DEPARTMENT OF AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCES

MASTER'S DEGREE COURSE IN FOOD AND BEVERAGE INNOVATION AND MANAGEMENT

COMPOSITIONAL QUALITY ANALYSES AND RESPONSE TO *BOTRYTIS* DISEASES OF DIFFERENT STRAWBERRY GENOTYPES

RESEARCH DISSERTATION

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Chapter 1
INTRODUCTION

The strawberry is an infructescence that belongs to the *Rosaceae* family, a perennial plant mistakenly considered to be of the herbaceous type.
Strawberry is one of the most cultivated and widespread fruits in the world and represents an important source of income for many countries, including Italy. According to data published by the Food and Agriculture Organization, the cultivation of strawberries worldwide covers 370,000 ha, with a production that exceeded 8.3 million tons in 2018 (Palmieri, 2020). Most of the production is concentrated in China and in the United States, which together account for about 50% of the total world production. Europe remains one of the main production areas, where most of the production is concentrated in Spain and Italy.
In Italy, in 2020 the cultivated areas to produce strawberries amounted to 3646 ha (in decrease of 4% compared to 2019), with an annual production of around 130,000 t. The Italian strawberry cultivation is mainly concentrated in the southern territories. Basilicata, Campania and Sicily represent about 50% of the total cultivated areas, while the complex of the 4 major northern regions slightly exceeds 22% (Palmieri, 2020).
Strawberry is a highly perishable fruit, characterized by a short shelf-life, which can be affected by numerous pathogenic species, such as fungi, bacteria, viruses and nematodes. The development of the disease can lead to the reduction in the commercial quality of the product, the development of numerous damages and, in the worst cases, even the death of the plant.
The most economically important pathogens of strawberry are fungi, and in particular the ascomycete *Botrytis cinerea* of the *Sclerotiniaceae* family, responsible for causing severe economic losses to the strawberry industry. To date, there is no variety that is immune or tolerant to the fungus. In the modern production, one of the most common management practices used for the prevention of postharvest rot is the use of fungicides, which are applied several times on the canopy of strawberry plants. However, the concern related to the presence of residues by the population, the growing resistance of the fungus and the legal restrictions related to the use of chemicals, have led to advanced studies to find new alternatives.

Numerous studies are evaluating the best less susceptible varieties on the market and experimenting new field instruments that can allow the identification of the disease directly in the field. Future research does not exclude the possibility of genetic modification and breeding techniques to produce commercial varieties characterized by a higher *B. cinerea* tolerance. Furthermore, the growing attention on qualitative and nutritional parameters, such as sugar content, acidity, and the content of bioactive substances, has led to an in-depth study of the varieties affected by the disease. Some studies were focused on the response to *botrytis* disease, on how the nutritional and qualitative content varies, to find the best and most resistant varieties, and to obtain high quality products.

The most used techniques to measure the parameters mentioned above can be destructive and non-destructive. The High-Performance Liquid Chromatography is a destructive method which therefore involves the loss of integrity of the fruit. However, is an easy-to-use tool that allows to investigate a wide range of substances, achieving complete separation of a mixture of compounds in a relatively short time.

1.1 **Historical traces and evolution of the hybrid *Fragaria x ananassa***

The strawberry is an infructescence that belongs to the *Rosaceae* family, the *Rosoidae* subfamily and the *Fragaria* genus.

Until the end of 1600, the cultivation of strawberries had not yet found an agronomic sense. The plants were used in the courtyards of the houses for decorative purposes, gardens, and flower beds, without any horticultural sense.

The first to devote himself to the study of strawberries with diligence and competence was a young Frenchman, a gardener at the court of the King of France, Antoine Nicolas Duchesne. Considered the progenitor of the modern strawberry farming, he was the first who followed and reported step by step the description of the whole genus *Fragaria*, with the general notions on the sexual characteristics of the flower, on flowering and fruiting, and the effects of the weather on the cultivation. His studies on the interspecific crossing between *F. chiloensis* and *F. moschata*, reported in the book *The natural history of the strawberry*, in which he describes each step in detail, allowed to lay the foundations for modern breeding techniques.

All the strawberries currently present on the market derive from the hybridization that happened by chance in 1766 of *F. virginiana*, coming from the eastern United States, with *F. chiloensis*, coming from the Chilean coast of the Pacific and imported into Europe for the first time by a French soldier in 1712, Amédée Francois Frézier, who collected and preserved the plants to bring them with him on his return home. The species obtained, called *Fragaria x ananassa*, was characterized by large fruits, whose seeds were able to produce plants of easy pollination and yield for a longer period. All the current cultivated varieties derive from this species.

1.2 **Genetic characteristics**

The numerous species belonging to the genus *Fragaria* differ in the number of chromosomes present in the nucleus of eukaryotic cells. Based on their ploidy level, the most common species are diploid, tetraploid, hexaploid, and octoploid.

Diploid species have chromosomes in the cell nucleus, each having two morphologically identical copies (2x=2n=14). Examples of diploid species are *F. vesca*, the most common type of wild strawberry, and *F. viridis*, a wild strawberry resistant to heat with a high commercial value.

Tetraploid species have chromosomes in the cell nucleus, each having four morphologically identical copies (4x=2n=28). Examples of tetraploid species are *F. orientalis* and *F. moupinensis*, both local species of low commercial value.

The hexaploid species have chromosomes in the cell nucleus, each having six morphologically identical copies (6x=2n=42). The only example of a hexaploid species is *F. moschata*, recognized for its heavy aromatic flavor and dark red fruits.
The octoploid species have chromosomes in the cell nucleus, each having eight morphologically identical copies (8x=2n=56). Examples of octoploid species are *F. virginiana* and *F. chiloensis*, from which the currently cultivated octoploid interspecific hybrid *Fragaria x ananassa* was created.

1.3 **Botanical aspects**

Strawberry is a perennial plant, mistakenly considered to be of the herbaceous type. Its structure can be divided into three different systems: the root, the stem or rhizome, and the leaf system (*figure 1*).
The root system allows the absorption and accumulation of reserve substances, as well as the absorption of water and the anchoring of the plant to the ground, necessary for the growth and the fruit production. The roots can reach depths of about 30 cm, depending on the type of soil in which the plants are grown. The collated-type system allows to distinguish the primary roots, originating directly from the crown, from the secondary roots, originating from the branching of the primaries. The latter have a period of activity between a few days and a few weeks and are constantly replaced.
The stem is a short and thickened rhizome, from which the leaves and the flower peduncles depart, up to 10 cm long. The main function performed by the rhizome is the accumulation of reserve substances, absorbed by the roots.
The leaf apparatus is composed of pinnate or palmate leaves inserted on petioles of variable length. The system allows the plant to capture the light and, through photosynthesis, to produce substances necessary for the growth and the maintenance of the plant. The buds are formed at the base of the leaves and based on the number of hours of daily light and the temperature values, they will be vegetative or reproductive. The vegetative buds give rise to stolon, the reproductive buds to inflorescences.
The flower can be hermaphroditic, called ‘perfect’, containing both male and female organs; or unisexual, containing only stamens (male organ) or pistils (female organ). The flower is typical of the *Rosaceae* family, consisting of a calyx with 5 sepals and a corolla composed of 5 white petals, and is carried on inflorescences in the primary, secondary, or tertiary axis, depending on the chronological order of formation. Generally, the inflorescences formed in the primary axes are the first to be formed and lead to the production of a high number of flowers and fruits.

