 10 sec. The instrument is equipped with an array of 32 MOS sensors (as described in paragraph 3.3.2.2).

The **volatile fraction** was evaluated using a Gas Chromatographer equipped with a Mass Spectrometer (GCMS QP2010, Shimadzu). The instrument was equipped with a SLB5-ms capillary column (30 m x 0,25 mm x 0,25 µm, Supelco), and settled as previously described by Costa et al. (2010), with some modifications (Cupane et al., 2012). Before analysis, samples were conditioned for 30 minutes at 60 °C, with the addition of salt in order to promote the removal of volatile molecules from the juice matrix. The volatile fraction was sampled with the headspace solid phase micro extraction (HS-SPME) technique using a Car/PDMS fiber (1 cm, Supelco).

The peaks identification was performed through comparison of the experimental mass spectra and those reported in the National Institute of Standards and Technologies (NIST) libraries incorporated in the instrument software (GCMS solution Library, Shimadzu) and by comparison with previous studies on citrus fruits (Yo and Lin, 2004; Allegrone et al., 2006; Dharmawan et al., 2007; Tounsi et al., 2010; Gonzalez-Mas et al., 2011; Saura et al., 2012). Libraries used were: NIST 21, NIST 107, and NIST 147. Only the molecules recognized with a percentage of

similarity greater than 90% were used for this study. All the analyses were performed for each sample in duplicate.

Statistical Analysis

Physical and chemical parameters data were submitted to two-way Analysis of Variance (ANOVA) using the software SYSTAT 13 (Systat Software Inc.) and analyzed for the effects of treatment and time. Means are separated using Tuckey Honestly Significant Difference Test.

For the e-nose sensors responses, a multivariate classification analysis with the software WEKA using different algorithms was performed. WEKA is an open source software issued under the GNU General Public License (Hall at al., 2009). The different algorithms applied were: two probabilistic models, Bayes Net and Naïve Bayes, that calculate a set of probabilities by counting the frequency and combination values on a given data set; two Artificial Neuron Networks (ANN), Multi-Layer Perceptron (MLP) and Radial Basis Function network (RBF), that permit to create a model, during a training test, that is able to classify the data (Batista et al., 2012); a classification tree, J48 that is a support system that uses a tree- like graph decisions and their possible after effect; and a lazy algorithm named IB1, that uses the technique of the 'Nearest Neighbour' to classify new instances using a similarity function to calculate the similarity between the training instance and the instances of the data set (Barrueta et al., 2007).

The classification analysis consists in the organization of data in classes, using given class labels to order the objects in the data collection. Classification approaches normally use a training set where all objects are already associated with known class labels. The classification algorithms learn from the training set to build a model. The model is used to classify new objects (Patil and Sherekar, 2013). ANNs learn from examples through iteration, without requiring a priori knowledge of the relationship among variables under investigation (Benedetti et al. 2004).

The results are illustrated in the confusion matrix that represents the accuracy of the solution of the classification problem. It allows the visualization of the performance of an algorithm. Each column of the matrix represents the instances in a predicted class, while each row represents the instances in the actual class. The ideal result is to

have all the samples end up on the diagonal cells of the matrix (Benedetti et al. 2004).

3.3 Results and Discussion

Physical and Chemical Parameters of Treated Orange Fruits

Carpometric parameters were significantly affected by storage time, but the interaction between treatment and storage time was not statistical significant, meaning that, regardless of the treatment applied, the variation of the carpometric parameters during storage remains unchanged (Tab. 1). Juice content was not altered by storage. Storage had no significant effect on TSS or TA values (Tab. 2). Regarding maturity index, it was significantly affected by storage time, generally increasing over time. This parameter allows noticing that there are slight differences in content of TSS and TA that cause the enhancement of this index. These results are in general agreement with those reported from previous work (Marcilla et al., 2006). Moreover, similar studies demonstrated that fruit quality can be properly preserved in cold conditions for long period of time, resulting only in a small reduction of flavor quality, and a small enhance in the VOCs content, weight loss and maturity index (Martínez Jávega et al., 1991; Baldwin et al., 1995).

Titratable Acidity: Acidity content decreased after 4 weeks and enhanced after 8 weeks. The storage week at 20 °C led to a decrease in its content. Ethylene treatment caused a lower level of TA that remains lower respect to the other treatments, as previously showed in experiment using ‘Mosambi’ oranges (Ladaniya and Singh, 2001).

Total Soluble Solids: Soluble solids content was found to be more stable during storage, with respect of TA content. This caused an increase of maturity index as consequence of the last storage week at 20 °C.

pH: pH values generally registered a slight increase with storage. Its content reflects the variations in total acidity content.

Tab. 1. Physical parameters of the analyzed fruits

Treatment	Time	Weight (g)	DL (mm)	DT (mm)	Juice % (w/w)
Control	T0	220,2±8,4	71,1±3,4	76,1±5,1	49,6
	T1	181,0±28,8	67,5±4,0	71,9±4,0	52,4
	T2	157,3±13,8	63,1±3,4	66,9±3,4	53,6
	T3	161,0±24,8	61,1±2,8	67,3±4,4	53,3
Chemical	T0	194,2±21,1	70,1±4,2	73,9±3,1	53,0
	T1	168,6±24,2	66,5±4,2	70,8±4,3	52,8
	T2	159,9±26,0	65,6±3,9	67,0±4,4	52,5
	T3	150,4±20,2	61,9±5,0	66,2±3,8	52,8
Ethylene	T0	221,0±17,3	69,9±4,8	77,5±2,4	52,3
	T1	211,1±25,1	68,6±6,4	75,2±3,8	52,1
	T2	204,0±34,0	67,9±5,4	74,7±5,7	51,1
	T3	183,0±33,0	63,3±3,9	70,5±5,1	54,2
Wax	T0	164,0±2,4	64,8±3,5	68,5±1,9	53,8
	T1	162,1±6,1	63,0±4,3	66,3±2,0	54,1
	T2	149,1±5,1	60,5±2,2	65,0±2,3	54,1
	T3	136,9±5,6	58,2±3,7	63,1±1,5	56,3
Treatment		**	**	**	***NS***
Time		**	**	**	***NS***

p-value is determined by ANOVA for P<0,05. For each parameter, *p* indicates differences among Treatments and Time.
Time T0: just harvest; T1: after 4 weeks at 4 °C; T2: after 8 weeks at 4 °C; T3: after an additional week at 20 °C. Main effects are indicated as non-significant (NS) or significant at either the * $p<0,05$ or ** $p<0,01$

Tab. 2. Chemical parameters of the analyzed fruit juices

Treatment	Time	TSS (°Brix)	TA (g/L citric acid)	pH	M.I.
Control	T0	9,4±0,1	10,7±0,1	3,6±0,0	8,8±0,1
	T1	9,9±0,1	11,3±0,1	3,5±0,0	8,8±0,1
	T2	10,3±0,1	11,7±0,0	3,6±0,0	8,8±0,1
	T3	10,2± 0,0	10,9±0,1	3,7±0,0	9,3±0,1
Chemical	T0	10,0±0,1	11,9±0,1	3,4±0,0	8,4±0,2
	T1	10,7±0,1	11,2±0,1	3,5±0,0	9,6±0,1
	T2	10,8±0,0	11,7±0,0	3,6±0,0	9,2±0,0
	T3	10,6±0,1	11,2±0,1	3,6±0,0	9,4±0,1
Ethylene	T0	10,2±0,1	10,7±0,0	3,6±0,0	9,5±0,1
	T1	10,4±0,0	10,5±0,0	3,6±0,0	9,9±0,0
	T2	9,9±0,0	10,9±0,1	3,6±0,0	9,1±0,1
	T3	10,12±0,0	10,3±0,0	3,7±0,0	9,8±0,0
Wax	T0	9,6±0,0	11,7±0,0	3,4±0,0	8,2±0,0
	T1	10,2±0,1	10,6±0,0	3,6±0,0	9,6±0,1
	T2	10,2±0,1	11,5±0,0	3,7±0,0	8,9±0,1
	T3	9,9±0,1	10,9±0,1	3,7±0,0	9,1±0,1
Treatment		*****	*****	***NS***	***NS***
Time		***NS***	***NS***	******	*****

p-value is determined by ANOVA for P<0,05. For each parameter, *p* indicates differences among Treatments and Time.
Time T0: after harvest; T1: after 4 weeks at 4 °C; T2: after 8 weeks at 4 °C; T3: after an additional week at 20 °C. Main effects are indicated as non-significant (NS) or significant at either the * $p<0,05$ or ** $p<0,01$

Aroma Pattern by Electronic Noses

At the beginning all the responses from the E.N. systems were used as inputs to the model that was build. The classification was performed separating one subset of samples for training and another for testing, using the cross-validation method. Tables 3 and 4 show the results obtained by the different algorithms to classify fruits from different treatment in each time. It was necessary to use algorithms to test the variables that most strongly influenced the classification. Variable selection consists in the selection of a subset of variables that are the most discriminating (Berrueta et al., 2007). For this purpose we used two different algorithms: CfsSubsetEval and GreedyStepWise. The stepwise selection is based on a greedy search that sequentially adds or deletes variables from the pool of total variables (Berrueta et al.,

2007). The addiction or deletion of a variable is determined based on the largest improvement in the classification, until the search finds the most influencing variables.

Tab. 3. Results of the used classification algorithms for the EOS835

Sample type	Classifier algorithm % accuracy					
	Bayes Net	Naive Bayes	MLP	RBF	J48	IB1
T0	20	35	40	25	30	40
T1	93,06	94,44	93,06	93,06	94,44	91,67
T2	95,77	88,73	92,96	90,14	92,96	94,37
T3	90,41	65,75	95,89	86,3	90,41	84,83

Tab. 4. Results of the used classification algorithms for the MOOSY32

Sample type	Classifier algorithm % accuracy					
	Bayes Net	Naive Bayes	MLP	RBF	J48	IB1
T0	92,5	95	95	97,5	88,75	97,5
T1	100	100	100	100	96,25	100
T2	97,5	97,5	98,75	98,75	91,25	97,5
T3	87,5	93,75	87,5	92,5	85	90

The results show as:

- EOS835 is not able to distinguish among the different juice samples at T0;
- General classification accuracy is better in MOOSY32 than in EOS835;
- In the MOOSY32, the algorithm J48 permitted the worse classification, and the percentage of accuracy decreased with time. Not the same for EOS835. It can be due to the diverse number of sensors, and information that had to be processed;
- The applied algorithms showed different performances;
- In the MOOSY32the classification accuracy decreased over time and it is maximum in storage time T2, after 8 weeks of storage at 4 °C;
- In the EOS835 system the classification accuracy was higher in T1, after 4 weeks of refrigerated storage, and decreased over time.

It is interesting to notice that, while in the other analysis time the classification was better in the MOOSY32, in T3 is the opposite with EOS835 having the best percentage of correct classifications.

Detailed confusion matrices of each algorithm applied in each time of storage are presented in Appendix 2 (Tab. 6).

Both instruments allowed a good classification of the analyzed juice samples according to the time of storage, with better results using the MOOSY32. It is possible to highlight a trend in the observed data:

- T0: this time gave the worst classification, especially for EOS835. The aromatic patterns were similar right after the treatments. The lower percentage of classification is caused by confusion between 'Control' and 'Chemical';
- T1 and T2: showed the greater percentage of correctly classified instances with some accuracy difference due to the different instruments and algorithms;
- T3: this time permits the worst classification with all the algorithms.

These results are consisting with the hypothesis that initially the aromatic patterns were quite similar (T0), but with slight differences that permitted the classification. Since fruits belonging to the same lot, the differences at T0 can only be due to the applied treatments. Major mistakes in classification were mainly due to un-correct classification of 'Control' and 'Chemical' treated samples.

With advancing of time the aromatic patterns became more different, resulting in an increase of the correctly classified instances. After 4 and 8 weeks of storage (T1 and T2) the instruments were able to better classify the samples, meaning that the major changes occurred during this period. It is possible to suppose that the different treatments caused variations in the internal atmosphere of fruits, probably due to metabolic changes and variations in the respiration rate, as reported in literature (Rodrigo and Zacaria, 2007; Mayuoni et al. 2011; Tietel et al., 2011), that led to different VOCs production and consequently to changes in the aromatic pattern.

The last analysis time (T3) gave the worst results. There were no more detectable differences in the aromatic patterns that became similar for all the treatments after a week of storage at 20 °C. All the changes that were stimulated by the applied treatments were amplified by the storage, and overall by the temperature of storage.

To confirm this hypothesis and to better understand the evolution of the aromatic pattern, juice samples were analyzed by GC-MS instrument.

VOCs Analysis of Orange Juices by GC-MS

The aroma characterization led to the detection and identification of 72 volatile molecules that can be divided in 6 principal chemical classes, as reported in Appendix 2 (Tables 3, and 4). The radar plots, in Figures 1, 2, 3 and 4, show qualitative analyses and comparison of the gas chromatographic peaks of the analyzed juices, divided into chemical classes and expressed as a percentage of area. To better highlight the small changes in the chemical classes, the plot scale used is logarithmic.

There was a general trend in the variation of several chemical classes over time, with aldehyde and alcohol contents tending to diminish, and ester and sesquiterpene contents increasing. Regarding aldehydes, *hexanal* was the major molecule that contributes to this class. It is an important aldehyde that characterize orange flavor, and its content dramatically decreased with storage and was not detectable after the week of simulated shelf life. Several molecules contribute to the alcohol content, and its variation depended on the variation of several molecules, such as *1-octanol*, *β-linalool*, *β-terpineol*, *nonanol*, *3-exen-1-ol*, and *4-terpineol*. For this chemical class, the variation was more accentuated between T0 and T1, and then the overall content remained stable. Especially *β-linalool* and *3-exen-1-ol* are important molecules, whose content makes a positive contribution to orange flavor in combination with other volatiles, being responsible respectively of a floral-woody odor with a faintly citrusy note (Arctander, 1969), and of a fruity-green flavor in fresh orange juice. *B-linalool* was present in major amount in 'Control' fruits, and *3-exen-1-ol* is detected only in 'Control' and 'Chemical' fruits. The decrease of both these compounds over time is consistent with the decrease of orange flavor in response to storage and to different treatments.

Ester and sesquiterpene contents tended to enhance with time. Fruits of the 'Control' were characterized by a lower content of esters, especially *ethyl butanoate* and *ethyl hexanoate*. As previously reported, its content increases with maturation (Selli et al., 2004). Sesquiterpene content increased after 4 weeks of storage, and then remained stable. The major contributors to this chemical class were *valencene*, whose content was higher in the ethylene and wax treatments, and *β-panasinsene*. Monoterpene content remained almost stable with time and treatments. Being esters and aldehydes the primary contributors to fresh orange flavor (Bruemmer, 1975) their content reflect the major changes that occur in fruit aroma as a consequence of the packing line and storage.

Analyzing each time specifically, it is interesting to notice that at T0 (Fig. 1), right after the treatments, the volatile composition was similar in groups of two: ‘Control+Chemical’ and ‘Ethylene+Wax’. This clusterization remained stable in T1 (Fig. 2), then the differences tended to flatten. After 8 weeks of refrigerated storage (T2, Fig. 3) the aromatic pattern of fruits submitted to chemical treatments became similar to that of ‘Ethylene+Wax’, while ‘Control’ remained different. The major variations were related to the aldehyde, ester and sesquiterpene contents. After another week of storage at 20 °C the volatile composition of all the fruits, regardless of treatment, was very similar (T3, Fig. 4). The only remarkable difference concerned the esters remained lower in ‘Control’ fruits, while it increased in the other treatments. Even if previous work showed a positive correlation among esters and orange flavor (Selli et al., 2004), this enhance over time is not necessarily positive for the aromatic pattern of the juice because it can lead to an altered balance in the fruit aroma, which is dependent on the correct proportion of the different compounds (Shaw, 1979). It was also reported that e*thyl butanoate* and *ethyl hexanoate* contents increase in concentration over the course of the storage in packed orange fruits, while it is not detected in fruits that did not pass through the packing line (Obenland et al., 2008).