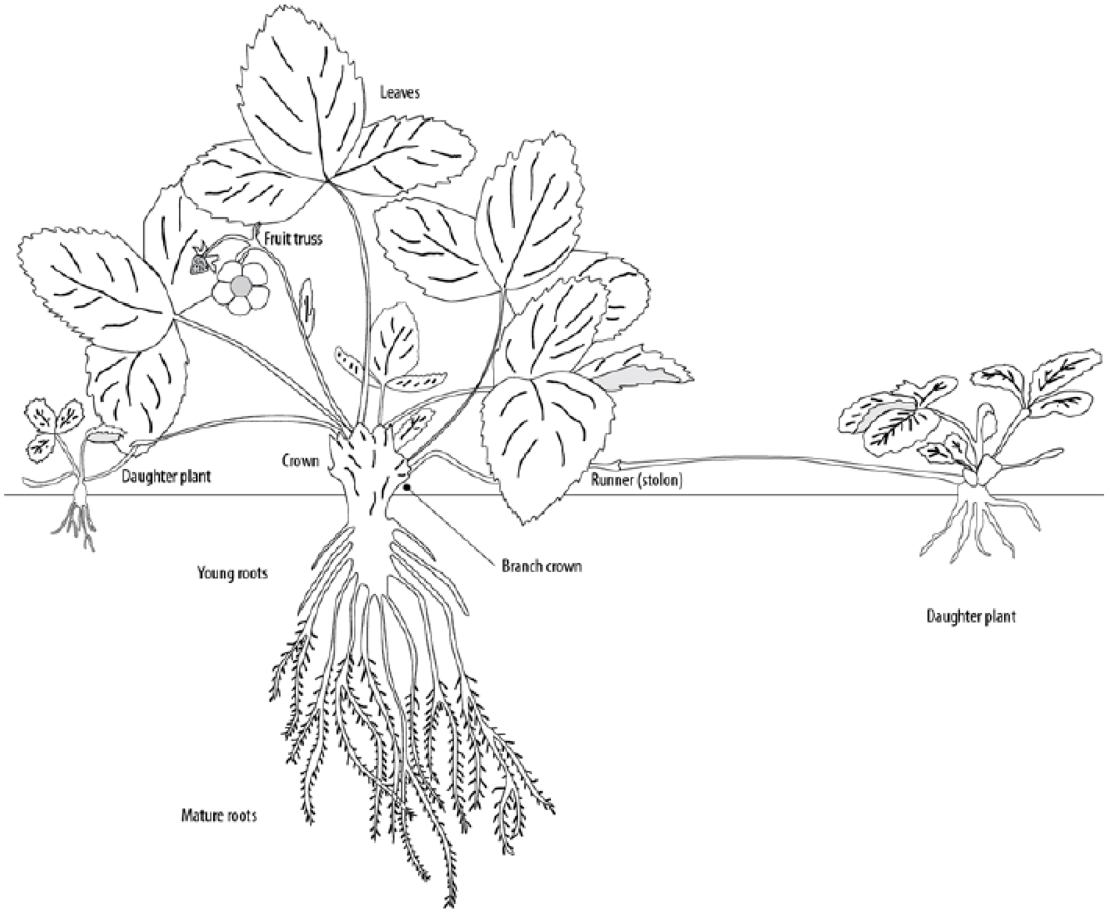


Figure 1: Morphology of a typical strawberry plant. Credits: www.researchgate.net.

The edible fruit is a false fruit, obtained from the enlargement of the receptacle, with many seeds imbedded in the surface. The numerous seeds, called achenes, present on the external surface in a variable number from 100 to 300, constitute the true fruit. The achenes can have a color that varies from light to dark brown, protruding from the receptacle.

Both the color and the weight of the fruit are characteristic for the different cultivars, and therefore extremely variable. The weight can vary from 10 to 100 grams, depending on whether the species is wild or domesticated; the color can vary from light orange to red-orange or deep red.

The size of the fruit can be very small, small, medium, large or very large; the fruit can have a kidney shape, spheroidal, conical-rounded, conical, conical-elongated, biconical, almost cylindrical, wedge-shaped, ovoid shape.

To obtain fruits of regular shape and size, it is necessary that all the pistils are fertilized. When conditions are not favorable for pollination, this leads to a reduced number of pollinated pistils, and consequently to malformed and deformed fruit, that do not meet the minimum quality criteria required by the market.

1.4 **Cultivar classification**

The cultivars, based on their ability to produce fruits during the year, are generally classified as:

- Short day cultivars/Floricanes: the plants bloom only once a year (in spring), following the differentiation of the flower buds in autumn. The differentiation of the flower needs a daily light length of less than 12 hours and temperatures below 15 ° C. The plant is planted in the summer, to enter the vegetative rest in the winter. The flowering of the buds occurs in spring. They represent 70% of the market because they are more productive, and the quality is higher.
- Day neutral cultivars/Remontant: the plants differentiate flower buds with various lighting conditions and differentiate the gems regardless of the length of the day. The crops belonging to this category are commercially important, as they can guarantee a continuous production and can be grown in tropical and sub-tropical area, where there is no winter, because the plant does not need to enter vegetative rest. The main limiting factor is represented by the low yield capacity and the short shelf-life.
- Long day cultivars/Remontant: For the differentiation of the flower, they require a daily light period longer than 14 hours, with the characteristic temperatures of temperate climates. The plants differentiate in summer and bear fruit from spring to autumn. They have a good spread at industrial scale, but they are used almost exclusively at familiar level for their slow reproduction.

1.5 **Global and Italian production**

Strawberry is one of the most cultivated and widespread fruits in the world and represents an important source of income for many countries, including Italy.

According to data published by the Food and Agriculture Organization, the cultivation of strawberries worldwide covers 370,000 ha, with a production that exceeded 8.3 million tons in 2018 (Palmieri, 2020).

Most of the production is concentrated in China and in the United States, which together account for about 50% of the total world production. Among the major world producers in the last decade, the greatest increase was recorded in Mexico, Turkey, and Egypt, now among the top 5 producers in the world.

Europe remains one of the main production areas, with approximately 105,000 ha currently cultivated. In Europe, the largest producer is Spain, followed by Italy.

According to the data published by the Centro Servizi Ortifrutticoli (CSO), an aggregation that associates many Italian companies to make the fruit and vegetable supply chain more efficient, in 2020 the cultivated areas for the production of strawberries amounted to 3646 ha (in decrease of 4% compared to 2019), with an annual production of around 130,000 t. In the totality of Italian production, 83% of the surface is invested in protected crops, while only 17% is used for open field cultivation.

The Italian strawberry cultivation is mainly concentrated in the southern territories. Basilicata, Campania and Sicily represent about 50% of the total cultivated areas, while the complex of the 4 major northern regions slightly exceeds 22% (Palmieri, 2020).

In the southern areas the production is finalized in the winter and spring periods, where 80% of the investments are dedicated to the Sabrosa, Sabrina, Melissa and Florida fortuna genotypes, and is mainly managed by medium-large or large companies.