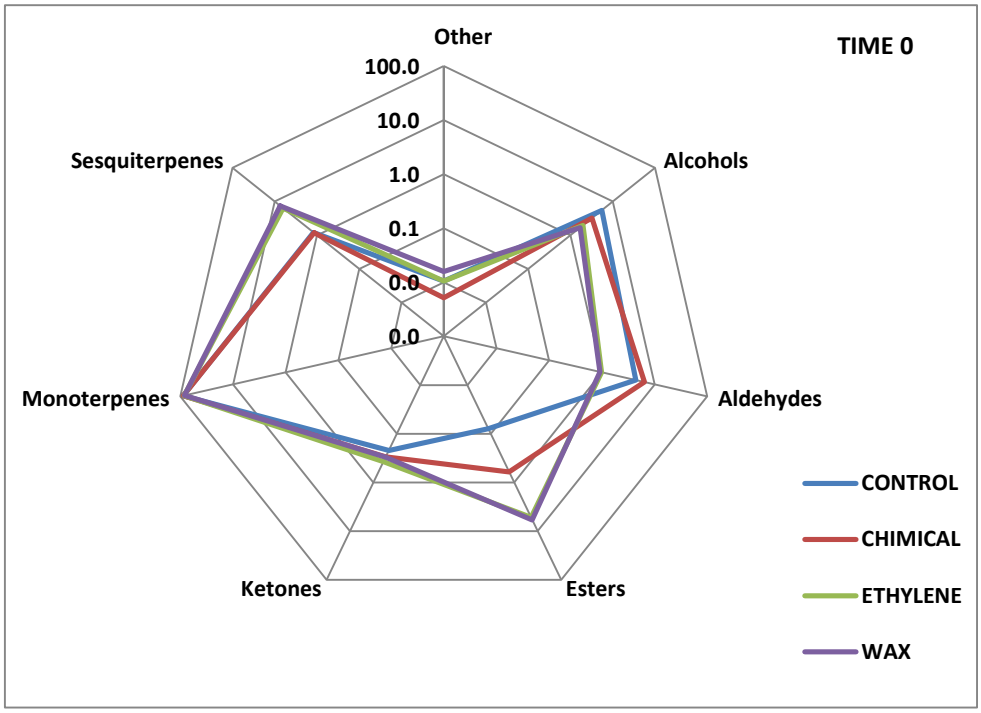

Fig.1. Chemical composition of the volatile fraction of the juices at Storage Time 0

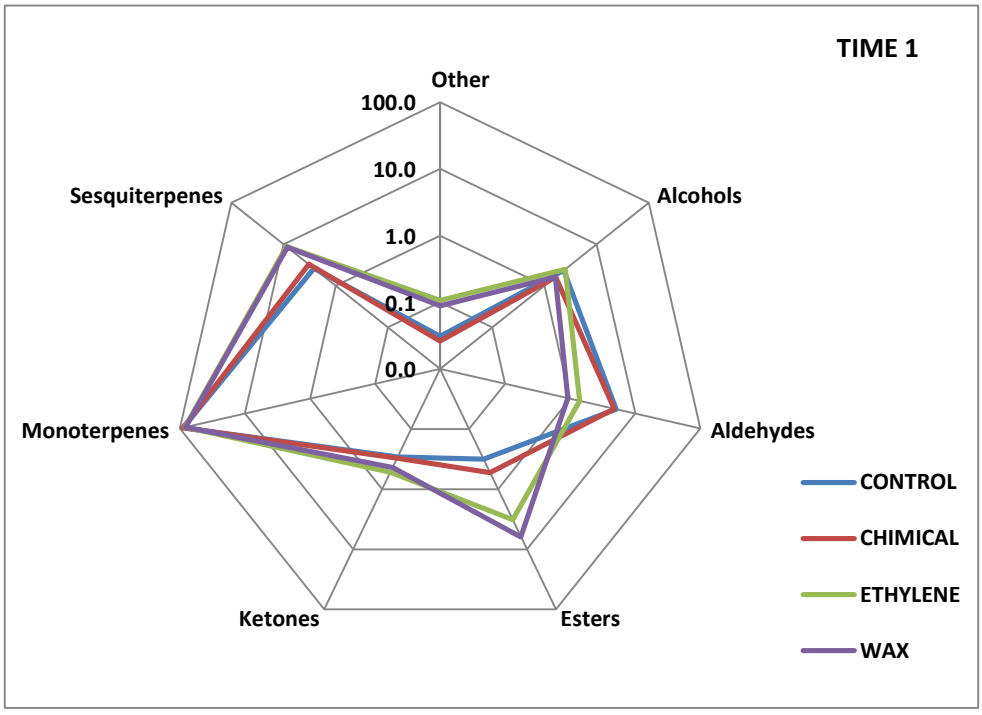

Fig.2. Chemical composition of the volatile fraction of the juices at Storage Time 1, after 4 weeks at 4°C

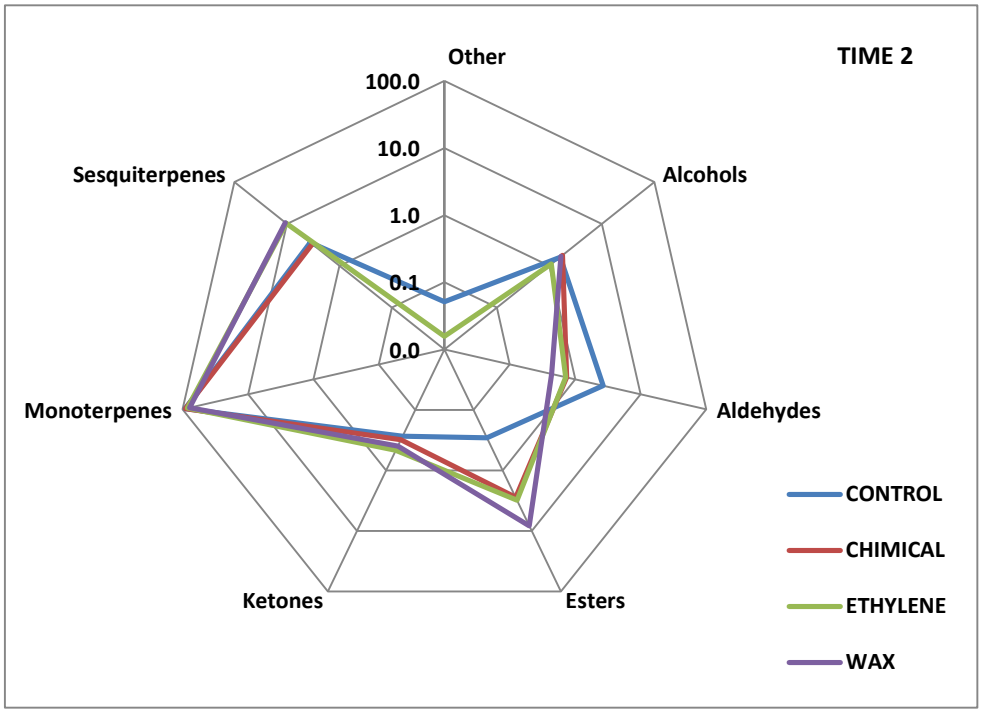

Fig.3. Chemical composition of the volatile fraction of the juices at Storage Time 2, after 8 weeks at 4°C

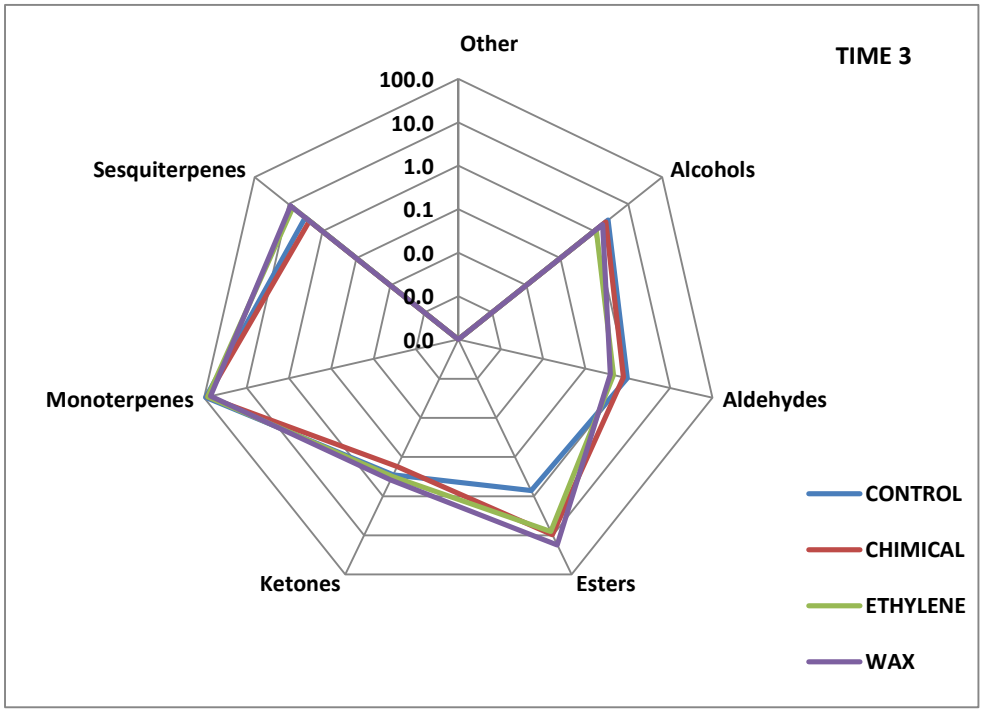

Fig.4. Chemical composition of the volatile fraction of the juices at Storage Time 3, after 8 weeks at 4°C + 1 week at 20°C

The PCA analysis of the chromatogram peaks is showed in Figure 5. This analysis confirmed that the biggest differences exist between the two groups 'Control+Chemical' and 'Ethylene+Wax', that forms two big clusters in the PCA loading plot, and that the overall aromatic pattern tends to change principally after 8 weeks of storage. Moreover, PCA analysis highlighted that the major variations determined by the storage are detectable in 'Control' fruits. Generally, the shift along PC1 describes the effect of the storage time that is the main effect observed, while the shift along PC2 describes the effect of the treatments. In fact, PC1 is the axis that covers the higher percentage of variance and describes the most significant effect, i.e. storage time and temperature effect. PC2, that cover the lower percentage of variance, describes the minor effect of the treatments applied to the fruits.

The major components responsible of the different volatile composition and of the positioning of the data in the PCA plot are:

- *Eudalene, 3-Carene,* and *nootkatone* the content of these molecules increased with time and was characteristics of T2 and T3, being absent in T0 and T1 in 'Control', 'Chemical', and 'Ethylene' treated fruits. These compounds determined the displacement of data toward the right of PC1 axis, and possess a sweet citrus odor type with a medium odor strength;
- *Levo-carvone*, *β-trans-ocimene*, and *4-acetyl-1-methylcyclohexene*, the content of these compounds was higher in T0 and tended to decrease with storage. These molecules are responsible of the shift along the left of the PC1 axis. *Levo-carvone* has a medium odor strength and a sweet minty herbal odor type (Luebke, 1993); while the ketone *4-acetyl-1-methylcyclohexene* is characterized by a spicy odor type, both of them are recognize to be important contributors to orange flavor; and *β-trans-ocimene* lends a sweet herbal note;
- *Cumaldehyde, Allo-aromadendrene, α-caryophyllene, α-gurjunene* and *β-panasinsene.* These compounds are responsible of the upward shift along PC2, and their content is higher in 'Ethylene' and 'Wax' treated fruits. *Cumaldehyde* has a spicy odor type with high odor strength, characterized by spicy-green odor (Mosciano, 1985); the other molecules that determined the displacement upward PC2 are sesquiterpene compounds that are characterized by a woody odor type

- *1-hexanol*, the variation of the content of this molecule determined the shift of the data downward along PC2 axis. It is characterized by herbal odor type of medium strength, with pungent fruity, sweet and alcoholic note (Mosciano, 1993).

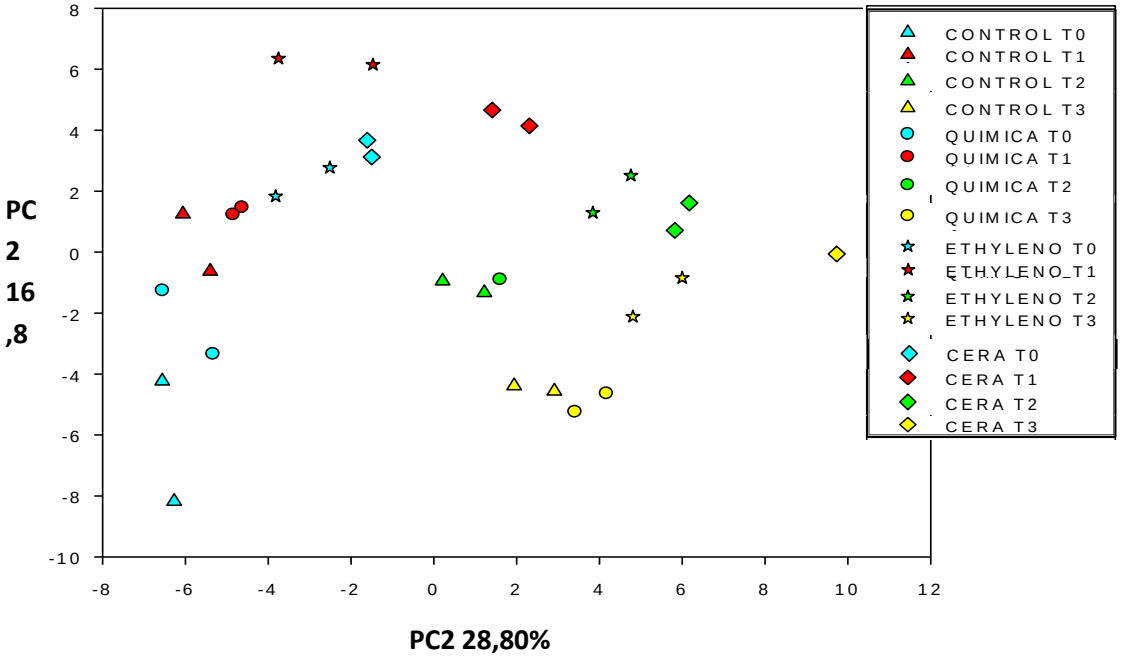

Fig.5. PCA analysis of the GC data

Relating the results of GC-MS analysis with those of the two E.N. instruments, it is interesting to point out that both electronic noses revealed differences in the aromatic pattern that are difficult to highlight from the GC analysis, confirming that the aromatic pattern derives from the complex combination of all the VOCs that compose the volatile fraction, and not of single compounds. Especially in T1, after 4 weeks at 4 °C, both E.N. detect differences that are quite difficult to notice with the GC-MS analysis. In fact, from the GC data seems that the volatile fractions are very similar between 'Control' and 'Chemical', and 'Ethylene' and 'Wax'.

However, E.N. results indicate that these slight differences revealed by the GC, even within the groups, are strong enough to discriminate among the different aromatic patterns.

To analyze GC-MS data with PCA methods seems to be useful to highlight the molecules that are effectively determining for the aromatic pattern changes.

The results obtained after a week of simulated shelf life at 20 °C are interesting. In this case, percentage of accuracy of both the instruments declined, being able to classify fruits from the end of the packing line ('Ethylene' and 'Wax') from the fruits that did not pass through the packing line ('Control' and 'Chemical'), and having difficulties to distinguish among fruits of the same group (like the volatile fraction of GC in T0 and T1).