In the northern areas the production is mainly concentrated in Veneto and Emilia-Romagna, where is finalized in the autumn and spring period; while, in Piemonte and Trentino-Alto Adige is finalized in the summer period. The production framework has a lower degree of specialization than the southern area, with numerous varieties produced, and is managed by small or small-medium companies.

The Italian territory is characterized by a very diversified environment, from the cold climate of the northern areas to the temperate climate of the southern areas. These parameters allow producers to diversify their commercial production, responding to market demand and covering almost the entire calendar year, not only in certain months of the year (from April to June), but distributed throughout seasons, avoiding accumulations or excessive concentrations of the product.

1.6 **Farming techniques**

The cultivation of plants through the use of nurseries has been established in Italy since the middle of the 60's, involving numerous cultivation techniques or strategies applicable to different varieties of strawberry plants.

Among the categories most used in the strawberry sector, we can distinguish frigo plants (A or A+) and fresh plants (bare root or rooted top).

The frigo plants are typical of the central-northern areas. They are cultivated in specialized nurseries, uprooted during the winter (from December to January), cleaned from the leaves, and placed in cold rooms at a temperature of 0/-2 ° C. During this period, which varies from 6 to 9 months, the plants are in the dormant phase and if necessary, they can be thawed and placed on

the market. The type of frigo plants is the most widespread in traditional crops, with a high vigor, large size and early production. According to the diameter at the base of the crown and the length of the roots, the frigo plants are in divided into plants of category A (8-12 mm; 10 cm) and A + (12-15 mm; 12 cm).
Category A plants are widespread in the northern regions of Italy. The plants are cultivated in specialized nurseries during the spring period, in sandy and well-drained soils, and mechanically uprooted in the winter, during the vegetative rest phase. They are removed from the nursery to be grouped in bunches inside boxes (600-700 plants), which are stored in cold rooms at a temperature of -2 ° C, before being sold.
Category A+ plants differ in their higher dimensions, favored by their homogeneous distribution within the nursery. They are refrigerated and used in the plants for summer and autumn productions.
The fresh bare root plants are typical of the southern areas. They are plants grown in sandy soils and are obtained in nurseries located in areas characterized by very early autumn colds, essential before their transfer to the southern strawberry groves in October. The plants can produce already after 2-3 months from when they were planted, with a prolonged production over time.
Fresh plants with rooted tops can be used as an alternative to frigo plants, to obtain early productions. The plants are placed to root inside polystyrene containers, in a controlled environment. After rooting, which takes about 30 days, the plants are transported to the southern territories during the autumn, to be transplanted.
Generally, fresh plants are characterized by a greater earliness of ripening, greater stress tolerance, lower production costs and higher quality of the fruits, which makes them advantageous compared to the frigo plants. The limiting factors linked to the use of this category of plants is the high percentage of losses that can occur during rooting, or the use of underdeveloped plants obtained from nurseries, due to an excessive plantation density.
The choice of the category of plants to be used is necessary to guarantee a good production. The selection of the producer in the pre-implantation phase is fundamental and must guarantee a plant suitable and resistant to the surrounding environment, certified, and which maintains the qualitative characteristics required by the market.
In recent years, the Italian territory has witnessed an evolution of cultivation techniques and nursery material, with a consequent change among the various regions. In southern environments a traditional cultivation protected with multiple tunnels is used, by planting the field between September and October, and harvesting in December, until May.

In the central-northern areas both traditional open field cultivation and protected cultivation with single or multiple tunnels are used, by planting the field between July and August, and harvesting in the following spring. In the Marche region, traditional open field cultivation is mostly used.

1.7 **Breeding and genetic improvement**

In recent years, the strawberry has been subjected to numerous studies and breeding activities, aimed at genetic improvement towards the search for resistance to the most widespread plant diseases, with the aim of obtaining interesting and improved selections and varieties, which can be exploited commercially.

Until now, the main focus of strawberry breeding programs has been geared towards the improvement the commercial and agronomic traits (Diamanti, et al., 2012). However, recently, strawberries received increasing attention thanks to the growing interest of consumers in beneficial products, both from a health and a nutritional point of view. Many programs aim to select new strawberry cultivars with fruits with high concentrations of antioxidant compounds. Furthermore, the variety of existing breeding programs around the world has led to the development of strawberry varieties that adapt to new market needs, farmers' demand, and sustainable farming practices.

The market has driven breeders to produce cultivars with high nutritional and sensorial traits even if, generally, the correlation between high production and high-quality fruit is difficult to obtain, and sometimes this challenge cause inefficiency in breeding programs.

The breeding program of the Department of Agricultural, Food and Environmental Sciences of the Università Politecnica delle Marche (UNIVPM) begun in 1993 with the aim of creating new cultivars, with an improved fruit quality, high adaptability to the cultivation locations, that are resistance to soil bound diseases and late ripening. The strawberry genetic improvement program in the Marche region is coordinated by Prof. Bruno Mezzetti, thanks to the contribution of the national project "Fruit growing", funded by the Ministry of Agriculture and Forestry. The program started by Prof. Pasquale Rosati at the Experimental Didactic Agricultural Company "P. Rosati "of Agugliano, obtained two late ripening varieties licensed in 2003, Adria and Sveva and in 2011 other two varieties, the first early ripening and the other late ripening, namely Romina and Cristina, suitable for cold temperate climates. Furthermore, in 2019, the same genetic improvement program led to the registration of 4 other cultivars: Dina, suitable for warm temperate climates, and Francesca (early ripening), Lauretta (intermediate ripening), and Silvia (late ripening), suitable for cold temperate climates.

1.8 ***Botrytis cinerea* and strawberry**

Strawberry is a highly perishable fruit, characterized by a short shelf-life, which can be affected by numerous pathogenic species, such as fungi, bacteria, viruses, and nematodes.
The development of the disease can lead to a reduction in the commercial quality of the product, the development of numerous damages and, in the worst cases, even the death of the plant.
The most economically important pathogens of strawberry are fungi, and in particular the Ascomycete *Botrytis cinerea* of the *Sclerotiniaceae* family, one of the main pathogens responsible to cause severe economic losses to the strawberry industry.
Botrytis cinerea is polyphagous, it has no apparent host specificity, and can infect more than 1000 plant species (Elad, et al., 2016), being the most impacting disease of numerous fruit and vegetable.
The inoculum can originate from dead leaves, flowers and mummified fruits that contain masses of mycelium, which grows as a saprophyte in plant material; or from spores released by the fungus, which are deposited directly on the plant.
The infection can occur both in the field and in the post-harvest period through flowers, natural openings, or mechanical wounds, present in the surface of the fruit. The infections can begin when the flowers bloom, or can be latent, responsible for rot developed near the harvest or in the post-harvest period. Often, *B. cinerea* can develop from rotten fruit next to the healthy ones by direct contact, causing extensive breakdown of the commodity, spoiling entire lots.
The first symptoms in the plants are manifested by the formation of darker and circular areas on the surface of the fruit. Subsequently, the area of interest becomes softer and eventually abundant sporification is formed, characterized by the formation of a velvety mold on the entire surface of the fruit.
The development of the fungus occurs at a temperature between 20 and 25°C, with a relative humidity greater than 90%, necessary for the germination of the conidia. The environmental factors that favor the development are stagnant air, canopy irrigation, moisture condensed in greenhouses, excessive nitrogen fertilization, high planting density, strawberry-soil contact and the presence of wounds, injuries or abrasions.
Gray mold is one of the most serious fungal disease that threaten the Italian strawberry cultivation. To date, there is no variety that is immune or tolerant to the fungus and, for this reason, it is necessary to implement prevention work that starts directly from the field, where it is possible to act on the climatic and logistical aspects, such as for example the removal of senescent plant material or selecting the right irrigation system, that do not allow the fungus to spread.