From the GC data, it was expected to have low classification in general, but high classification accuracy for 'Control' fruits. Except for T0, 'Control' fruits classification always had higher classification rate being always recognized as different. As confirmed by GC data.

Considering that both the instruments are able to correctly classified the fruits on the basis of treatment and that the principal chemical classes responsible for these differences are aldehyde, ester, sesquiterpene, and alcohols, it is possible to suppose that sensors are mostly sensible to those compounds, in general. Different sensibility anyway exists among the sensors of the two instruments. The MOOSY32 is always able to correctly classify a high percentage of the instances, while EOS835 is less stable in the accuracy of classification.

3.4 Conclusions

Electronic noses revealed different aromatic pattern of the fruit juices, with differences in the performance of the two instruments. The MOOSY32 was able to detect variation in the juice aroma even when juices were analyzed right after each treatment (T0). Treatments caused changes in the 'flavor' of orange fruit juices. These variations seem to be easier detected by electronic nose instruments, than in the direct analysis of the composition of volatile fractions by GC-MS. This confirming that even slight changes in the chemical composition of the volatile fraction can lead to changes in the perceived aroma.

Moreover, the E.N. results showed that something changed in the aromatic pattern of orange fruits due to the packing line, and that the pattern of variation is different on the basis of the applied storage conditions. Furthermore, there is a major effect due to ethylene degreening and coating, but also control fruits changed their aromatic pattern in response to the storage time and temperature. Storage amplified the effect of each treatment, making possible to distinguish among them.

The E.N. results showed that not only coating or degreening caused changes in the aromatic pattern, but also volatile composition is altered as well by passage through the drenching system.

This study suggests that the packing line itself is able to affect the aromatic pattern of oranges, and that this effect is more pronounced when fruits are stored. Particularly, major changes were caused by ethylene and wax applications. Take care in the manipulation of fruits and to minimize the other treatments and the storage times would be an appropriate suggestion in trying to preserve flavor quality of Salustiana orange juice. However, panel test to assess the effect of these variations on consumer acceptance would be useful to confirm these findings.

4. GENERAL CONCLUSIONS

- Storage time influence the carpometric parameters of lemon and orange fruits, while it has no influence on chemical characteristics.
- In lemon fruits storage, temperature affects total polyphenols and vitamin C contents that increase with refrigerated storage.
- Lemon juice flavor remains unchanged during 4 weeks of refrigerated storage. Moreover, this kind of storage allows an increase in bioactive compounds of the juices.
- The chemical compositions and the volatile fraction analysis of fruits permit the discrimination of the cultivar 'Femminello Santa Teresa'. This variety is characterized by a higher content in total soluble solids, titratable acidity, and vitamin C. Furthermore, it possesses different volatile composition.
- Orange juice flavor changes even when fruits are stored under refrigerated conditions. These changes are easier detectable with E.N. systems, than using GC-MS analysis.
- Electronic nose instruments equipped with MOS sensors confirm their ability to monitor the changes in the aromatic pattern of citrus juice during storage. Moreover, it seems able to reveal differences in the maturity stage of lemon fruits.
- Between the two E.N. instruments tested, the MOOSY32 provided better classification of the juices according to the treatments applied, being able to distinguish the different aromatic pattern of the juices in every step of the packing process.
- The chemical classes that better describe the variations of the aromatic pattern of lemon and orange juices are aldehyde and ester, the first decreasing and the second increasing over time, describing the loss of freshness and of typical citrusy-green odors and the increasing of sweet floral flavor.

Future perspective could be to establish a definitive correlation between the changes perceived by the electronic nose with those in the chemical composition of the volatile fraction of citrus fruits, and then relates these results with the consumer acceptance.

According to these results, and after other desirable investigations, electronic noses could be widely applicable by growers and processors to make the quality control simple and highly sensitive, directly in the field, monitoring fruits maturity, as well as during packing processes.

5. Appendix 1

Tab. 1. Variation of chemical composition of the volatile fraction of the juices of the three cvs at ST0) Storage Time 0; ST28 4°C) after 4 weeks at 4°C; ST28 18°C) after 4 weeks at 18°C. Year 2012.

Data are arranged according to Chemical Groups, and represents the mean relative percentage of individual compounds from duplicate experiments. Family code: AC, alcohols; AD, aldehydes; ES, esters; K, ketones; MT, monoterpenes; SQ, sesquiterpenes.

Chemical Classes	2012								
	F.C.			Z.B.			S.T.		
	ST0	**ST28 4°C**	**ST28 18°C**	**ST0**	**ST28 4°C**	**ST28 18°C**	**ST0**	**ST28 4°C**	**ST28 18°C**
AC	3,93±1,51	6,04±0,96	8,49±1,08	5,30±1,81	4,69±0,38	8,57±1,65	6,86±1,07	6,98±1,38	6,22±1,53
AD	9,11±1,23	10,14±0,93	11,01±1,92	13,07±1,62	10,40±1,68	11,46±1,92	18,32±1,46	13,71±1,15	13,12±0,79
ES	7,40±0,69	10,87±1,82	9,33±0,96	4,86±0,20	6,81±1,32	8,05±1,81	12,83±0,66	11,96±0,80	11,91±2,06
K	0,05±0,03	0,02±0,01	0,06±0,09	0,06±0,04	0,04±0,01	0,08±0,02	0,03±0,01	0,08±0,02	0,01±0,01
MT	73,82±2,49	65,70±1,15	65,60±1,67	72,72±4,18	71,63±1,82	66,89±3,83	56,62±1,58	61,80±2,48	65,24±1,79
SQ	5,43±0,18	6,82±0,11	4,60±0,56	3,77±0,54	6,13±1,21	3,17±0,59	5,02±065	4,97±1,33	2,89±0,62
Others	0,26±0,07	0,40±0,08	0,91±0,42	0,22±0,05	0,30±0,02	1,79±0,82	0,32±0,06	0,51±0,09	0,61±0,11

Tab. 2. Variation of chemical composition of the volatile fraction of the juices of the three cvs at ST0) Storage Time 0; ST28 4°C) after 4 weeks at 4°C; ST28 18°C) after 4 weeks at 18°C. Year 2013.

Data are arranged according to Chemical Groups, and represents the mean relative percentage of individual compounds from duplicate experiments. Family code: AC, alcohols; AD, aldehydes; ES, esters; K, ketones; MT, monoterpenes; SQ, sesquiterpenes.

Chemical Classes	2013								
	F.C.			Z.B.			S.T.		
	ST0	**ST28 4°C**	**ST28 18°C**	**ST0**	**ST28 4°C**	**ST28 18°C**	**ST0**	**ST28 4°C**	**ST28 18°C**
AC	5,34±0,32	6,03±1,26	5,02±2,32	5,31±1,07	4,45±0,44	6,45±0,91	5,93±1,65	6,88±1,33	4,92±1,51
AD	3,10±2,14	5,02±2,54	2,04±1,75	2,26±1,57	5,22±1,97	0,31±0,07	1,91±1,51	1,62±1,42	0,86±0,38
ES	7,20±1,38	11,32±4,50	5,20±3,21	4,09±1,67	8,22±0,98	4,55±0,77	6,34±2,03	6,55±2,04	5,98±1,62
K	0,01±0,00	0,02±0,01	0,01±000	0,01±0,01	0,02±0,01	0,01±0,00	0,01±0,00	0,01±0,01	0,01±0,01
MT	80,65±3,76	73,20±7,73	84,17±4,48	86,36±3,52	77,80±2,99	84,88±0,34	83,09±4,87	82,38±3,23	84,82±2,11
SQ	3,49±0,42	4,04±1,46	3,27±1,84	1,72±0,35	4,09±0,32	3,58±1,00	2,32±0,55	2,24±0,14	3,14±0,76
Others	0,20±0,03	0,36±0,11	0,29±0,00	0,26±0,04	0,20±0,04	0,21±0,02	0,41±0,09	0,31±0,12	0,26±0,01

Tab. 2. Chemical composition of the volatile fraction of the juices of the three cvs at ST0) Storage Time 0; ST28 4°C) after 4 weeks at 4°C; ST28 18°C) after 4 weeks at 18°C. Year 2012.
Data are arranged according to Chemical Groups, and represents the mean relative percentage of individual compounds from duplicate experiments. Family code: AC, alcohols; AD, aldehydes; ES, esters; K, ketones; MT, monoterpenes; SQ, sesquiterpenes.

Molecules	Chemical Classes	2012								
		F.C.			Z.B			S.T.		
		ST 0	ST28 4°	ST28 18°	ST 0	ST28 4°	ST28 18°	ST 0	ST28 4°	ST28 18°
1-Octanol	AC	0,04 ± 0,01	0,10 ± 0,04	0,16 ± 0,02	0,03 ± 0,01	0,03 ± 0,01	0,30 ± 0,04	0,10 ± 0,02	0,25 ± 0,21	0,37 ± 0,19
beta-Linalool	AC	0,24 ± 0,11	0,40 ± 0,05	0,32 ± 0,05	0,39 ± 0,16	0,28 ± 0,05	0,32 ± 0,02	0,41 ± 0,04	0,35 ± 0,07	0,32 ± 0,07
Fenchol	AC	0,09 ± 0,03	0,16 ± 0,05	0,33 ± 0,11	0,10 ± 0,06	0,10 ± 0,01	0,38 ± 0,14	0,16 ± 0,05	0,23 ± 0,04	0,24 ± 0,04
beta-Terpinol	AC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nonanol	AC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Terpineol	AC	1,85 ± 0,75	2,22 ± 0,41	2,81 ± 0,09	2,84 ± 0,76	2,37 ± 0,25	2,97 ± 0,10	2,99 ± 0,23	2,13 ± 0,28	1,92 ± 0,35
alpha-Terpineol	AC	1,56 ± 0,58	2,81 ± 0,83	4,64 ± 1,10	1,91 ± 0,84	1,87 ± 0,08	4,61 ± 1,38	2,91 ± 0,73	4,14 ± 1,07	3,70 ± 0,85
cis-Geraniol	AC	0,13 ± 0,05	0,30 ± 0,05	0,18 ± 0,04	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,27 ± 0,03	0,17 ± 0,14	0,26 ± 0,07
beta-Citronellol	AC	0,02 ± 0,01	0,05 ± 0,02	0,06 ± 0,06	0,03 ± 0,01	0,03 ± 0,01	0,00 ± 0,00	0,03 ± 0,01	0,03 ± 0,01	0,01 ± 0,00
trans-Geraniol	AC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1-Decanol	AC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
alpha-Bisabolol	AC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Heptenal, (Z)-	AD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Octanal	AD	0,07 ± 0,12	0,18 ± 0,10	0,98 ± 0,13	0,07 ± 0,03	0,11 ± 0,06	1,29 ± 0,34	0,48 ± 0,75	0,73 ± 0,02	0,99 ± 0,17
Benzaldehyde, 4-methyl-	AD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nonanal	AD	0,86 ± 0,05	0,99 ± 0,15	1,59 ± 0,12	1,01 ± 0,07	0,93 ± 0,14	1,83 ± 0,38	1,47 ± 0,18	0,96 ± 0,22	1,17 ± 0,17
Decanal	AD	0,41 ± 0,03	0,54 ± 0,07	0,60 ± 0,13	0,38 ± 0,06	0,43 ± 0,03	0,59 ± 0,22	0,93 ± 0,08	0,56 ± 0,05	0,56 ± 0,07
Benzaldehyde, 3,4-dimethyl-	AD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
beta-Citral	AD	2,28 ± 0,35	2,53 ± 0,26	2,23 ± 0,74	3,70 ± 0,52	2,71 ± 0,45	2,22 ± 0,61	4,46 ± 0,13	2,99 ± 0,34	2,93 ± 0,23
alpha-Citral	AD	5,22 ± 0,80	5,46 ± 0,81	5,31 ± 1,44	7,60 ± 1,04	5,94 ± 1,03	5,20 ± 0,96	10,46 ± 0,46	7,75 ± 0,71	6,93 ± 0,45
Perillaldehyde	AD	0,06 ± 0,04	0,08 ± 0,06	0,08 ± 0,08	0,07 ± 0,01	0,04 ± 0,01	0,17 ± 0,07	0,10 ± 0,02	0,14 ± 0,06	0,07 ± 0,04
Undecanal	AD	0,21 ± 0,01	0,36 ± 0,14	0,22 ± 0,05	0,25 ± 0,03	0,26 ± 0,04	0,16 ± 0,07	0,41 ± 0,06	0,22 ± 0,05	0,15 ± 0,05
n-Octyl acetate	ES	0,06 ± 0,01	0,10 ± 0,02	0,08 ± 0,04	0,04 ± 0,00	0,06 ± 0,01	0,09 ± 0,06	0,14 ± 0,02	0,10 ± 0,01	0,10 ± 0,01
Bergamiol	ES	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,01 ± 0,00	0,00 ± 0,00	0,01 ± 0,00
Bornyl acetate	ES	0,01 ± 0,00	0,01 ± 0,00	0,02 ± 0,01	0,01 ± 0,00	0,02 ± 0,01	0,02 ± 0,01	0,01 ± 0,00	0,02 ± 0,01	0,01 ± 0,01
n-Nonyl acetate	ES	0,04 ± 0,01	0,05 ± 0,02	0,05 ± 0,01	0,00 ± 0,00	0,03 ± 0,01	0,04 ± 0,01	0,00 ± 0,00	0,06 ± 0,01	0,06 ± 0,02
Methyl geranate	ES	0,01 ± 0,01	0,02 ± 0,00	0,03 ± 0,01	0,01 ± 0,00	0,02 ± 0,01	0,02 ± 0,01	0,03 ± 0,00	0,03 ± 0,01	0,03 ± 0,02
Decanoic acid, methyl ester	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Citronellol acetate	ES	0,38 ± 0,06	0,55 ± 0,11	0,58 ± 0,19	0,24 ± 0,01	0,40 ± 0,08	0,47 ± 0,13	0,32 ± 0,03	0,40 ± 0,03	0,37 ± 0,08
Nerol acetate	ES	2,95 ± 0,21	4,70 ± 0,41	4,89 ± 0,59	2,34 ± 0,12	3,26 ± 0,46	4,45 ± 0,98	6,23 ± 0,28	5,57 ± 0,53	5,54 ± 0,97
Geraniol acetate	ES	3,94 ± 0,69	5,44 ± 1,29	3,68 ± 0,40	2,22 ± 0,09	3,04 ± 0,76	2,96 ± 0,65	6,09 ± 0,43	5,66 ± 0,52	4,87 ± 1,02
Geranyl propionate	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
d-Camphor	K	0,01 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,02 ± 0,01	0,01 ± 0,00	0,01 ± 0,00	0,01 ± 0,00	0,01 ± 0,00	0,00 ± 0,00
m-Methylacetophenone	K	0,02 ± 0,02	0,02 ± 0,01	0,06 ± 0,09	0,02 ± 0,02	0,01 ± 0,01	0,04 ± 0,02	0,01 ± 0,01	0,04 ± 0,02	0,00 ± 0,00
Levo-carvone	K	0,02 ± 0,01	0,00 ± 0,00	0,00 ± 0,00	0,03 ± 0,02	0,01 ± 0,01	0,03 ± 0,01	0,01 ± 0,00	0,02 ± 0,01	0,02 ± 0,01
alpha-Thujene	MT	0,13 ± 0,05	0,22 ± 0,07	0,19 ± 0,04	0,19 ± 0,18	0,12 ± 0,07	0,15 ± 0,07	0,26 ± 0,07	0,17 ± 0,09	0,10 ± 0,03
alpha-Pinene	MT	1,83 ± 0,08	1,55 ± 0,42	1,31 ± 0,26	1,67 ± 0,93	1,80 ± 0,31	1,10 ± 0,24	2,11 ± 0,20	1,13 ± 0,41	0,87 ± 0,26
Camphene	MT	0,02 ± 0,01	0,06 ± 0,06	0,08 ± 0,01	0,04 ± 0,04	0,03 ± 0,02	0,06 ± 0,01	0,04 ± 0,04	0,06 ± 0,02	0,06 ± 0,02
beta-Phellandrene	MT	0,06 ± 0,04	0,02 ± 0,02	0,02 ± 0,01	0,16 ± 0,16	0,05 ± 0,03	0,00 ± 0,00	0,02 ± 0,01	0,02 ± 0,03	0,00 ± 0,00

Continue

Molecules	Chemical Classes	2012								
		F.C.			Z.B.			S.T.		
		ST0	ST28 4°C	ST28 18°C	ST0	ST28 4°C	ST28 18°C	ST0	ST28 4°C	ST28 18°C
beta-Pinene	**MT**	9,95 ± 0,33	7,29 ± 0,72	6,97 ± 0,56	10,20 ± 1,42	8,11 ± 1,18	6,37 ± 1,15	11,16 ± 0,58	6,16 ± 1,82	5,16 ± 1,01
beta-Myrcene	**MT**	3,33 ± 0,21	2,29 ± 1,36	4,37 ± 0,26	1,54 ± 1,50	1,86 ± 0,48	4,82 ± 0,57	1,70 ± 1,39	3,39 ± 0,50	4,13 ± 0,35
alpha-Phellandrene	**MT**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Carene	**MT**	0,07 ± 0,06	0,09 ± 0,04	0,06 ± 0,03	0,14 ± 0,04	0,06 ± 0,03	0,21 ± 0,22	0,02 ± 0,02	0,35 ± 0,20	0,39 ± 0,26
alpha-Terpinene	**MT**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
D-Limonene	**MT**	44,17 ± 1,49	40,01 ± 0,73	37,97 ± 0,83	44,39 ± 1,94	44,11 ± 1,24	39,17 ± 1,95	29,77 ± 0,44	38,53 ± 3,08	41,84 ± 2,21
beta-trans-Ocimene	**MT**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
beta-cis-Ocimene	**MT**	0,45 ± 0,19	0,45 ± 0,17	0,46 ± 0,07	0,35 ± 0,02	0,38 ± 0,04	0,44 ± 0,04	0,35 ± 0,04	0,52 ± 0,09	0,58 ± 0,05
gamma-Terpinene	**MT**	12,46 ± 0,60	12,13 ± 0,90	12,02 ± 0,28	12,69 ± 0,45	13,47 ± 0,21	11,75 ± 0,60	9,94 ± 0,62	10,51 ± 0,59	11,14 ± 0,60
(+)-4-Carene	**MT**	1,30 ± 0,18	1,51 ± 0,15	1,90 ± 0,18	1,29 ± 0,10	1,58 ± 0,04	2,41 ± 0,49	1,20 ± 0,08	1,40 ± 0,06	1,58 ± 0,11
Isopropenyltoluene	**MT**	0,16 ± 0,04	0,28 ± 0,06	0,75 ± 0,32	0,16 ± 0,03	0,19 ± 0,03	1,53 ± 0,63	0,22 ± 0,04	0,42 ± 0,08	0,58 ± 0,09
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	**MT**	0,02 ± 0,02	0,03 ± 0,03	0,10 ± 0,03	0,02 ± 0,00	0,04 ± 0,01	0,14 ± 0,05	0,02 ± 0,00	0,03 ± 0,01	0,09 ± 0,02
2,6-Dimethyl-1,3,5,7-octatetraene, E	**MT**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2,4,6-Octatriene, 2,6-dimethyl	**MT**	0,03 ± 0,02	0,05 ± 0,02	0,16 ± 0,06	0,03 ± 0,01	0,03 ± 0,01	0,25 ± 0,09	0,02 ± 0,01	0,05 ± 0,01	0,15 ± 0,03
Elixene	**SQ**	0,02 ± 0,01	0,03 ± 0,02	0,03 ± 0,01	0,02 ± 0,01	0,05 ± 0,01	0,02 ± 0,01	0,03 ± 0,01	0,03 ± 0,02	0,01 ± 0,01
alpha.-Bergamotene	**SQ**	0,11 ± 0,01	0,14 ± 0,01	0,09 ± 0,02	0,06 ± 0,01	0,11 ± 0,03	0,06 ± 0,01	0,10 ± 0,02	0,08 ± 0,03	0,06 ± 0,02
beta.-Caryophyllene	**SQ**	0,76 ± 0,16	0,96 ± 0,06	0,85 ± 0,21	0,81 ± 0,16	1,29 ± 0,25	0,91 ± 0,13	0,73 ± 0,09	0,56 ± 0,08	0,42 ± 0,06
trans-.alpha.-Bergamotene	**SQ**	1,44 ± 0,09	1,86 ± 0,09	1,32 ± 0,25	0,83 ± 0,12	1,45 ± 0,31	0,90 ± 0,14	1,30 ± 0,19	1,17 ± 0,36	0,78 ± 0,21
Caryophyllene	**SQ**	0,07 ± 0,00	0,10 ± 0,02	0,07 ± 0,02	0,04 ± 0,01	0,07 ± 0,02	0,05 ± 0,02	0,06 ± 0,01	0,05 ± 0,02	0,04 ± 0,01
Allo-Aromadendrene	**SQ**	0,01 ± 0,01	0,03 ± 0,01	0,03 ± 0,03	0,02 ± 0,01	0,04 ± 0,01	0,02 ± 0,02	0,04 ± 0,02	0,02 ± 0,02	0,01 ± 0,00
beta.-Farnesene	**SQ**	0,14 ± 0,00	0,16 ± 0,02	0,06 ± 0,02	0,07 ± 0,01	0,13 ± 0,03	0,02 ± 0,01	0,13 ± 0,03	0,09 ± 0,04	0,03 ± 0,01
alpha.-Caryophyllene	**SQ**	0,06 ± 0,02	0,08 ± 0,01	0,07 ± 0,02	0,06 ± 0,01	0,10 ± 0,03	0,07 ± 0,02	0,05 ± 0,01	0,04 ± 0,01	0,04 ± 0,02
beta.-Santalene	**SQ**	0,08 ± 0,00	0,11 ± 0,01	0,09 ± 0,02	0,05 ± 0,01	0,08 ± 0,01	0,07 ± 0,01	0,07 ± 0,01	0,07 ± 0,02	0,06 ± 0,01
beta.-Himachalene	**SQ**	0,03 ± 0,01	0,03 ± 0,01	0,01 ± 0,01	0,01 ± 0,01	0,03 ± 0,01	0,01 ± 0,00	0,03 ± 0,01	0,02 ± 0,01	0,01 ± 0,01
alpha.-Curcumene	**SQ**	0,02 ± 0,01	0,04 ± 0,01	0,04 ± 0,02	0,01 ± 0,01	0,04 ± 0,04	0,03 ± 0,02	0,02 ± 0,00	0,03 ± 0,01	0,02 ± 0,02
Isocaryophyllene	**SQ**	0,09 ± 0,01	0,11 ± 0,01	0,08 ± 0,01	0,05 ± 0,01	0,09 ± 0,03	0,05 ± 0,01	0,00 ± 0,00	0,08 ± 0,02	0,06 ± 0,01
Valencene	**SQ**	0,14 ± 0,06	0,27 ± 0,04	0,48 ± 0,21	0,11 ± 0,03	0,28 ± 0,06	0,24 ± 0,05	0,12 ± 0,03	0,37 ± 0,01	0,24 ± 0,04
Eremophilene	**SQ**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Germacrene B	**SQ**	0,13 ± 0,02	0,17 ± 0,05	0,10 ± 0,02	0,17 ± 0,03	0,23 ± 0,04	0,08 ± 0,02	0,13 ± 0,01	0,08 ± 0,10	0,04 ± 0,01
.beta.-Bisabolene	**SQ**	2,24 ± 0,05	2,65 ± 0,17	1,24 ± 0,32	1,39 ± 0,15	2,08 ± 0,41	0,62 ± 0,17	2,14 ± 0,31	1,84 ± 0,59	0,83 ± 0,27
(-)-.alpha.-Panasinsen	**SQ**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Humulene	**SQ**	0,08 ± 0,01	0,08 ± 0,02	0,04 ± 0,02	0,05 ± 0,01	0,07 ± 0,01	0,02 ± 0,01	0,06 ± 0,01	0,06 ± 0,02	0,03 ± 0,01
Bornyl chloride	**OTHER**	0,04 ± 0,02	0,06 ± 0,03	0,11 ± 0,10	0,01 ± 0,01	0,04 ± 0,02	0,21 ± 0,22	0,06 ± 0,02	0,09 ± 0,02	0,04 ± 0,02
Tridecane	**OTHER**	0,01 ± 0,00	0,00 ± 0,00	0,01 ± 0,00	0,01 ± 0,00	0,02 ± 0,01	0,01 ± 0,00	0,01 ± 0,01	0,00 ± 0,00	0,00 ± 0,00
7-Tetradecene, (E)	**OTHER**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tetradecane	**OTHER**	0,04 ± 0,01	0,06 ± 0,02	0,04 ± 0,01	0,03 ± 0,01	0,06 ± 0,01	0,03 ± 0,01	0,03 ± 0,01	0,03 ± 0,03	0,02 ± 0,01
Hexadecane	**OTHER**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Tab. 3. Chemical composition of the volatile fraction of the juices of the three cvs at ST0) Storage Time 0; ST28 4°C) after 4 weeks at 4°C; ST28 18°C) after 4 weeks at 18°C. Year 2013.
Data are arranged according to Chemical Groups, and represents the mean relative percentage of individual compounds from duplicate experiments. Family code: AC, alcohols; AD, aldehydes; ES, esters; K, ketones; MT, monoterpenes; SQ, sesquiterpenes.

Molecules	Chemical Classes	2013								
		F.C.			Z.B.			S.T.		
		ST 0	ST28 4°	ST28 18°	ST 0	ST28 4°	ST28 18°	ST 0	ST28 4°	ST28 18°
1-Octanol	AC	0,24 ± 0,06	0,17 ± 0,06	0,13 ± 0,04	0,15 ± 0,05	0,15 ± 0,07	0,25 ± 0,06	0,41 ± 0,14	0,46 ± 0,08	0,25 ± 0,10
beta-Linalool	AC	0,34 ± 0,03	0,38 ± 0,12	0,41 ± 0,23	0,39 ± 0,16	0,26 ± 0,03	0,45 ± 0,10	0,55 ± 0,12	0,67 ± 0,26	0,41 ± 0,16
Fenchol	AC	0,09 ± 0,02	0,18 ± 0,02	0,08 ± 0,03	0,12 ± 0,02	0,12 ± 0,03	0,14 ± 0,03	0,07 ± 0,03	0,09 ± 0,02	0,08 ± 0,03
beta-Terpinol	AC	0,02 ± 0,01	0,03 ± 0,02	0,04 ± 0,02	0,04 ± 0,01	0,01 ± 0,00	0,03 ± 0,01	0,03 ± 0,02	0,03 ± 0,02	0,02 ± 0,01
Nonanol	AC	0,23 ± 0,04	0,23 ± 0,17	0,14 ± 0,01	0,18 ± 0,04	0,21 ± 0,06	0,23 ± 0,06	0,21 ± 0,10	0,25 ± 0,08	0,14 ± 0,05
4-Terpineol	AC	1,11 ± 0,06	1,45 ± 0,30	0,90 ± 0,29	1,12 ± 0,23	1,36 ± 0,19	1,09 ± 0,11	0,87 ± 0,31	0,97 ± 0,17	0,75 ± 0,27
alpha-Terpineol	AC	1,58 ± 0,11	2,47 ± 0,63	1,94 ± 0,95	2,17 ± 0,25	1,47 ± 0,24	2,33 ± 0,48	1,82 ± 0,82	2,22 ± 0,62	1,75 ± 0,60
cis-Geraniol	AC	0,53 ± 0,16	0,33 ± 0,07	0,45 ± 0,27	0,33 ± 0,15	0,25 ± 0,07	0,69 ± 0,14	0,61 ± 0,05	0,65 ± 0,11	0,58 ± 0,14
beta-Citronellol	AC	0,13 ± 0,03	0,10 ± 0,03	0,13 ± 0,08	0,09 ± 0,05	0,07 ± 0,02	0,16 ± 0,03	0,15 ± 0,03	0,14 ± 0,08	0,11 ± 0,04
trans-Geraniol	AC	0,98 ± 0,27	0,61 ± 0,20	0,77 ± 0,52	0,68 ± 0,38	0,47 ± 0,20	1,02 ± 0,19	1,15 ± 0,21	1,31 ± 0,26	0,78 ± 0,16
1-Decanol	AC	0,07 ± 0,01	0,02 ± 0,02	0,04 ± 0,02	0,01 ± 0,01	0,04 ± 0,03	0,05 ± 0,02	0,06 ± 0,03	0,07 ± 0,04	0,04 ± 0,02
alpha-Bisabolol	AC	0,03 ± 0,01	0,05 ± 0,03	0,02 ± 0,01	0,01 ± 0,00	0,03 ± 0,01	0,01 ± 0,00	0,01 ± 0,01	0,02 ± 0,01	0,01 ± 0,01
2-Heptenal, (Z)-	AD	0,01 ± 0,01	0,03 ± 0,03	0,01 ± 0,01	0,02 ± 0,02	0,01 ± 0,01	0,01 ± 0,01	0,02 ± 0,00	0,03 ± 0,03	0,03 ± 0,02
Octanal	AD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzaldehyde, 4-methyl-	AD	0,00 ± 0,01	0,00 ± 0,00	0,06 ± 0,02	0,02 ± 0,01	0,00 ± 0,00	0,04 ± 0,01	0,00 ± 0,01	0,00 ± 0,00	0,04 ± 0,08
Nonanal	AD	0,12 ± 0,01	0,30 ± 0,12	0,06 ± 0,02	0,11 ± 0,12	0,35 ± 0,16	0,06 ± 0,01	0,06 ± 0,02	0,10 ± 0,05	0,10 ± 0,05
Decanal	AD	0,12 ± 0,05	0,27 ± 0,17	0,04 ± 0,02	0,07 ± 0,08	0,27 ± 0,11	0,02 ± 0,00	0,05 ± 0,01	0,07 ± 0,06	0,05 ± 0,03
Benzaldehyde, 3,4-dimethyl-	AD	0,00 ± 0,00	0,03 ± 0,03	0,00 ± 0,00	0,00 ± 0,00	0,01 ± 0,01	0,00 ± 0,00	0,00 ± 0,00	0,06 ± 0,01	0,01 ± 0,02
beta-Citral	AD	0,77 ± 0,55	1,09 ± 0,60	0,47 ± 0,41	0,55 ± 0,40	1,24 ± 0,47	0,05 ± 0,02	0,49 ± 0,43	0,34 ± 0,35	0,16 ± 0,08
alpha-Citral	AD	1,97 ± 1,48	3,09 ± 1,60	1,35 ± 1,21	1,40 ± 1,00	3,14 ± 1,22	0,14 ± 0,05	1,25 ± 1,02	0,99 ± 1,03	0,46 ± 0,21
Perillaldehyde	AD	0,06 ± 0,04	0,09 ± 0,03	0,06 ± 0,05	0,05 ± 0,02	0,09 ± 0,02	0,00 ± 0,00	0,04 ± 0,02	0,02 ± 0,03	0,01 ± 0,01
Undecanal	AD	0,05 ± 0,02	0,11 ± 0,06	0,00 ± 0,00	0,02 ± 0,03	0,12 ± 0,04	0,00 ± 0,00	0,01 ± 0,01	0,01 ± 0,01	0,00 ± 0,01
n-Octyl acetate	ES	0,03 ± 0,02	0,06 ± 0,02	0,03 ± 0,01	0,02 ± 0,01	0,05 ± 0,01	0,02 ± 0,01	0,04 ± 0,02	0,04 ± 0,02	0,03 ± 0,01
Bergamiol	ES	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bornyl acetate	ES	0,00 ± 0,00	0,01 ± 0,01	0,01 ± 0,01	0,00 ± 0,01	0,01 ± 0,00	0,01 ± 0,00	0,00 ± 0,01	0,00 ± 0,01	0,00 ± 0,00
n-Nonyl acetate	ES	0,02 ± 0,01	0,04 ± 0,01	0,01 ± 0,01	0,01 ± 0,00	0,03 ± 0,01	0,01 ± 0,00	0,01 ± 0,00	0,03 ± 0,02	0,01 ± 0,00
Methyl geranate	ES	0,03 ± 0,01	0,03 ± 0,01	0,03 ± 0,01	0,01 ± 0,00	0,02 ± 0,01	0,02 ± 0,00	0,02 ± 0,01	0,02 ± 0,00	0,02 ± 0,01
Decanoic acid, methyl ester	ES	0,01 ± 0,01	0,00 ± 0,00	0,01 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,01 ± 0,00	0,01 ± 0,01	0,01 ± 0,00	0,01 ± 0,01
Citronellol acetate	ES	0,50 ± 0,05	0,69 ± 0,37	0,44 ± 0,30	0,26 ± 0,09	0,56 ± 0,08	0,38 ± 0,06	0,26 ± 0,05	0,27 ± 0,06	0,30 ± 0,08
Nerol acetate	ES	3,33 ± 0,49	5,08 ± 1,91	2,27 ± 1,51	2,03 ± 0,86	3,79 ± 0,50	2,27 ± 0,48	3,19 ± 0,95	2,96 ± 0,84	2,87 ± 0,77
Geraniol acetate	ES	3,24 ± 0,82	5,37 ± 2,26	2,37 ± 1,32	1,74 ± 0,73	3,72 ± 0,69	1,82 ± 0,25	2,78 ± 1,03	3,19 ± 1,10	2,74 ± 0,78
Geranyl propionate	ES	0,03 ± 0,01	0,04 ± 0,03	0,03 ± 0,04	0,02 ± 0,01	0,05 ± 0,01	0,02 ± 0,00	0,03 ± 0,01	0,03 ± 0,03	0,00 ± 0,00
d-Camphor	K	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
m-Methylacetophenone	K	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Levo-carvone	K	0,01 ± 0,00	0,02 ± 0,01	0,01 ± 0,00	0,01 ± 0,01	0,02 ± 0,01	0,01 ± 0,00	0,01 ± 0,00	0,01 ± 0,01	0,01 ± 0,01
alpha-Thujene	MT	0,10 ± 0,03	0,19 ± 0,12	0,08 ± 0,01	0,17 ± 0,09	0,18 ± 0,11	0,05 ± 0,01	0,05 ± 0,02	0,09 ± 0,03	0,04 ± 0,01
alpha-Pinene	MT	0,59 ± 0,16	1,40 ± 0,71	0,61 ± 0,03	1,49 ± 0,82	1,20 ± 0,58	0,55 ± 0,05	0,36 ± 0,08	0,64 ± 0,13	0,43 ± 0,07
Camphene	MT	0,03 ± 0,01	0,07 ± 0,03	0,05 ± 0,00	0,06 ± 0,03	0,07 ± 0,03	0,04 ± 0,01	0,02 ± 0,01	0,04 ± 0,01	0,03 ± 0,01
beta-Phellandrene	MT	0,06 ± 0,02	0,02 ± 0,02	0,02 ± 0,01	0,02 ± 0,04	0,03 ± 0,02	0,02 ± 0,02	0,01 ± 0,01	0,03 ± 0,03	0,00 ± 0,00