In the modern production, one of the most common management practices used for the prevention of postharvest rot is the use of fungicides, which are applied several times on the canopy of strawberry plants. However, the concern related to the presence of residues by the population, the growing resistance of the fungus and the legal restrictions related to the use of chemicals, have led to advanced studies to find new alternatives. The use of ecological treatments and breeding activity are some examples that aim at a reduction in the use of fungicides and an increase in plant tolerance to the fungus. Numerous studies are evaluating the best less susceptible varieties on the market and experimenting new field instruments that can allow the identification of the disease directly in the field.

Future research does not exclude the possibility of genetic modification and breeding techniques to produce commercial varieties characterized by a higher *B. cinerea* tolerance, to achieve the maximum control of the disease.

1.9 **The quality of strawberry fruit**

The concept of food quality is complex and difficult to define, as it is influenced by contextual factors, different measurement indices and varies according to the opinion of the designated individual. In the past, the quality of fruits was associated with the absence of defects, while today the individual perceives the quality of food based on the organoleptic and nutritional properties and the possible benefits that the consumption of the food can exert.

The perception of the quality of a product varies according to the subject involved: the quality perceived by consumers differs from the one perceived by manufacturers.

From the manufacturer's point of view, a quality product is associated with the efficiency, the productivity, and the resistance of the cultivar, together with the appearance and shelf-life of the final product. The concept of quality also varies according to the final destination of the product: fresh consumption or industrial process. Strawberries intended for fresh consumption must have a regular size and shape, with a bright red color of the epidermis and a good consistency that allows the product to be preserved longer, avoiding the development of rot.

From the consumer's point of view, the concept of food quality involves visual, olfactory, tactile, and organoleptic stimuli, together with the expectations regarding the possible beneficial effects that the consumption of the product can bring to human health, thanks to the nutraceutical substances it contains.

The color is generally the first characteristic that the consumer perceives, which makes the product attractive, and allows to appreciate the state of freshness of strawberries. Generally, the skin tones of bright red color and a green and turgid calyx are appreciated.

Concerning the shape and the size of strawberries, varieties of conical, regular, slightly elongated shape and medium-large size (25-30 g), of high consistency (600-800g), are preferred.
The appreciation of the refractometric dry residue and acidity of strawberries is influenced by the eating habits of the different geographical areas. However, a balanced acid/sugar ratio positively characterize the flavor of the fruit and makes them more appreciated by very large groups of consumers.
The aroma of strawberries is determined by the presence of volatile substances, which reach the olfactory cells by inhaling or chewing the product. It consists of numerous compounds included in the chemical categories of esters, aldehydes, alcohols, funar and sulfur compounds. The sweet and fruity aromatic notes imparted by the methyl and ethyl esters of acetate, furanone and linalool, make strawberries one of the most requested fruits in the market.
Consumer health expectations are linked to the intrinsic characteristics of the product, such as the presence of bioactive compounds, with a nutraceutical effect. Many epidemiologic studies have shown that a diet rich in fruits and vegetables is often associated with a lower incidence of several chronic pathologies, including obesity, infections, cancer and cardiovascular and neurologic diseases. Berries, including strawberries, have an important role among fruits because of their high phytochemical content (Giampieri, et al., 2012). Numerous scientific studies confirm that the strawberry contains bioactive molecules with antioxidant power, such as ascorbic acid, polyphenolic compounds such as ellagic acid, ferulic acid and some flavonoids (anthocyanins, catechins, phenolic acids etc.). These compounds exhibit a nutraceutical effect, exerting beneficial and protective properties on the human body.

1.10 The nutritional characteristics of strawberry fruit

The strawberry is a fruit characterized by a high content in water (about 91%), a high content in dietary fiber (2.0g/100g), soluble carbohydrates (fructose content 2.44g/100 in fresh matter), vitamins and minerals. The phytochemical compounds present determine its nutritional profile, by giving it different nutraceutical properties and making it a highly recommended healthy choice. The high-water content makes the product susceptible and perishable, characterized by a limited shelf-life. Moreover, its dietary fiber and fructose contents may contribute in regulating blood sugar levels by slowing digestion, with its fiber content also contributing to control calorie intake by its satiating effect (Giampieri, et al., 2012).
The fruit is an important source of vitamin supply for humans. The high content of vitamin C (58.8 mg/100g), capable of fulfilling the recommended daily allowance, makes the strawberry a food with high antioxidant power. Furthermore, 250 g of strawberries contain 60µg of folate on

average, which can supply 30% of the daily European and U.S. folate recommended daily allowances (Giampieri, et al., 2012). To a lesser extent, strawberry is a source of other vitamins such as thiamin, riboflavin, niacin, vitamin B6, vitamin K, vitamin A and vitamin E; and is also rich in calcium (16 mg/100 g), potassium (153 mg/100 g), phosphorus (24 mg/100 g) and magnesium (13 mg/100 g). Sodium, iron, zinc and selenium are also present in significant quantities.

The total antioxidant capacity (TAC) is a common parameter used for determining nutritional quality, as it is directly related to the quantity of bioactive compounds in the fruits. The strawberry is a fruit with a high total antioxidant capacity, with levels up to 4 times greater than other fruits, 10 times greater than vegetables, and 40 times greater than cereals (Halvorsen, et al., 2002).

The activity is not only associated with the high content of vitamin C but depends also on the presence of bioactive substances represented by phenolic compounds, such as anthocyanins (Pelargonidin-3-glucoside and cyanidin-3-glucoside), hydrolysable tannins (ellagitannins and gallotannins), phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids) and carotenoids. The bioactive compounds present in combined action, are responsible for contrasting the activity of reactive oxygen species, counteracting damages, or the development of severe diseases, caused by oxidative stress.

1.10.1 *Vitamin C*

Vitamins are organic compounds of different chemical nature, needed in small amounts for the development of cellular metabolism, and therefore indispensable for the growth and maintenance of vital functions. They are not bio-synthesized by the human organism, and for this reason they are introduced from sources as food into the body. Vitamins are generally classified according to their solubility, namely fat soluble and water-soluble vitamins. Liposoluble vitamins are stored in fat tissue, especially in the liver, while water-soluble vitamins do not accumulate in the body, but are eliminated through urine and sweating.

Water soluble vitamins are composed of B vitamins (B1, B2, B3, B5, B6, B7, B9, B12) and vitamin C. Many of them play important roles in human metabolism, some of them act as coenzyme taking part to biochemical reactions, and others protect components from oxidation, thanks to their antioxidant capacity.

Vitamins are essential, which means that in the event of a shortage in the human body system, they can lead to specific states of deficiency (pellagra, scurvy, anemia etc.).

Vitamin C (*figure 2*), also known as L-ascorbic acid, is a water-soluble essential vitamin, naturally occurring organic compound, which exerts antioxidant properties.