Continue

Molecules	Chemical Classes	2013								
		F.C.			Z.B.			S.T.		
		ST0	ST28 4°C	ST28 18°C	ST0	ST28 4°C	ST28 18°C	ST0	ST28 4°C	ST28 18°C
beta-Pinene	MT	4,31 ± 0,80	7,25 ± 1,75	3,56 ± 0,17	6,73 ± 2,15	7,79 ± 2,68	3,36 ± 0,41	2,36 ± 0,71	3,93 ± 1,10	2,37 ± 0,39
beta-Myrcene	MT	3,59 ± 0,47	3,76 ± 0,81	5,03 ± 0,59	5,44 ± 0,92	3,99 ± 0,31	5,03 ± 0,17	4,26 ± 0,20	4,91 ± 0,67	4,45 ± 0,12
alpha-Phellandrene	MT	0,16 ± 0,02	0,05 ± 0,10	0,18 ± 0,01	0,12 ± 0,07	0,03 ± 0,06	0,15 ± 0,03	0,17 ± 0,03	0,20 ± 0,01	0,17 ± 0,03
2-Carene	MT	0,00 ± 0,00	0,35 ± 0,25	0,00 ± 0,00	0,39 ± 0,18	0,27 ± 0,20	0,54 ± 0,06	0,00 ± 0,00	0,12 ± 0,20	0,33 ± 0,21
alpha-Terpinene	MT	0,40 ± 0,08	0,02 ± 0,03	0,58 ± 0,06	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,42 ± 0,07	0,37 ± 0,32	0,11 ± 0,19
D-Limonene	MT	58,41 ± 5,23	46,19 ± 7,35	61,62 ± 4,96	59,20 ± 4,13	48,94 ± 5,14	62,89 ± 1,19	64,39 ± 6,68	59,94 ± 6,06	66,10 ± 2,33
beta-trans-Ocimene	MT	0,00 ± 0,00	0,00 ± 0,00	0,09 ± 0,13	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,09 ± 0,11	0,05 ± 0,09	0,21 ± 0,05
beta-cis-Ocimene	MT	0,42 ± 0,05	0,41 ± 0,08	0,58 ± 0,01	0,37 ± 0,04	0,42 ± 0,13	0,55 ± 0,11	0,41 ± 0,07	0,62 ± 0,18	0,50 ± 0,05
gamma-Terpinene	MT	11,50 ± 1,36	12,40 ± 0,57	10,47 ± 1,14	11,17 ± 0,77	13,42 ± 0,84	10,30 ± 0,57	9,54 ± 0,96	10,24 ± 1,74	8,98 ± 0,16
(+)-4-Carene	MT	0,99 ± 0,15	1,00 ± 0,68	1,21 ± 0,01	1,13 ± 0,10	1,38 ± 0,20	1,30 ± 0,10	0,90 ± 0,12	1,08 ± 0,22	1,01 ± 0,15
Isopropenyltoluene	MT	0,17 ± 0,02	0,21 ± 0,05	0,26 ± 0,00	0,18 ± 0,02	0,15 ± 0,03	0,18 ± 0,01	0,35 ± 0,02	0,25 ± 0,09	0,25 ± 0,01
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	MT	0,03 ± 0,01	0,02 ± 0,02	0,05 ± 0,01	0,02 ± 0,01	0,03 ± 0,01	0,04 ± 0,01	0,04 ± 0,01	0,07 ± 0,02	0,04 ± 0,01
2,6-Dimethyl-1,3,5,7-octatetraene, E	MT	0,01 ± 0,00	0,02 ± 0,01	0,01 ± 0,00	0,01 ± 0,00	0,01 ± 0,00	0,01 ± 0,01	0,01 ± 0,01	0,01 ± 0,01	0,01 ± 0,00
2,4,6-Octatriene, 2,6-dimethyl	MT	0,03 ± 0,01	0,04 ± 0,01	0,06 ± 0,01	0,03 ± 0,00	0,04 ± 0,01	0,05 ± 0,01	0,06 ± 0,01	0,06 ± 0,01	0,03 ± 0,01
Elixene	SQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
alpha.-Bergamotene	SQ	0,05 ± 0,01	0,07 ± 0,03	0,03 ± 0,03	0,03 ± 0,01	0,07 ± 0,01	0,06 ± 0,02	0,04 ± 0,01	0,05 ± 0,02	0,05 ± 0,02
beta.-Caryophyllene	SQ	0,41 ± 0,13	0,51 ± 0,26	0,39 ± 0,23	0,27 ± 0,10	0,74 ± 0,07	0,39 ± 0,07	0,18 ± 0,04	0,26 ± 0,08	0,21 ± 0,05
trans-.alpha.-Bergamotene	SQ	0,79 ± 0,07	1,01 ± 0,36	0,52 ± 0,28	0,44 ± 0,11	0,97 ± 0,11	0,89 ± 0,33	0,62 ± 0,16	0,74 ± 0,28	0,74 ± 0,30
Caryophyllene	SQ	0,04 ± 0,01	0,05 ± 0,02	0,03 ± 0,02	0,02 ± 0,00	0,05 ± 0,01	0,05 ± 0,02	0,03 ± 0,01	0,04 ± 0,02	0,04 ± 0,02
Allo-Aromadendrene	SQ	0,01 ± 0,01	0,01 ± 0,01	0,00 ± 0,00	0,00 ± 0,00	0,02 ± 0,01	0,00 ± 0,01	0,00 ± 0,00	0,00 ± 0,01	0,00 ± 0,00
beta.-Farnesene	SQ	0,07 ± 0,01	0,09 ± 0,04	0,05 ± 0,03	0,03 ± 0,01	0,09 ± 0,01	0,08 ± 0,03	0,05 ± 0,01	0,06 ± 0,03	0,07 ± 0,03
alpha.-Caryophyllene	SQ	0,03 ± 0,01	0,04 ± 0,02	0,03 ± 0,03	0,02 ± 0,01	0,06 ± 0,01	0,02 ± 0,01	0,01 ± 0,00	0,01 ± 0,01	0,01 ± 0,01
beta.-Santalene	SQ	0,05 ± 0,01	0,06 ± 0,02	0,04 ± 0,03	0,03 ± 0,00	0,06 ± 0,01	0,07 ± 0,03	0,04 ± 0,01	0,05 ± 0,02	0,05 ± 0,02
beta.-Himachalene	SQ	0,01 ± 0,01	0,02 ± 0,01	0,00 ± 0,00	0,01 ± 0,00	0,02 ± 0,01	0,01 ± 0,01	0,01 ± 0,01	0,01 ± 0,01	0,01 ± 0,01
alpha.-Curcumene	SQ	0,02 ± 0,01	0,02 ± 0,01	0,03 ± 0,01	0,01 ± 0,01	0,02 ± 0,01	0,03 ± 0,01	0,03 ± 0,01	0,02 ± 0,01	0,03 ± 0,01
Isocaryophyllene	SQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Valencene	SQ	0,45 ± 0,11	0,29 ± 0,17	0,99 ± 0,54	0,14 ± 0,06	0,21 ± 0,07	0,38 ± 0,11	0,38 ± 0,32	0,26 ± 0,09	0,48 ± 0,09
Eremophilene	SQ	0,04 ± 0,01	0,04 ± 0,02	0,07 ± 0,04	0,00 ± 0,00	0,06 ± 0,02	0,04 ± 0,01	0,04 ± 0,02	0,03 ± 0,01	0,04 ± 0,01
Germacrene B	SQ	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
.beta.-Bisabolene	SQ	1,45 ± 0,16	1,77 ± 0,62	1,02 ± 0,56	0,69 ± 0,13	1,67 ± 0,10	1,47 ± 0,50	0,85 ± 0,37	0,64 ± 0,49	1,36 ± 0,41
(-)-.alpha.-Panasinsen	SQ	0,02 ± 0,01	0,02 ± 0,01	0,05 ± 0,03	0,01 ± 0,01	0,01 ± 0,01	0,02 ± 0,01	0,02 ± 0,02	0,02 ± 0,01	0,02 ± 0,01
Humulene	SQ	0,04 ± 0,01	0,06 ± 0,02	0,03 ± 0,01	0,02 ± 0,01	0,06 ± 0,01	0,06 ± 0,02	0,03 ± 0,01	0,04 ± 0,02	0,04 ± 0,01
Bornyl chloride	OTHER	0,00 ± 0,00	0,11 ± 0,08	0,03 ± 0,01	0,07 ± 0,04	0,00 ± 0,01	0,00 ± 0,00	0,01 ± 0,01	0,02 ± 0,02	0,00 ± 0,00
Tridecane	OTHER	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,01 ± 0,01	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00
7-Tetradecene, (E)	OTHER	0,01 ± 0,00	0,02 ± 0,01	0,00 ± 0,00	0,00 ± 0,01	0,02 ± 0,01	0,01 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,01 ± 0,01
Tetradecane	OTHER	0,02 ± 0,01	0,02 ± 0,01	0,01 ± 0,01	0,01 ± 0,01	0,02 ± 0,01	0,01 ± 0,00	0,04 ± 0,07	0,04 ± 0,04	0,01 ± 0,00
Hexadecane	OTHER	0,00 ± 0,01	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,00 ± 0,00	0,01 ± 0,01	0,01 ± 0,03	0,00 ± 0,00	0,00 ± 0,00

5. Appendix 2

Tab. 1. Confusion matrices generated by the different algorithms applied for the treatments of EOS835 data

True/ Predicted Class	Bayes Net – Time 0			
	Control	Ethylene	Wax	Chemical
Control	3	1	0	1
Ethylene	0	0	5	0
Wax	0	5	0	0
Chemical	0	3	1	1
Correctly Classified Instances			20%	

True/ Predicted Class	Naive Bayes– Time 0			
	Control	Ethylene	Wax	Chemical
Control	4	0	0	1
Ethylene	0	2	2	1
Wax	0	3	0	2
Chemical	0	2	2	1
Correctly Classified Instances			35%	

True/ Predicted Class	MLP – Time 0			
	Control	Ethylene	Wax	Chemical
Control	5	0	0	0
Ethylene	1	0	3	1
Wax	0	4	1	0
Chemical	1	1	1	2
Correctly Classified Instances			40%	

True/ Predicted Class	RBF Network – Time 0			
	Control	Ethylene	Wax	Chemical
Control	3	1	1	0
Ethylene	1	1	3	0
Wax	0	4	1	0
Chemical	0	4	1	0
Correctly Classified Instances			25%	

True/ Predicted Class	J48 – Time 0			
	Control	Ethylene	Wax	Chemical
Control	4	0	1	0
Ethylene	1	0	3	1
Wax	0	4	1	0
Chemical	1	3	0	1
Correctly Classified Instances			30%	

True/ Predicted Class	IB1 – Time 0			
	Control	Ethylene	Wax	Chemical
Control	5	0	0	0
Ethylene	1	0	3	1
Wax	0	3	2	0
Chemical	1	1	2	1
Correctly Classified Instances			40%	

True/ Predicted Class	Bayes Net – Time 1			
	Control	Ethylene	Wax	Chemical
Control	20	0	0	0
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	1	0	0	19
Correctly Classified Instances			97,25%	

True/ Predicted Class	Naive Bayes– Time 1			
	Control	Ethylene	Wax	Chemical
Control	18	1	0	0
Ethylene	1	15	0	2
Wax	0	0	17	0
Chemical	0	0	0	18
Correctly Classified Instances			94,44%	

True/ Predicted Class	MLP – Time 1			
	Control	Ethylene	Wax	Chemical
Control	19	0	0	0
Ethylene	0	15	0	3
Wax	0	0	17	0
Chemical	0	2	0	16
Correctly Classified Instances			93,06%	

True/ Predicted Class	RBF Network – Time 1			
	Control	Ethylene	Wax	Chemical
Control	18	1	0	0
Ethylene	1	16	0	1
Wax	0	0	17	0
Chemical	0	2	0	16
Correctly Classified Instances			93,06%	