The vitamin C content is documented in many plant species, cell compartments and tissues. Fruits and vegetables represent the major sources of vitamin C, such as tomato, red and green peppers, broccoli, melon, and strawberry. In plants, L-ascorbic acid is responsible for three main functions: enzyme cofactor, radical scavenger, and a donor/acceptor in electron transport, either in the plasma membrane or in the chloroplasts, besides other minor functions (Davey, et al., 2000).
The biosynthesis of vitamin C in plants depends on various factors and can vary between organs. Considering the variety of functions that ascorbate exerts in plant cells and its tight regulation in green tissues, it is remarkable how variable the content of ascorbate can be among the fruits of different species, or between varieties from the same species (Davey, et al., 2000). There are both pre- and post-harvest factors that can affect the content of ascorbic acid in plants, and they include the environmental conditions during cultivation, such as temperatures, quality of light and the number of hours of light per day; but also, cultural practices and handling conditions, such as pruning, cutting, washing, use of fertilizers or irrigation procedures.

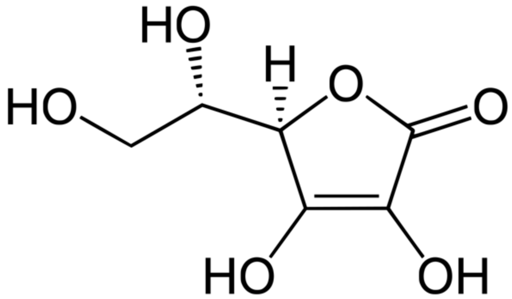


Figure 2: Chemical formula of vitamin C. Credits: www.wikipedia.org.

Vitamin C plays a vital role for human health. Its supplementation has been shown to reduce levels of oxidative stress, thereby reducing potential damage to tissue (Schlueter, et al., 2010). It acts as a powerful antioxidant able to boost the immune system, and to scavenge reactive oxygen and nitrogen species produced by cellular and environmental stresses, that cause oxidative damage to cells, lipids, and nucleic acids. It also plays biological role in the body, such as the synthesis of bile acids, carnitine, and steroid hormones.
In humans, vitamin C is absorbed in the small intestine and partly stored in the liver, while the excess is eliminated through urine and sweat.
The recommended daily intake is 90 mg/day in adults and 120 mg/day in smokers, which ensure antioxidant effect and enhancement of the immune system. Excessive doses of vitamin C (500 mg/day) can lead to gastrointestinal disorders, formation of kidney stones and pro-oxidizing effect, while insufficient doses can lead to the development of deficiencies states.
Many disease states have been studied in relationship to vitamin C, including cardiovascular diseases, cancer, and diabetes, even if no link has been established yet. Human beings need

vitamin C to ensure the collagen and neurotransmitter (serotonin and norepinephrine) biosynthesis, and to enhance the bioavailability of iron. Mild vitamin C deficiencies can lead to fatigue, asthenia, loss of appetite and insomnia; severe deficiencies can lead to scurvy and, eventually, death.

Great interest has developed in strawberries because of their extremely high content of vitamin C, which makes them an important source of this vitamin for human nutrition (Giampieri, et al., 2012). Strawberries contain on average 58.8 mg/100 g of fresh weight , making them one of the main sources able to satisfy in small handfuls the vitamin C daily recommended intake.

1.10.2 *Polyphenolic compounds*

Besides the nutritive compounds, strawberries contain also a variety of phytochemicals, mainly represented by the class of polyphenolic compounds, which contribute to the TAC of strawberry fruits.

Polyphenols are a diverse group of plant substances that contain one or more benzene ring and varying number of hydroxy, carbonyl and carboxylic groups. They are secondary metabolites produced by the plant as defensive system, in response to stresses of different nature (biological, physical, or chemical), and they are responsible for many physiochemical properties.

As non-essential metabolites, they play an important role for defense against UV radiation or aggression by pathogens, they are responsible for plant colouration (yellow, red, blue, and purple pigments), taste (bitterness and astringency), and they are extremely potent odorants.

Polyphenols are present in all plants with a high variability, and fruits and vegetables are the main sources. There are many factors that can influence the polyphenols content in fruits, both in the pre- and post-harvest stages, such as the environmental conditions at the time of cultivation, the ripeness and handling conditions at the time of harvest, the storage, and possible further industrial food processing.

Polyphenols can be divided in 3 main classes, distinguish in further sub-classes, as described in *Figure 3*. The major class of phenolic compounds is represented by the flavonoids (mainly anthocyanins, with flavonols and flavanols providing a minor contribution), followed by hydrolyzable tannins (ellagitannins and gallotannins) and phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), with condensed tannins (proanthocyanidins) being the minor constituents (Kähkönen, et al., 2001; Määttä-Riihinen, et al., 2004; Aaby, et al., 2005). Anthocyanins and phenolic acids were part of the analysis of this work and are described in detail below.

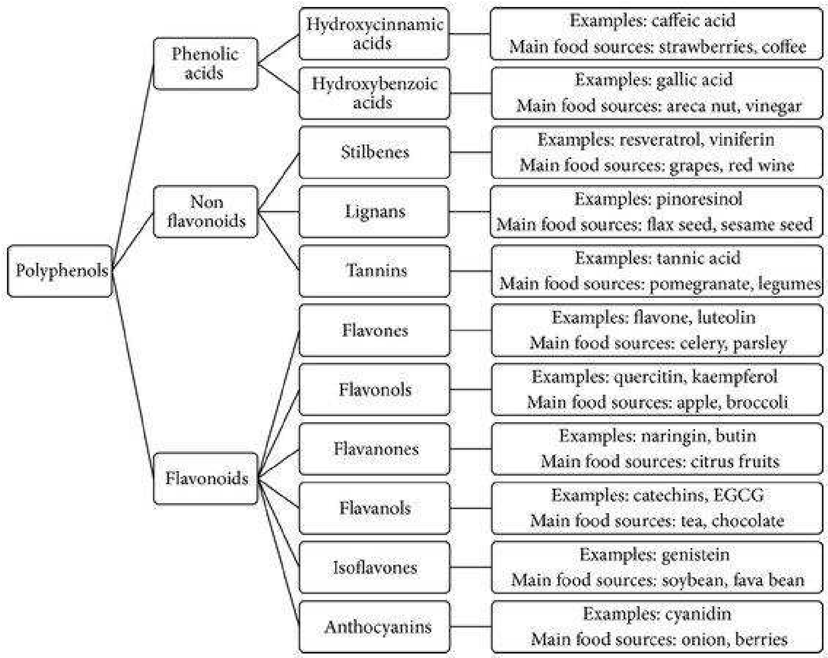


Figure 3: Classification of polyphenols. Credits: www.researchgate.net.

Anthocyanins

The strawberry is a rich source of anthocyanins, a subclass of flavonoids that represent the biggest class of polyphenols. They are small component highly soluble in water, which consist of two aromatic C6 rings, bound together by three carbon atoms, that form an oxygenated heterocycle.

Anthocyanins are the most important group of water-soluble pigment in plants. These pigments are responsible for the red, blue, orange, and purple coloration of flowers and fruits of the plants, which allow to attract animals as pollinators or seed disperser. They also play a vital role in protecting plants from ultra-violet light damages or aggression by pathogens and insects.

Plants' anthocyanin synthesis is dependent on several factors, among those genetics, soil composition, ultraviolet radiation, and stress such as pathogenic attack or cold temperatures (Chalker-Scott, 1999).