True/ Predicted Class	J48 – Time 1			
	Control	Ethylene	Wax	Chemical
Control	18	1	0	0
Ethylene	0	17	0	1
Wax	0	0	17	0
Chemical	0	1	1	16
Correctly Classified Instances			94,44%	

True/ Predicted Class	IB1 – Time 1			
	Control	Ethylene	Wax	Chemical
Control	19	0	0	0
Ethylene	0	15	0	3
Wax	0	0	16	1
Chemical	0	2	0	16
Correctly Classified Instances			91.67%	

True/ Predicted Class	Bayes Net – Time 2			
	Control	Ethylene	Wax	Chemical
Control	18	0	0	1
Ethylene	0	17	0	0
Wax	0	0	17	1
Chemical	0	0	1	16
Correctly Classified Instances			95,77%	

True/ Predicted Class	Naive Bayes– Time 2			
	Control	Ethylene	Wax	Chemical
Control	17	0	2	0
Ethylene	0	16	1	0
Wax	0	0	15	3
Chemical	0	0	2	15
Correctly Classified Instances			88,73%	

True/ Predicted Class	MLP – Time 2			
	Control	Ethylene	Wax	Chemical
Control	17	0	1	1
Ethylene	0	17	0	0
Wax	0	0	17	1
Chemical	1	0	1	15
Correctly Classified Instances			92,96%	

True/ Predicted Class	RBF Network – Time 2			
	Control	Ethylene	Wax	Chemical
Control	18	0	1	0
Ethylene	0	14	2	1
Wax	0	0	16	2
Chemical	0	0	1	16
Correctly Classified Instances			90,14%	

True/ Predicted Class	J48 – Time 2			
	Control	Ethylene	Wax	Chemical
Control	18	0	0	1
Ethylene	0	15	2	0
Wax	0	0	16	2
Chemical	0	0	0	17
Correctly Classified Instances			92,96%	

True/ Predicted Class	IB1 – Time 2			
	Control	Ethylene	Wax	Chemical
Control	17	0	1	1
Ethylene	0	17	0	0
Wax	0	0	17	1
Chemical	0	0	1	16
Correctly Classified Instances			94.37%	

True/ Predicted Class	Bayes Net – Time 3			
	Control	Ethylene	Wax	Chemical
Control	15	2	1	0
Ethylene	2	17	0	0
Wax	1	1	16	0
Chemical	0	0	0	18
Correctly Classified Instances			90,41%	

True/ Predicted Class	Naive Bayes– Time 3			
	Control	Ethylene	Wax	Chemical
Control	17	0	0	1
Ethylene	14	4	0	1
Wax	1	0	17	0
Chemical	7	1	0	10
Correctly Classified Instances			65,75%	

True/ Predicted Class	MLP – Time 3			
	Control	Ethylene	Wax	Chemical
Control	17	0	0	1
Ethylene	0	19	0	0
Wax	1	0	17	0
Chemical	1	0	0	17
Correctly Classified Instances			95,89%	

True/ Predicted Class	RBF Network – Time 3			
	Control	Ethylene	Wax	Chemical
Control	15	2	0	1
Ethylene	3	15	0	1
Wax	1	0	17	0
Chemical	1	1	0	16
Correctly Classified Instances			86,30%	

True/ Predicted Class	J48 – Time 3			
	Control	Ethylene	Wax	Chemical
Control	14	3	0	1
Ethylene	2	17	0	0
Wax	1	0	17	0
Chemical	0	0	0	18
Correctly Classified Instances			90,41%	

True/ Predicted Class	IB1 – Time 3			
	Control	Ethylene	Wax	Chemical
Control	11	7	0	0
Ethylene	1	17	0	1
Wax	0	1	17	0
Chemical	0	1	0	17
Correctly Classified Instances			84.93%	

Tab. 2. Confusion matrices generated by the different algorithms applied for the treatments of MOOSY32 data

True/ Predicted Class	Bayes Net – Time 0			
	Control	Ethylene	Wax	Chemical
Control	16	0	1	3
Ethylene	0	20	0	0
Wax	1	0	19	0
Chemical	1	0	0	19
Correctly Classified Instances			92,5%	

True/ Predicted Class	Naive Bayes– Time 0			
	Control	Ethylene	Wax	Chemical
Control	18	0	0	2
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	2	0	0	18
Correctly Classified Instances			95%	

True/ Predicted Class	MLP – Time 0			
	Control	Ethylene	Wax	Chemical
Control	17	0	0	3
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	1	0	0	19
Correctly Classified Instances			95%	

True/ Predicted Class	RBF Network – Time 0			
	Control	Ethylene	Wax	Chemical
Control	19	0	0	1
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	1	0	0	19
Correctly Classified Instances			97,5%	

True/ Predicted Class	J48 – Time 0			
	Control	Ethylene	Wax	Chemical
Control	15	0	1	4
Ethylene	0	20	0	0
Wax	0	1	18	1
Chemical	2	0	0	18
Correctly Classified Instances			88.75%	

True/ Predicted Class	IB1 – Time 0			
	Control	Ethylene	Wax	Chemical
Control	19	0	0	1
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	1	0	0	19
Correctly Classified Instances			97.5%	

True/ Predicted Class	Bayes Net – Time 1			
	Control	Ethylene	Wax	Chemical
Control	20	0	0	0
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	0	0	0	20
Correctly Classified Instances			100%	

True/ Predicted Class	Naive Bayes– Time 1			
	Control	Ethylene	Wax	Chemical
Control	20	0	0	0
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	0	0	0	20
Correctly Classified Instances			100%	

True/ Predicted Class	MLP – Time 1			
	Control	Ethylene	Wax	Chemical
Control	20	0	0	0
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	0	0	0	20
Correctly Classified Instances			100%	

True/ Predicted Class	RBF Network – Time 1			
	Control	Ethylene	Wax	Chemical
Control	20	0	0	0
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	0	0	0	20
Correctly Classified Instances			100%	

True/ Predicted Class	J48 – Time 1			
	Control	Ethylene	Wax	Chemical
Control	19	0	0	1
Ethylene	1	19	0	0
Wax	0	0	20	0
Chemical	0	0	1	19
Correctly Classified Instances			96,25%	

True/ Predicted Class	IB1 – Time 1			
	Control	Ethylene	Wax	Chemical
Control	20	0	0	0
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	1	0	0	20
Correctly Classified Instances			100%	

True/ Predicted Class	Naive Bayes – Time 2			
	Control	Ethylene	Wax	Chemical
Control	20	0	0	0
Ethylene	0	19	0	1
Wax	0	0	20	0
Chemical	0	0	1	19
Correctly Classified Instances			97,5%	

True/ Predicted Class	Bayes Net – Time 2			
	Control	Ethylene	Wax	Chemical
Control	20	0	0	0
Ethylene	0	19	0	1
Wax	1	0	19	0
Chemical	0	0	0	20
Correctly Classified Instances			97,5%	

True/ Predicted Class	RBF Network – Time 2			
	Control	Ethylene	Wax	Chemical
Control	20	0	0	0
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	0	1	0	19
Correctly Classified Instances			98,75%	

True/ Predicted Class	MLP – Time 2			
	Control	Ethylene	Wax	Chemical
Control	20	0	0	0
Ethylene	0	19	0	1
Wax	0	0	20	0
Chemical	0	0	0	20
Correctly Classified Instances			98,75%	

True/ Predicted Class	J48 – Time 2			
	Control	Ethylene	Wax	Chemical
Control	19	0	1	0
Ethylene	0	18	0	2
Wax	1	0	19	0
Chemical	2	1	0	17
Correctly Classified Instances			91,25%	

True/ Predicted Class	IB1 – Time 2			
	Control	Ethylene	Wax	Chemical
Control	19	0	0	1
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	1	0	0	19
Correctly Classified Instances			97.5%	

True/ Predicted Class	Bayes Net – Time 3			
	Control	Ethylene	Wax	Chemical
Control	14	0	1	5
Ethylene	1	19	0	0
Wax	1	0	19	0
Chemical	2	0	0	18
Correctly Classified Instances			87,5%	

True/ Predicted Class	Naive Bayes – Time 3			
	Control	Ethylene	Wax	Chemical
Control	18	0	0	2
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	3	0	0	17
Correctly Classified Instances			93,75%	

True/ Predicted Class	MLP – Time 3			
	Control	Ethylene	Wax	Chemical
Control	15	0	0	5
Ethylene	0	20	0	0
Wax	1	0	19	0
Chemical	4	0	0	16
Correctly Classified Instances			87,5%	

True/ Predicted Class	RBF Network – Time 3			
	Control	Ethylene	Wax	Chemical
Control	18	0	0	2
Ethylene	0	20	0	0
Wax	1	0	19	0
Chemical	3	0	0	17
Correctly Classified Instances			92,5%	

True/ Predicted Class	J48 – Time 3			
	Control	Ethylene	Wax	Chemical
Control	14	2	0	4
Ethylene	2	18	0	0
Wax	0	0	20	0
Chemical	4	0	0	16
Correctly Classified Instances			85%	

True/ Predicted Class	IB1 – Time 3			
	Control	Ethylene	Wax	Chemical
Control	16	0	0	4
Ethylene	0	20	0	0
Wax	0	0	20	0
Chemical	4	0	0	16
Correctly Classified Instances			90%	

Tab. 3. Variation of chemical composition of the volatile fraction of the juices in the four treatments at T0) after harvest; T1) after 4 weeks at 4°C; T2) after 8 weeks at 4°C; T3) after an additional week at 20 °C.

Data are arranged according to Chemical Groups, and represents the mean relative percentage of individual compounds from duplicate experiments.

		CHEMICAL CLASSES						
TREATMENTS	**TIME**	**Alcohols**	**Aldehydes**	**Esters**	**Ketones**	**Monoterpenes**	**Sesquiterpenes**	**Others**
CONTROL	**T0**	5,46±1,27	4,41±2,79	0,08±0,01	0,22±0,03	88,81±4,03	1,21±0,02	0,01±0,01
	T1	2,35±0,35	5,08±0,64	0,32±0,04	0,29±0,02	89,33±1,80	2,61±0,76	0,03±0,00
	T2	1,61±0,50	2,69±1,17	029±0,06	0,27±0,03	91,52±1,50	3,57±0,27	0,05±0,01
	T3	2,56±0,02	0,98±0,30	0,72±0,21	0,29±0,01	92,24±0,38	3,22±0,12	0,00±0,00
CHEMICAL	**T0**	3,22±0,35	6,42±0,51	0,61±0,09	0,30±0,07	88,27±1,08	1,17±0,06	0,01±0,01
	T1	1,63±0,17	4,78±0,21	0,53±008	030±0,01	89,45±0,20	3,28±0,12	0,03±0,01
	T2	1,79±0,00	0,74±0,00	2,80±0,00	0,31±0,00	91,08±0,00	3,28±0,00	0,00±0,00
	T3	2,18±0,19	0,78±0,16	9,66±0,53	0,18±0,04	84,80±1,27	2,40±0,35	0,00±0,00
EHYLENE	**T0**	1,96±0,19	0,99±0,06	5,23±0,44	0,37±004	85,04±0,37	6,39±1,01	0,01±0,00
	T1	2,50±0,34	1,40±0,33	3,22±0,53	0,52±0,03	83,49±0,38	8,77±1,57	0,11±0,02
	T2	1,10±0,19	0,71±0,08	3,12±0,49	0,46±0,03	84,22±116	10,37±1,97	0,02±0,02
	T3	1,15±0,09	0,46±0,11	8,05±2,34	0,32±0,08	82,53±3,37	7,49±1,13	0,00±0,00
WAX	**T0**	1,68±0,33	1,68±0,05	5,88±0,40	0,30±0,02	83,74±0,74	7,46±0,04	0,02±0,02
	T1	1,62±0,27	0,92±0,13	6,19±0,55	0,43±0,02	82,30±0,68	8,45±0,32	0,09±0,02
	T2	1,67±0,07	0,43±0,08	8,27±0,14	0,40±0,05	78,55±0,62	10,68±0,55	0,00±0,00
	T3	1,75±0,00	0,39±0,00	17,49±0,00	0,39±0,00	71,35±0,00	8,63±0,00	0,00±0,00

Tab. 4. Variation of chemical composition of the volatile fraction of the juices in the four treatments at T0) after harvest; T1) after 4 weeks at 4°C; T2) after 8 weeks at 4°C; T3) after an additional week at 20 °C.
Data are arranged according to Chemical Groups, and represents the mean relative percentage of individual compounds from duplicate experiments. Family code: AC, alcohols; AD, aldehydes; ES, esters; K, ketones; MT, monoterpenes; SQ, sesquiterpenes.

Molecules	Chemical Classes	CONTROL								CHEMICAL							
		T 0		T1		T2		T3		T 0		T1		T2		T3	
1-Butanol, 3-methyl	**AC**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,04 ± 0,02		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,06 ± 0,01	
4-Hexen-1-ol, (Z)	**AC**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,35 ± 0,02		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,48 ± 0,01	
3-Hexen-1-ol, (Z)	**AC**	0,27 ± 0,25		0,11 ± 0,01		0,12 ± 0,04		0,00 ± 0,00		0,41 ± 0,02		0,07 ± 0,01		0,06 ± 0,00		0,00 ± 0,00	
2-Hexen-1-ol, (Z)	**AC**	0,19 ± 0,09		0,04 ± 0,01		0,07 ± 0,02		0,10 ± 0,01		0,08 ± 0,01		0,02 ± 0,00		0,04 ± 0,00		0,06 ± 0,01	
1-Hexanol	**AC**	0,35 ± 0,30		0,15 ± 0,01		0,16 ± 0,05		0,47 ± 0,02		0,39 ± 0,02		0,11 ± 0,01		0,13 ± 0,00		0,52 ± 0,04	
1-Octanol	**AC**	1,83 ± 0,04		0,53 ± 0,06		0,39 ± 0,11		0,42 ± 0,07		0,77 ± 0,08		0,41 ± 0,02		0,58 ± 0,00		0,29 ± 0,04	
beta-Linalool	**AC**	1,83 ± 0,52		0,57 ± 0,13		0,29 ± 0,13		0,33 ± 0,01		0,76 ± 0,19		0,42 ± 0,07		0,43 ± 0,00		0,29 ± 0,04	
beta-Terpineol	**AC**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,01 ± 0,01		0,01 ± 0,01		0,00 ± 0,00		0,00 ± 0,00	
Nonanol	**AC**	0,05 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
4-Terpineol	**AC**	0,91 ± 0,11		0,00 ± 0,00		0,56 ± 0,16		0,85 ± 0,01		0,75 ± 0,15		0,00 ± 0,00		0,54 ± 0,00		0,47 ± 0,04	
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	**AC**	0,00 ± 0,00		0,92 ± 0,12		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,57 ± 0,07		0,00 ± 0,00		0,00 ± 0,00	
beta-Citronellol	**AC**	0,02 ± 0,02		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,01 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
Carvacrol	**AC**	0,01 ± 0,01		0,03 ± 0,01		0,03 ± 0,01		0,00 ± 0,00		0,04 ± 0,01		0,03 ± 0,01		0,00 ± 0,00		0,00 ± 0,00	
Pentanal	**AD**	0,11 ± 0,10		0,25 ± 0,02		0,10 ± 0,02		0,08 ± 0,00		0,20 ± 0,01		0,14 ± 0,01		0,07 ± 0,00		0,05 ± 0,00	
Hexanal	**AD**	3,57 ± 2,73		3,93 ± 0,46		1,89 ± 0,94		0,00 ± 0,00		5,14 ± 0,27		3,57 ± 0,19		0,00 ± 0,00		0,00 ± 0,00	
2-Hexenal, (E)	**AD**	0,16 ± 0,03		0,08 ± 0,02		0,05 ± 0,01		0,32 ± 0,06		0,16 ± 0,03		0,09 ± 0,00		0,00 ± 0,00		0,29 ± 0,07	
Heptanal	**AD**	0,21 ± 0,06		0,30 ± 0,06		0,14 ± 0,11		0,26 ± 0,15		0,46 ± 0,11		0,28 ± 0,04		0,09 ± 0,00		0,12 ± 0,02	
Nonanal	**AD**	0,14 ± 0,02		0,08 ± 0,02		0,08 ± 0,04		0,13 ± 0,08		0,10 ± 0,01		0,08 ± 0,01		0,10 ± 0,00		0,09 ± 0,04	
2-Nonenal, (E)	**AD**	0,00 ± 0,00		0,03 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,02 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
Benzaldehyde, 2,4-dimethyl	**AD**	0,00 ± 0,00		0,00 ± 0,00		0,37 ± 0,02		0,17 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,38 ± 0,00		0,23 ± 0,01	
Benzaldehyde, 3,4-dimethyl	**AD**	0,11 ± 0,05		0,25 ± 0,02		0,00 ± 0,00		0,00 ± 0,00		0,21 ± 0,04		0,45 ± 0,02		0,00 ± 0,00		0,00 ± 0,00	
3-Cyclohexene-1-acetaldehyde, .alpha.,4-dimethyl	**AD**	0,03 ± 0,04		0,05 ± 0,01		0,01 ± 0,01		0,00 ± 0,00		0,06 ± 0,01		0,05 ± 0,00		0,04 ± 0,00		0,00 ± 0,00	
Cumaldehyde	**AD**	0,00 ± 0,00		0,04 ± 0,00		0,03 ± 0,01		0,00 ± 0,00		0,01 ± 0,01		0,04 ± 0,01		0,02 ± 0,00		0,00 ± 0,00	
alpha-Citral	**AD**	0,04 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
Perillaldehyde	**AD**	0,05 ± 0,02		0,07 ± 0,01		0,03 ± 0,01		0,01 ± 0,02		0,08 ± 0,02		0,06 ± 0,00		0,03 ± 0,00		0,01 ± 0,01	
Propanoic acid, ethyl ester	**ES**	0,00 ± 0,00		0,00 ± 0,00		0,02 ± 0,01		0,02 ± 0,01		0,00 ± 0,00		0,01 ± 0,01		0,01 ± 0,00		0,03 ± 0,04	
Butanoic acid, methyl ester	**ES**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,03 ± 0,01		0,00 ± 0,00		0,01 ± 0,01		0,02 ± 0,00		0,07 ± 0,01	
Butanoic acid, ethyl ester	**ES**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		2,41 ± 0,00		8,68 ± 0,36	
2-Butenoic acid, ethyl ester	**ES**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,04 ± 0,02		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,09 ± 0,03	
Butanoic acid, 2-methyl-, ethyl ester	**ES**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,03 ± 0,04	
Butanoic acid, propyl ester	**ES**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
Hexanoic acid, ethyl ester	**ES**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,42 ± 0,17		0,29 ± 0,04		0,05 ± 0,07		0,00 ± 0,00		0,46 ± 0,01	
Ethyl 2-hexenoate	**ES**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,01 ± 0,02		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,02 ± 0,00	
n-Octyl acetate	**ES**	0,06 ± 0,02		0,24 ± 0,02		0,18 ± 0,03		0,10 ± 0,02		0,25 ± 0,09		0,36 ± 0,02		0,24 ± 0,00		0,15 ± 0,01	
Linalyl anthranilate	**ES**	0,02 ± 0,01		0,03 ± 0,01		0,04 ± 0,01		0,04 ± 0,01		0,06 ± 0,02		0,04 ± 0,00		0,05 ± 0,00		0,07 ± 0,02	
n-Decyl acetate	**ES**	0,00 ± 0,00		0,05 ± 0,01		0,06 ± 0,01		0,06 ± 0,01		0,02 ± 0,02		0,06 ± 0,00		0,07 ± 0,00		0,06 ± 0,01	
4-Acetyl-1-methylcyclohexene	**K**	0,04 ± 0,00		0,04 ± 0,01		0,01 ± 0,01		0,00 ± 0,00		0,05 ± 0,01		0,03 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
p-Methylacetophenone	**K**	0,00 ± 0,00		0,04 ± 0,01		0,06 ± 0,01		0,04 ± 0,01		0,05 ± 0,02		0,04 ± 0,01		0,07 ± 0,00		0,02 ± 0,02	
Dihydrocarvone	**K**	0,01 ± 0,01		0,03 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,04 ± 0,01		0,03 ± 0,00		0,02 ± 0,00		0,00 ± 0,00	
Levo-carvone	**K**	0,14 ± 0,02		0,13 ± 0,00		0,09 ± 0,01		0,06 ± 0,01		0,15 ± 0,04		0,12 ± 0,01		0,08 ± 0,00		0,04 ± 0,01	

Molecules	Chemical Classes	ETHYLENE								WAX							
		T 0		T1		T2		T3		T 0		T1		T2		T3	
1-Butanol, 3-methyl	**AC**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,02 ± 0,02		0,01 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,27 ± 0,00	
4-Hexen-1-ol, (Z)	**AC**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
3-Hexen-1-ol, (Z)	**AC**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,06 ± 0,08		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
2-Hexen-1-ol, (Z)	**AC**	0,07 ± 0,00		0,10 ± 0,02		0,00 ± 0,00		0,12 ± 0,01		0,03 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,04 ± 0,00	
1-Hexanol	**AC**	0,12 ± 0,01		0,20 ± 0,05		0,04 ± 0,01		0,33 ± 0,05		0,09 ± 0,01		0,07 ± 0,01		0,05 ± 0,01		0,18 ± 0,00	
1-Octanol	**AC**	0,39 ± 0,05		0,90 ± 0,05		0,00 ± 0,00		0,10 ± 0,14		0,33 ± 0,03		0,58 ± 0,07		0,58 ± 0,03		0,38 ± 0,00	
beta-Linalool	**AC**	0,60 ± 0,07		0,59 ± 0,12		0,47 ± 0,07		0,23 ± 0,03		0,43 ± 0,10		0,34 ± 0,08		0,50 ± 0,01		0,40 ± 0,00	
beta-Terpineol	**AC**	0,01 ± 0,01		0,01 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,01 ± 0,02		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
Nonanol	**AC**	0,00 ± 0,00		0,03 ± 0,00		0,02 ± 0,02		0,00 ± 0,00		0,00 ± 0,00		0,01 ± 0,00		0,03 ± 0,00		0,02 ± 0,00	
4-Terpineol	**AC**	0,72 ± 0,03		0,58 ± 0,07		0,55 ± 0,06		0,30 ± 0,00		0,00 ± 0,00		0,60 ± 0,12		0,51 ± 0,03		0,45 ± 0,00	
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	**AC**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,75 ± 0,17		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
beta-Citronellol	**AC**	0,00 ± 0,00		0,04 ± 0,01		0,01 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
Carvacrol	**AC**	0,04 ± 0,01		0,05 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,03 ± 0,00		0,02 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
Pentanal	**AD**	0,12 ± 0,01		0,13 ± 0,08		0,07 ± 0,00		0,04 ± 0,02		0,21 ± 0,06		0,07 ± 0,01		0,03 ± 0,01		0,00 ± 0,00	
Hexanal	**AD**	0,00 ± 0,00		0,08 ± 0,02		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
2-Hexenal, (E)	**AD**	0,15 ± 0,00		0,00 ± 0,00		0,03 ± 0,04		0,11 ± 0,15		0,07 ± 0,02		0,01 ± 0,01		0,00 ± 0,00		0,11 ± 0,00	
Heptanal	**AD**	0,22 ± 0,04		0,28 ± 0,14		0,16 ± 0,11		0,12 ± 0,10		0,22 ± 0,05		0,16 ± 0,06		0,08 ± 0,02		0,06 ± 0,00	
Nonanal	**AD**	0,09 ± 0,01		0,22 ± 0,08		0,14 ± 0,08		0,07 ± 0,01		0,08 ± 0,02		0,09 ± 0,02		0,08 ± 0,00		0,10 ± 0,00	
2-Nonenal, (E)	**AD**	0,01 ± 0,01		0,01 ± 0,02		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
Benzaldehyde, 2,4-dimethyl	**AD**	0,00 ± 0,00		0,00 ± 0,00		0,25 ± 0,05		0,13 ± 0,05		0,00 ± 0,00		0,00 ± 0,00		0,24 ± 0,05		0,12 ± 0,00	
Benzaldehyde, 3,4-dimethyl	**AD**	0,20 ± 0,00		0,52 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,14 ± 0,00		0,50 ± 0,02		0,00 ± 0,00		0,00 ± 0,00	
3-Cyclohexene-1-acetaldehyde, .alpha.,4-dimethyl	**AD**	0,06 ± 0,00		0,06 ± 0,02		0,04 ± 0,02		0,00 ± 0,00		0,08 ± 0,01		0,04 ± 0,00		0,02 ± 0,03		0,00 ± 0,00	
Cumaldehyde	**AD**	0,04 ± 0,01		0,05 ± 0,01		0,01 ± 0,01		0,00 ± 0,00		0,04 ± 0,01		0,04 ± 0,01		0,00 ± 0,00		0,00 ± 0,00	
alpha-Citral	**AD**	0,01 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
Perillaldehyde	**AD**	0,10 ± 0,01		0,04 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,08 ± 0,01		0,02 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
Propanoic acid, ethyl ester	**ES**	0,00 ± 0,00		0,02 ± 0,01		0,02 ± 0,02		0,04 ± 0,00		0,08 ± 0,07		0,05 ± 0,01		0,04 ± 0,01		0,05 ± 0,00	
Butanoic acid, methyl ester	**ES**	0,03 ± 0,00		0,02 ± 0,00		0,05 ± 0,01		0,06 ± 0,08		0,00 ± 0,00		0,07 ± 0,01		0,06 ± 0,00		0,14 ± 0,00	
Butanoic acid, ethyl ester	**ES**	4,47 ± 0,42		2,58 ± 0,47		2,79 ± 0,51		6,98 ± 1,83		5,13 ± 0,26		5,57 ± 0,47		7,64 ± 0,15		14,60 ± 0,00	
2-Butenoic acid, ethyl ester	**ES**	0,02 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,12 ± 0,08		0,03 ± 0,02		0,04 ± 0,01		0,09 ± 0,01		0,46 ± 0,00	
Butanoic acid, 2-methyl-, ethyl ester	**ES**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,16 ± 0,15		0,00 ± 0,00		0,02 ± 0,00		0,00 ± 0,00		0,27 ± 0,00	
Butanoic acid, propyl ester	**ES**	0,00 ± 0,00		0,01 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,03 ± 0,01		0,03 ± 0,00		0,04 ± 0,00	
Hexanoic acid, ethyl ester	**ES**	0,28 ± 0,02		0,18 ± 0,01		0,03 ± 0,04		0,50 ± 0,21		0,29 ± 0,03		0,16 ± 0,01		0,22 ± 0,01		1,70 ± 0,00	
Ethyl 2-hexenoate	**ES**	0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,02 ± 0,01		0,00 ± 0,00		0,00 ± 0,00		0,08 ± 0,00	
n-Octyl acetate	**ES**	0,36 ± 0,02		0,29 ± 0,02		0,13 ± 0,02		0,08 ± 0,02		0,24 ± 0,00		0,17 ± 0,03		0,08 ± 0,00		0,05 ± 0,00	
Linalyl anthranilate	**ES**	0,05 ± 0,01		0,06 ± 0,02		0,05 ± 0,01		0,08 ± 0,02		0,05 ± 0,01		0,04 ± 0,01		0,06 ± 0,00		0,04 ± 0,00	
n-Decyl acetate	**ES**	0,03 ± 0,00		0,07 ± 0,00		0,06 ± 0,01		0,04 ± 0,00		0,04 ± 0,01		0,05 ± 0,00		0,05 ± 0,01		0,04 ± 0,00	
4-Acetyl-1-methylcyclohexene	**K**	0,04 ± 0,00		0,05 ± 0,01		0,01 ± 0,01		0,00 ± 0,00		0,03 ± 0,01		0,03 ± 0,00		0,00 ± 0,00		0,00 ± 0,00	
p-Methylacetophenone	**K**	0,08 ± 0,01		0,07 ± 0,00		0,06 ± 0,01		0,02 ± 0,02		0,05 ± 0,00		0,07 ± 0,00		0,04 ± 0,01		0,03 ± 0,00	
Dihydrocarvone	**K**	0,03 ± 0,00		0,03 ± 0,00		0,00 ± 0,00		0,00 ± 0,00		0,03 ± 0,00		0,03 ± 0,01		0,00 ± 0,00		0,00 ± 0,00	
Levo-carvone	**K**	0,15 ± 0,00		0,15 ± 0,00		0,07 ± 0,01		0,03 ± 0,00		0,10 ± 0,01		0,12 ± 0,01		0,06 ± 0,01		0,04 ± 0,00	