Anthocyanins play a significant role for human health. They act as powerful antioxidants, able to boost the immune system and to prevent cancer, diabetes, neuronal and cardiovascular illnesses (Konczak, et al., 2004); they are free radical scavengers, so that foods with a high anthocyanin content have a high antioxidant potential.

More than 25 different anthocyanin pigments have been described in strawberries of different varieties and selections (Lopes de Silva, et al., 2007). Pelargonidin-3-glucoside (*Figure 4*) is the major anthocyanin in strawberries, independent from genetic and environmental factors, together with cyanidin-3-glucoside, which seems to be constant in strawberries, although only in smaller proportions (Bridle, et al., 1997; Hong, et al., 1990; Lukton, et al., 1955). Studies have determined the total anthocyanin content in strawberries, reporting values from 150 to 600 mg/kg of fresh weight (Clifford, 2000; Lopes-da-Silva, et al., 2002).

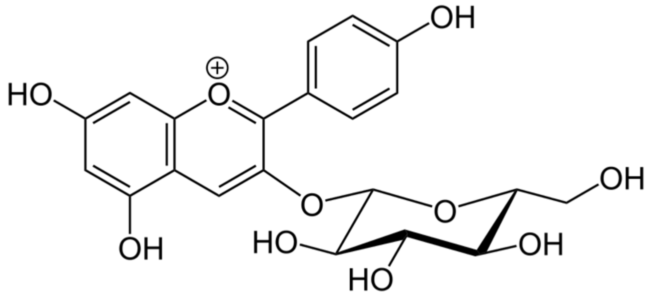


Figure 4: Structural formula of pelargonidin-3-glucoside. Credits: www.wikipedia.org.

<u>Phenolic acids</u>

The strawberry is rich in phenolic acids, one of the three main classes of phenolic compounds. They are aromatic secondary plant metabolites that can be distinguished, based on their structure, in derivatives of benzoic acid and derivatives of cinnamic acids. These groups consist in a benzene ring as a basis, bond to a carboxylic group (benzoic acid) or to a propenoic acid (cinnamic acids), as shown in *figure 5*.

In strawberries it is possible to find both derivatives from hydroxycinnamic acid (such as caffeic, chlorogenic, ferulic, and coumaric acids), and from hydroxybenzoic acid (such as gallic acid or ellagic acid). In particular, the *p*-coumaric acid content varies between 2.9 and 4.9 mg/100 g of fresh fruit, caffeic acid between 0.139 and 0.42 mg/100 g of fresh fruit, ferulic acid between 0.20 and 0.25 mg/100 g of fresh fruit, p-hydroxybenzoic acid between 4.4 and 6.3 mg/100 g of fresh fruit and gallic acid between 2.1 and 5.3 mg/100 g of fresh fruit (Mattila, et al., 2004). Strawberries also contain a relevant quantity of ellagic acid, a gallic dimer rarely present in its free form, known to play an important role as antimicrobial compound against human pathogens. More often, ellagic acids are occurring bound in ellagitannins, which belong to the phenolic groups of hydrolysable tannins. Gallic acid is, together with ellagic acid, the basic monomer of hydrolyzable tannins, that, together with anthocyanins, are the most abundant phenolic compounds in strawberries.

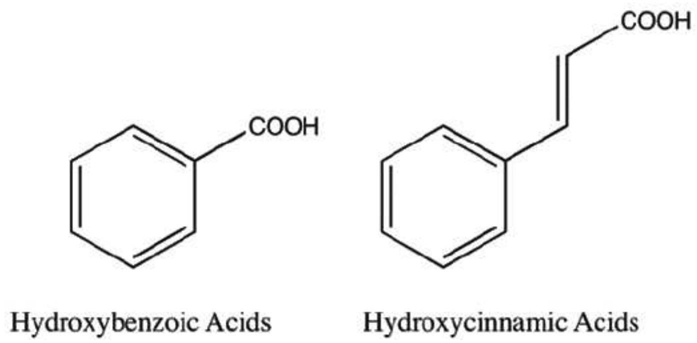


Figure 5: Basic structures of phenolic acids. Credits: www.researchgate.net

Among the variety of phenolic compounds, phenolic acids have attracted considerable interest in the past few years due to their many potential health benefits. They are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anti-inflammatory and vasodilatory activities (Duthie, et al., 2000; Breinholt, 1999; Shahidi, et al., 1995). Their biological actions are ascribed to the peculiar antioxidant and anti-inflammatory properties, but they also possess anti-mutagenic, anti-carcinogenic and anti-allergy activities ((Koponen, et al., 2007; Giampieri, et al., 2012; Giampieri, et al., 2013). More epidemiological and clinical studies should be performed, to fully clarify their beneficial effects on human health.

1.11 **Factors that influence strawberries quality**

The qualitative and nutritional characteristics of strawberries are influenced by a multitude of genetic, environmental, and agronomic factors. The genetic background is the main factor that determines the potential yield and the quality of the fruit, while the full capacity is determined by the interaction with the environmental conditions and the cultivation systems adopted (Di Vittori, et al., 2018). The interaction between pre-, post-harvest and genetic factors, determine the final quality of the product.

The genetic background is the first factor defining the capacity of a fruit to accumulate bioactive compounds (Di Vittori, et al., 2018). Studies conducted on seedlings obtained from combinations of crosses between genotypes, characterized by different total polyphenols content (TPC), ascorbic acid content (AA) and total antioxidant capacity (TAC), have shown that by arranging suitable crosses between parents with fruits of high nutritional characteristics, it is possible to increase the quantity contained in the fruits (Battino, et al., 2004; Capocasa, et al., 2008). In particular, it has been shown that using *Fragaria virginiana glauca* genotypes in the cross selection increases the TAC of strawberry fruits (Mezzetti, et al., 2005).

Nutritional quality is also influenced by environmental factors, including exposure to light and radiation, harvest season, growing location, cultivation temperatures and techniques.

The nutritional quality and yield of strawberries can be influenced by the cultivation location. A study conducted on strawberries grown in southern European environments, has shown how cultivating the same species in the north can lead to production of fruit with higher commercial and organoleptic traits, such as increased dry matter, total acidity, and soluble solids content (Krüger, et al., 2012).

The climatic conditions that occur in the growing area are another fundamental parameter that affects the aroma of strawberries. The content of total soluble solids is more influenced by the environmental conditions, while the acidity is more linked to the genetic characteristics of the variety. High temperature values, around 30/22 °C, in the period following flowering until harvest, result in poor contents of soluble solids and acidity of the fruits, while temperature values ≤ 25 ° C, but with an excursion temperature between day and night equal to 7 ÷ 8 ° C, favor the increase of both parameters (Wang, et al., 2000).

Studies on strawberries demonstrated how the exposure to sunlight, or shading, significantly affect the content of flavonoid compounds. Shading slightly decreased the anthocyanin content, while in fruits grown at 100% light level the content was 9% higher (Anttonen, et al., 2006). The quality of light and temperature are environmental factors that vary with the season and that can greatly influence the quality of strawberries.