Continue

Molecules	Chemical Classes	CONTROL												CHEMICAL											
		T 0			T1			T2			T3			T 0			T1			T2			T3		
Nootkatone	**K**	0,03	±	0,01	0,05	±	0,00	0,11	±	0,00	0,19	±	0,01	0,02	±	0,00	0,08	±	0,00	0,13	±	0,00	0,12	±	0,01
alpha thujene	**MT**	0,04	±	0,02	0,06	±	0,01	0,07	±	0,02	0,06	±	0,01	0,07	±	0,01	0,03	±	0,01	0,03	±	0,00	0,03	±	0,00
alpha-Pinene	**MT**	0,10	±	0,06	0,07	±	0,00	0,07	±	0,01	0,07	±	0,01	0,08	±	0,00	0,08	±	0,01	0,06	±	0,00	0,08	±	0,00
beta-Phellandrene	**MT**	0,20	±	0,17	0,11	±	0,01	0,15	±	0,04	0,25	±	0,11	0,08	±	0,00	0,05	±	0,01	0,06	±	0,00	0,09	±	0,00
2-Menthene	**MT**	0,21	±	0,29	0,07	±	0,01	0,03	±	0,04	0,00	±	0,00	0,10	±	0,04	0,04	±	0,01	0,00	±	0,00	0,00	±	0,00
3-Menthene	**MT**	0,98	±	1,39	1,04	±	0,13	0,51	±	0,19	0,38	±	0,07	1,40	±	0,51	0,73	±	0,09	0,31	±	0,00	0,35	±	0,01
beta-Myrcene	**MT**	3,98	±	0,09	2,95	±	0,25	2,52	±	0,70	2,88	±	0,86	2,72	±	0,22	3,64	±	0,41	2,76	±	0,00	2,98	±	0,04
3-Carene	**MT**	0,00	±	0,00	0,00	±	0,00	1,09	±	0,11	1,41	±	0,22	0,00	±	0,00	0,00	±	0,00	1,07	±	0,00	1,20	±	0,03
2-Carene	**MT**	0,00	±	0,00	2,88	±	2,77	4,16	±	1,10	3,22	±	0,31	0,00	±	0,00	2,29	±	2,41	3,78	±	0,00	2,70	±	0,30
D-Limonene	**MT**	73,61	±	2,43	72,98	±	1,37	72,73	±	0,10	74,16	±	1,97	73,93	±	1,11	73,10	±	2,69	72,13	±	0,00	69,48	±	0,85
β-cis-Ocimene	**MT**	0,09	±	0,01	0,07	±	0,01	0,06	±	0,01	0,07	±	0,03	0,06	±	0,01	0,07	±	0,01	0,07	±	0,00	0,06	±	0,00
Cyclopentene, 3-isopropenyl-5,5-dimethyl	**MT**	0,00	±	0,00	0,00	±	0,00	0,08	±	0,02	0,08	±	0,01	0,00	±	0,00	0,00	±	0,00	0,08	±	0,00	0,07	±	0,00
β trans Ocimene	**MT**	0,08	±	0,01	0,09	±	0,01	0,00	±	0,00	0,00	±	0,00	0,07	±	0,01	0,08	±	0,01	0,00	±	0,00	0,00	±	0,00
gamma-Terpinene	**MT**	1,21	±	0,27	0,99	±	0,00	0,72	±	0,09	0,79	±	0,19	0,72	±	0,02	0,86	±	0,04	0,76	±	0,00	0,61	±	0,04
4-Carene	**MT**	5,56	±	0,55	5,00	±	0,32	4,15	±	1,02	4,25	±	0,35	3,65	±	0,44	4,92	±	0,22	4,67	±	0,00	3,66	±	0,29
Styrene, 2,6-dimethyl	**MT**	2,38	±	0,87	2,95	±	0,17	5,07	±	0,32	4,53	±	0,23	5,22	±	0,82	3,42	±	0,16	5,10	±	0,00	3,43	±	0,21
2,4,6-Octatriene, 2,6-dimethyl	**MT**	0,09	±	0,03	0,00	±	0,00	0,04	±	0,01	0,00	±	0,00	0,10	±	0,00	0,07	±	0,01	0,07	±	0,00	0,07	±	0,00
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)	**MT**	0,07	±	0,02	0,06	±	0,00	0,08	±	0,00	0,09	±	0,02	0,08	±	0,01	0,07	±	0,00	0,10	±	0,00	0,00	±	0,00
Copaene	**SQ**	0,03	±	0,00	0,04	±	0,02	0,04	±	0,02	0,01	±	0,02	0,01	±	0,01	0,07	±	0,02	0,02	±	0,00	0,02	±	0,02
beta-Elemene, (-)	**SQ**	0,02	±	0,01	0,05	±	0,01	0,07	±	0,00	0,06	±	0,00	0,02	±	0,00	0,06	±	0,00	0,05	±	0,00	0,05	±	0,01
Caryophyllene	**SQ**	0,03	±	0,00	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,05	±	0,01	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00
Alloaromadendrene	**SQ**	0,03	±	0,01	0,02	±	0,02	0,04	±	0,00	0,02	±	0,03	0,01	±	0,01	0,04	±	0,01	0,03	±	0,00	0,03	±	0,00
alpha-Caryophyllene	**SQ**	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00
alpha-Gurjunene	**SQ**	0,00	±	0,00	0,03	±	0,04	0,04	±	0,01	0,03	±	0,00	0,01	±	0,01	0,04	±	0,01	0,03	±	0,00	0,01	±	0,01
beta-Selinene	**SQ**	0,00	±	0,00	0,13	±	0,01	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,14	±	0,00	0,00	±	0,00	0,00	±	0,00
Valencene	**SQ**	0,99	±	0,02	1,94	±	0,53	2,76	±	0,23	2,54	±	0,07	0,95	±	0,01	2,49	±	0,10	2,58	±	0,00	1,92	±	0,32
beta-Humulene	**SQ**	0,00	±	0,00	0,00	±	0,00	0,03	±	0,00	0,02	±	0,02	0,00	±	0,00	0,00	±	0,00	0,03	±	0,00	0,00	±	0,00
beta-Panasinsene	**SQ**	0,11	±	0,02	0,38	±	0,09	0,52	±	0,05	0,45	±	0,02	0,13	±	0,01	0,43	±	0,00	0,46	±	0,00	0,30	±	0,02
Selina-3,7(11)-diene	**SQ**	0,00	±	0,00	0,02	±	0,03	0,05	±	0,01	0,03	±	0,00	0,00	±	0,00	0,02	±	0,00	0,03	±	0,00	0,03	±	0,01
Eudalene	**SQ**	0,00	±	0,00	0,00	±	0,00	0,03	±	0,01	0,05	±	0,01	0,00	±	0,00	0,00	±	0,00	0,04	±	0,00	0,05	±	0,00
Styrene	**OTHER**	0,00	±	0,00	0,00	±	0,00	0,04	±	0,02	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00
Benzene, 1,3-bis(1,1-dimethylethyl)	**OTHER**	0,01	±	0,01	0,03	±	0,00	0,02	±	0,01	0,00	±	0,00	0,01	±	0,01	0,03	±	0,01	0,00	±	0,00	0,00	±	0,00

Molecules	Chemical Classes	ETHYLENE												WAX											
		T 0			T1			T2			T3			T 0			T1			T2			T3		
Nootkatone	**K**	0,07	±	0,03	0,22	±	0,04	0,33	±	0,03	0,26	±	0,06	0,09	±	0,01	0,18	±	0,01	0,30	±	0,03	0,31	±	0,00
alpha thujene	**MT**	0,06	±	0,00	0,04	±	0,01	0,05	±	0,01	0,03	±	0,00	0,05	±	0,01	0,04	±	0,01	0,03	±	0,00	0,00	±	0,00
alpha-Pinene	**MT**	0,09	±	0,02	0,07	±	0,00	0,06	±	0,01	0,05	±	0,00	0,07	±	0,01	0,07	±	0,00	0,05	±	0,00	0,03	±	0,00
beta-Phellandrene	**MT**	0,12	±	0,04	0,06	±	0,01	0,12	±	0,02	0,13	±	0,02	0,07	±	0,01	0,08	±	0,01	0,07	±	0,02	0,04	±	0,00
2-Menthene	**MT**	0,04	±	0,01	0,02	±	0,02	0,03	±	0,04	0,03	±	0,04	0,07	±	0,01	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00
3-Menthene	**MT**	0,60	±	0,02	0,50	±	0,22	0,57	±	0,31	0,41	±	0,27	0,99	±	0,06	0,37	±	0,06	0,32	±	0,02	0,24	±	0,00
beta-Myrcene	**MT**	3,19	±	0,33	3,32	±	0,12	2,84	±	0,44	2,50	±	0,92	2,11	±	0,33	2,49	±	0,10	2,35	±	0,06	1,78	±	0,00
3-Carene	**MT**	0,00	±	0,00	0,00	±	0,00	1,16	±	0,05	1,09	±	0,14	0,00	±	0,00	1,21	±	0,09	1,04	±	0,00	1,07	±	0,00
2-Carene	**MT**	0,00	±	0,00	0,00	±	0,00	3,53	±	0,19	3,19	±	1,59	0,29	±	0,41	2,04	±	2,88	2,96	±	0,27	0,92	±	0,00
D-Limonene	**MT**	72,07	±	0,06	70,36	±	0,54	66,13	±	1,11	70,76	±	8,24	71,06	±	1,50	66,24	±	3,24	62,93	±	1,33	59,40	±	0,00
β-cis-Ocimene	**MT**	0,05	±	0,01	0,06	±	0,00	0,07	±	0,02	0,02	±	0,02	0,05	±	0,01	0,07	±	0,01	0,06	±	0,01	0,06	±	0,00
Cyclopentene, 3-isopropenyl-5,5-dimethyl	**MT**	0,00	±	0,00	0,00	±	0,00	0,08	±	0,01	0,03	±	0,04	0,00	±	0,00	0,07	±	0,01	0,07	±	0,01	0,05	±	0,00
β trans Ocimene	**MT**	0,05	±	0,00	0,07	±	0,01	0,00	±	0,00	0,00	±	0,00	0,07	±	0,00	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00
gamma-Terpinene	**MT**	0,56	±	0,02	0,58	±	0,03	0,62	±	0,09	0,21	±	0,30	0,77	±	0,09	0,73	±	0,16	0,59	±	0,05	0,43	±	0,00
4-Carene	**MT**	2,99	±	0,02	3,49	±	0,01	3,93	±	0,03	1,67	±	2,36	4,38	±	0,20	3,89	±	1,00	3,65	±	0,19	2,87	±	0,00
Styrene, 2,6-dimethyl	**MT**	5,16	±	0,10	4,81	±	0,25	4,88	±	0,07	2,34	±	3,31	3,69	±	0,21	4,83	±	0,10	4,25	±	0,11	4,36	±	0,00
2,4,6-Octatriene, 2,6-dimethyl	**MT**	0,00	±	0,00	0,00	±	0,00	0,06	±	0,04	0,00	±	0,00	0,00	±	0,00	0,08	±	0,04	0,09	±	0,06	0,00	±	0,00
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)	**MT**	0,06	±	0,00	0,10	±	0,01	0,09	±	0,01	0,08	±	0,01	0,05	±	0,00	0,09	±	0,01	0,09	±	0,01	0,09	±	0,00
Copaene	**SQ**	0,02	±	0,00	0,06	±	0,02	0,03	±	0,01	0,02	±	0,00	0,01	±	0,01	0,02	±	0,00	0,02	±	0,00	0,02	±	0,00
beta-Elemene, (-)	**SQ**	0,14	±	0,00	0,16	±	0,04	0,18	±	0,09	0,13	±	0,01	0,15	±	0,04	0,10	±	0,00	0,13	±	0,02	0,10	±	0,00
Caryophyllene	**SQ**	0,12	±	0,01	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,14	±	0,00	0,17	±	0,00	0,00	±	0,00	0,00	±	0,00
Alloaromadendrene	**SQ**	0,13	±	0,02	0,16	±	0,02	0,17	±	0,05	0,11	±	0,03	0,13	±	0,01	0,14	±	0,01	0,18	±	0,01	0,13	±	0,00
alpha-Caryophyllene	**SQ**	0,02	±	0,01	0,03	±	0,00	0,03	±	0,01	0,01	±	0,01	0,02	±	0,00	0,02	±	0,00	0,01	±	0,02	0,02	±	0,00
alpha-Gurjunene	**SQ**	0,03	±	0,01	0,06	±	0,01	0,04	±	0,00	0,02	±	0,00	0,06	±	0,01	0,06	±	0,01	0,04	±	0,01	0,03	±	0,00
beta-Selinene	**SQ**	0,16	±	0,03	0,23	±	0,03	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,21	±	0,01	0,00	±	0,00	0,00	±	0,00
Valencene	**SQ**	5,17	±	0,80	6,87	±	1,30	8,55	±	1,60	6,34	±	0,96	6,05	±	0,13	6,53	±	0,21	8,89	±	0,42	7,22	±	0,00
beta-Humulene	**SQ**	0,00	±	0,00	0,18	±	0,06	0,19	±	0,08	0,11	±	0,04	0,04	±	0,06	0,15	±	0,04	0,21	±	0,01	0,14	±	0,00
beta-Panasinsene	**SQ**	0,60	±	0,14	1,00	±	0,19	1,08	±	0,14	0,67	±	0,10	0,80	±	0,07	0,97	±	0,04	1,11	±	0,08	0,87	±	0,00
Selina-3,7(11)-diene	**SQ**	0,02	±	0,02	0,00	±	0,00	0,05	±	0,00	0,03	±	0,00	0,05	±	0,00	0,06	±	0,00	0,06	±	0,01	0,04	±	0,00
Eudalene	**SQ**	0,00	±	0,00	0,01	±	0,01	0,05	±	0,00	0,05	±	0,01	0,00	±	0,00	0,03	±	0,01	0,04	±	0,02	0,05	±	0,00
Styrene	**OTHER**	0,00	±	0,00	0,09	±	0,01	0,02	±	0,02	0,00	±	0,00	0,02	±	0,02	0,08	±	0,02	0,00	±	0,00	0,00	±	0,00
Benzene, 1,3-bis(1,1-dimethylethyl)	**OTHER**	0,01	±	0,00	0,02	±	0,01	0,00	±	0,00	0,00	±	0,00	0,00	±	0,00	0,01	±	0,00	0,00	±	0,00	0,00	±	0,00

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8. Scientific skills

8.1 List of paper and reports

- Characterization of Fruits of Four Different Lemon Cultivars, Collected in the Northern Coast of Sicily.

  Authors: Cupane M., Guarrasi V., Palazzolo E., San Biagio P.L. and Germanà M.A. Acta Horticolturae in press. Proceedings of International Citrus Congress – Valencia 2012.
- Contenuto in Polifenoli Totali del Succo di Tre Varietà di Limone (*Citrus limon* L. Burm) Coltivate in Sicilia.

  Authors: Cupane M., Guarrasi V., Germanà M.A. e San Biagio P.L. Meeting IBIM-STEBICEF – Palermo 2013.
- Electronic Nose to detect off-flavor of drinking water.

  Authors: Cupane M., Pelegrì -Sebastia J., Guarrasi V., Chilo J. and Sogorb T. SIBPA – Palermo 2014.
- Contenuto in polifenoli totali di tre varietà di limone (Citrus limon L.Burm) coltivate in Sicilia.

  Authors: Cupane M., Guarrasi V., Germanà M.A. and San Biagio P.L. Agricoltura, Cibo e Salute – Orto Botanico di Palermo, 2014.
- Postharvest life and aroma quality of four lemon cultivars grown in Sicily.

  Authors: Cupane M., Guarrasi V., Palazzolo E., San Biagio P.L. and Germanà M.A. Agricoltura, Cibo e Salute – Orto Botanico di Palermo, 2014.

8.2 Stage and Collaborations

- CRA – ACM. Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee. Acireale, Catania. Acquisition of techniques to evaluate the antioxidant potential of citrus fruits: Vitamin C content and ORAC assay.
- Univesitat Politecnica de Valencia, Dept. Enginyeria Electrònica. Institut d'Investigaciò per a la Gestiò Integrada de Zones Costaneres – IGIC. Escola Politècnica Superior de Gandia. Improvement of a new Multysensory Odor Olfactory System. Application on citrus quality control and water pollution.

8.3 Seminar participations

- Thermo Scientific – Palermo. Sicurezza alimentare e difesa dell'autenticità delle produzioni nazionali. Orto Botanico, Palermo, 2014
- Epigenetics and hormone dynamics during pollen embryogenesis in crop species and fruit tree. Dr. María-Carmen Risueño. Facoltà di Agraria, Palermo, maggio 2013.
- Dissecting pollen embryogenesis: a biotechnological tool for crop breeding, potentials and limitations. Dr. Pilar S. Testillano. Facoltà di Agraria, Palermo, maggio 2013.
- Citrus breeding and the problem of HLB in Florida. Prof. F.G. Gmitter Jr. Facoltà di Agraria, Palermo, 2013.
- On farm water management for fruit crops in Mediterranean area. Prof. Ahmed El-Araby. Facoltà di Agraria, Palermo, ottobre 2013.
- Evoluzioni metodologiche sull'incapsulamento dell'olivo. Dr. Maurizio Micheli. Facoltà di Agraria, Palermo, marzo 2014.
- Crioconservazione e crioterapia per la salvaguardia della biodiversità vegetale. Dr. M. Lambardi. Facoltà di Agraria, Palermo, aprile 2013.
- Le malattie del legno della vite e il complesso di funghi associate: sintomi, interazione ospite-patogeno, lotta e diagnosi. Prof. L. Mugnai. Facoltà di Agraria, Palermo, aprile 2013.
- La struttura delle argille e la sua influenza sui prodotti agricoli e alimentari. Prof.ssa S. Petit. Facoltà di Agraria, Palermo, aprile 2013.
- Fruit growth and root development relationships in loquat. Dr.ssa C. Reig. Facoltà di Agraria, Palermo, gennaio 2013.
- Do gibberellins regulate flowering in citrus?. Prof. M. Agustí. Facoltà di Agraria, Palermo, gennaio 2013.
- Aspetti nutrizionali e salutistici delle arance rosse. Dr. P. Rapisarda. Facoltà di Agraria, Palermo, dicembre 2012.
- Nutrizione degli agrumi in ambito biologico. Dr. G. Roccuzzo. Facoltà di Agraria, Palermo, dicembre 2012.
- Aspetti tecnici e fisiologici della nutrizione e della potatura. Dr. F. Intrigliolo. Facoltà di Agraria, Palermo, dicembre 2012.

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