Fertilization can significantly influence the phytochemical content, the yield and the dimension of strawberries. The data presented in the study of Anttonen et al. (2006) shows that increased fertilization in strawberries reduced the content of flavonols and ellagic acid. Recently, some authors demonstrated that a reduction in iron and phosphorus intake increases the quality of the strawberry (Valentinuzzi, et al., 2015). Iron deficiency unbalances the micronutrient content (Pestana, et al., 2012), causing an accumulation of zinc and copper in strawberry fruits, total phenols and anthocyanin content, without affecting sweetness, acidity and compactness of fruit (Valentinuzzi, et al., 2015).

The size of the fruit varies according to the genotype, the position occupied in the inflorescence (the fruits with the highest size are obtained from the primary axis), and according to the population density in the field.

The consistency of the fruit is a character that tends to decrease in adverse weather conditions, characterized by rainy pre-harvest periods, while it increases in periods of low light and low temperatures.

1.12 **Main methods of qualitative analysis for strawberries**

The qualitative characteristics of the fruit can be determined by means of destructive and non-destructive analysis.

Destructive analyzes guarantee clear and reliable results, even if they have limitations both in terms of quantity of sample that can be analyzed, and in terms of time required for the analysis. Since these are destructive methodologies, it is possible to apply them only to a certain representative percentage of fruits. Furthermore, the examination will take longer than the non-destructive methods. Some examples for destructive analyses are:

- Penetrometer: provides an index for determining the most appropriate period for fruit harvesting, measuring the hardness of the fruit pulp (kg, g, N);
- Refractometer: measures the refractive index of a sample. A few microliters of fruit juice are needed to measure the refractometric dry residue (° Brix);
- Titratable acidity: allows the titration of the organic acids present in the fruit, from a small quantity of fruit juice;
- High Performance Liquid Chromatography: allows to separate and identify two or more compounds present in the sample to be analyzed.

To deal with destructive analyses problems and analyze the quality parameters of the strawberry, non-destructive techniques can be used. Non-destructive analyzes are much faster than destructive ones and can be performed on all samples, not just on a representative percentage of the fruit. This makes them statistically more reliable. Examples of non-destructive tools are:

- Colorimeter: measures the absorption of particular wavelengths of light by the fruit;
- Caliber and balance: allow to detect the size of the fruit in terms of its diameter and weight.

Over the years, new non-destructive techniques have been developed that are increasingly successful as qualitative analysis methods, such as electromagnetic technologies: NIR, fluorescence and X and γ rays; and electrochemical technologies such as the electronic nose. All these methods of analysis make it possible to identify one or more parameters without damaging the integrity of the fruit.

1.12.1 *High performance liquid chromatography (HPLC)*

Chromatography is a chemical-physical process that allows the constituents of a mixture to be separated, purified, and quantified, by exploiting the balance between two immiscible phases: a stationary phase, placed inside the chromatographic column, and a mobile phase, which flows through it.

The stationary phase is a supported or chemically bonded liquid on a solid inside a steel column, in which the solute binds. It is typically a granular material consisting of solid particles (for example silica, polymers, etc.), with a size of 2-50 micrometers.
The mobile phase is represented by pure solvents, or mixtures of several solvents (for example water, acetonitrile, or methanol) that, with the sample, pass through the column pushed by great pressures. Its composition and temperature plays an important role in the separation process, by influencing the interactions that occur between the sample and sorbent components.
The instrument includes a sampler, pumps, a detector, and a digital microprocessor, as shown in *figure 6*.
The sampler carries the sample mixture into the mobile phase stream, which carries it into the column.
The pumps deliver the desired flow rate and composition of the mobile phase through the column.
The detector is a calculator that allows a continuous analysis of the column output and the identification of the injected substances. It generates a signal proportional to the quantity of sample component emerging from the column, allowing a quantitative analysis. Various detectors are in common use, such as absorbance, fluorescence, and mass spectrometer detectors.
Finally, a digital microprocessor and user software allows to control the HPLC instrument, by programming and providing data analysis.

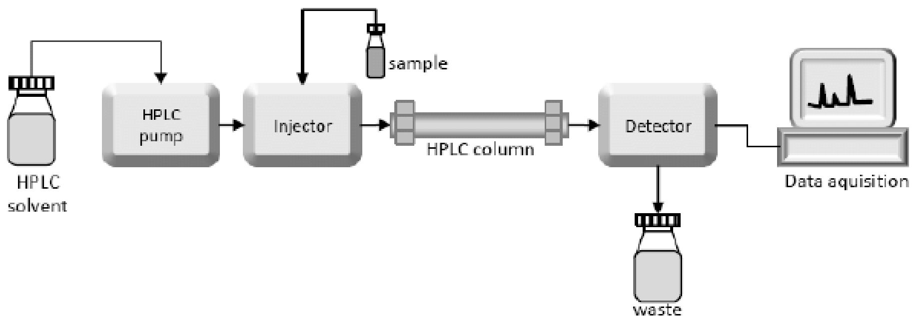


Figure 6: schematic diagram of HPLC system. Credits: www.researchgate.net.

Fundamental to this technique is the preparation of the sample that is introduced into the machine, to avoid incorrect results. The preparation procedure varies depending on the nature of the matrix of interest: a solid matrix such as strawberries must be homogenized, mixed with special solvents, and then filtered to be inserted into the vial.
During the analysis, a small amount of sample is introduced with a special syringe into the column, and, through a valve, the solvent (mobile phase) is channeled to push the sample into the column (stationary phase).

As the analysis proceeds, the optical detectors record the variations in intensity of the light and transform them into electrical impulses transmitted to a computer that provides a graph, called chromatogram. The chromatogram shows the time expressed in minutes on the abscissa and the absorbance on the ordinate, and the separated substances are graphically represented as peaks.
This chromatographic technique can be used to separate complex mixtures and to determine the quantitative composition, as well as to obtain information on the chemical nature of the substances subjected to analysis. HPLC is an easy-to-use tool that allows to investigate a wide range of substances, achieving complete separation of a mixture of compounds in a relatively short time.

Chapter 2
AIM OF THE THESIS

For the present study, four well-defined strawberry cultivars (Romina, Cristina, Silvia and Sibilla) were selected in the 2020 harvest season and tested under treatment with *Botrytis cinerea*, the fungus that causes gray mold. The aim is to determine the nutritional and qualitative changes, evaluating the content of soluble solids, titratable acidity, the content of vitamin C and phenolic acids; and to evaluate the response and susceptibility of each cultivar, in response to the disease caused by the fungal pathogen.

The study involves the use of traditional and destructive techniques for the evaluation of the nutritional and qualitative status, which allow us to obtain accurate results from a wide range of samples.

Chapter 3
MATERIALS AND METHODS

3.1 **Description of the field and cultivation technique adopted**

The production of strawberries took place in the experimental field of the didactic-experimental agricultural company ''P. Rosati'' of the Università Politecnica delle Marche, in the municipality of Agugliano (AN). The farm covers a total area of 125 hectares, about 20 km away from the sea and at an altitude of 80 meters above the sea level, invested in arboreal and herbaceous crops, research, experimentation projects, and evaluation of the local Marche germplasm.

The field is located in a flat area, with a loam-clay soil texture, characterized by sub-alkaline pH, high content of active limestone (12.1%), low content of organic matter (1.14%), low content of total N and assimilable P, and high content in exchange bases (Mg and K) with high cation exchange capacity (CSC).

The climate of Agugliano, according to the classification of Köppen and Geiger, can be classified as humid temperate, with very hot summers (Cfa) (http://it.climate-data.org/). The average temperature of the coldest month oscillates between −3 ° C and +18 ° C, while the average temperature of the warmest month is above 22 ° C.

Rainfall is abundant in the spring and autumn months, and scarce in the winter and summer months. The annual average is 739 mm, distributed over 83 days, with maximum peaks in autumn and relative minimums in winter.

The annual average relative humidity records the value of 75.7%, with a minimum of 71% in July and a maximum of 82% in November; on average there are 36 days a year with foggy episodes (www.clima.meteoam.it/AtlanteClimatico/).

The cultivation technique applied is typical of the Marche region and predominant in the Cesenate area in Romagna: it involves the use of type A refrigerated plants, planted in the open field in the third decade of July, which represents the optimal period to obtain production in the spring of the following year.

3.2 **Plant material**

The evaluation of the qualitative parameters and response to *Botrytis* disease in the year 2020 were carried out on four strawberry cultivars: Cristina, Silvia, Sibilla and Romina. *Table 1* describes the breeder, the country of origin and the main characteristics for each genotype.

CULTIVAR	**BREEDER PATENT**	**PLACE OF ORIGIN**	**CHARACTERIZATION**
CRISTINA	D3A-UNIVPM	ITALY	Late ripening cultivar characterized by high productivity, large fruit size and conical shape, with an orange-red color, more or less bright. The pulp has a good consistency with a medium-high flavor. It is not particularly sensitive to the main pathogens.
SILVIA	D3A-UNIVPM	ITALY	Late ripening cultivar characterized by conical-short, regular shape, with an intense red color. Plant of high hardiness and high cold temperatures requirement; selected in conditions of non-fumigated soil, with medium density of foliage and medium-high productivity. The flavor of the fruit is medium, with a medium sugar content and titratable acidity.
SIBILLA	CIV – Consorzio Italiano Vivaisti	ITALY	Late ripening cultivar characterized by conical-elongated shape, regular size with a bright red color. The flesh is well colored internally. Uniform cultivar with high cold temperatures requirement, suitable

			for continental environments. Characterized by good tolerance to diseases and stresses. The taste is pleasant and of high sweetness.
ROMINA	D3A-UNIVPM	ITALY	Early ripening cultivar characterized by conical or biconical shape, regular size, with a bright red color. Uniform cultivar suitable for non-fumigated soils. Sweet taste, with good sugar content and low acidity. The high consistency makes it a suitable fruit for marketing in large-scale distribution

Table 1: Strawberry genotypes analyzed

3.3 **Fruit collection and sampling method**

The strawberry harvest takes place between the end of April and the end of June, selecting only the fruits that have reached commercial ripeness from each genotype, characterized by a uniform red color, absence of damages that could alter their conservation and typical consistency of the cultivar. The fruits are selected from various plots to obtain a statistically valid data and are collected during the third or fourth harvest, to avoid analyzing primary fruits or fruits produced at the end of the vegetative cycle, that would result different from the standard of the cultivar.
At the third and fourth harvest, 108 fruits were sampled for the Cristina, Silvia, Sibilla and Romina genotypes, for a total of 432 strawberries then used for the different analyses.

3.4 **Analysis protocol and parameters examined**

The work started in 2020 harvesting season, in which 108 fruits were examined for the Cristina, Silvia, Sibilla and Romina genotypes respectively, carrying out two treatments of 54 fruits each, 27 for control and 27 for *Botrytis* inoculation.
The treatments for each genotype were carried out over a week, from Monday (t0) to Friday (t4). The fruits harvested in the field at t0 were brought to the laboratory the same day, selected, numbered, sterilized, and treated (50% contaminated, 50% put in water without spores of

Botrytis). After a daily visual assessment of the presence of *Botrytis*, strawberries are then frozen at -80°C.
The same operations were carried out every week: at t0 an evaluation of the phytosanitary status of the fruits, with the freezing of the first 3 strawberries at a temperature of -80°C. The remaining 96 fruits (48 per test, 24 control and 24 treated) were divided and destined for the remaining four days of the week, keeping them in a climatic cell at 25 °C and about 90% humidity.
At t4 the analysis ends with the freezing at -80 ° C of the last 24 fruits (12 per test, 6 controls and 6 treated).
The study in question starts from the thawing of strawberries subjected to the analyzes described in more detail in the following paragraphs. The parameters considered were: content in soluble solids and acidity, which together influence the flavor of the product; and content in vitamin C and phenolic compounds, which influence the nutritional quality.

3.5 **Sterilization and contamination protocol**

The contamination procedure applied to the genotypes of interest is based on the preparation of a suspension with sterilized water and conidia of *Botrytis cinerea*, previously grown in Petri dishes. A volume between 50 and 100 ml is needed, sufficient to completely immerse the strawberries to be contaminated, with a concentration of conidia that must be of 10^6 /ml of solution. To ensure that the measurement is correct, before preparing the suspension, the CFU are counted under an optical microscope and the concentration is increased or decreased, to achieve the desired level of conidia.
For the Cristina, Silvia, Sibilla and Romina genotypes, two tests of 54 fruits each were carried out, 27 for control and 27 for contamination: for both tests, 3 beakers with distilled water and 5% bleach are used. The fruits are immersed one after the other inside each beaker, to obtain 3 washes lasting 30 seconds each. Subsequently, the fruits for the control are immersed for 30 seconds in a beaker containing distilled water, while the fruits to be treated are immersed for 30 seconds in the conidial suspension previously obtained.
Finally, the fruits were numbered, assessed for the level of fungal infection, closed separately in transparent plastic bags and frozen at a temperature of -80 °C.

3.5.1 *Assessment of damage from botrytis cinerea*

Based on the protocol, every day (from t0 to t4), a visual assessment was made of the phytosanitary status and the damage caused by the infection of *Botrytis cinerea* in the treated strawberries, which was followed by freezing.

The severity of the infection was assessed according to an empirical scale with six degrees:

- 0: healthy fruit
- 1: 1% to 20% fruit surface infected
- 2: 21% to 40% fruit surface infected
- 3: 41% to 60% fruit surface infected
- 4: 61% to 80% fruit surface infected
- 5: 81% to 100% fruit surface infected and showing sporulation (Feliziani, et al., 2015)

The decay index were calculated based on I= $\left[\sum(\frac{(d \times f)}{(N \times D)})\right]$*100, where *d* is the category of rot intensity scored on the fruit, and *f* is its frequency, N is the total number of examined fruit (i.e., healthy and infected), and D is the highest category of decay intensity that occurred on the empirical scale (Mckinney, 1923).

3.6 Compositional quality analyses

The parameters to assess the quality and response to *Botrytis* disease are: soluble solids content and titratable acidity, to indicate the organoleptic quality of strawberry fruits through the quantity of soluble solids sugars and free acids present, which together influence the aroma and flavor of the final product; and the nutritional parameters, such as the determination of the content of vitamin C and phenolic acids, which influence the quality of the product from a nutritional point of view.

Strawberries closed inside the plastic bags were handled with care, thawed, and individually recorded, before proceeding with the analyses. From each strawberry 1g and 5g were taken, to determine the content of vitamin C and phenolic compounds, respectively; while, with the remainders of the sample of interest, the analyses of soluble solids content and titratable acidity were carried out. All the phases presented in *figure 6* have been described in detail in the following paragraphs.

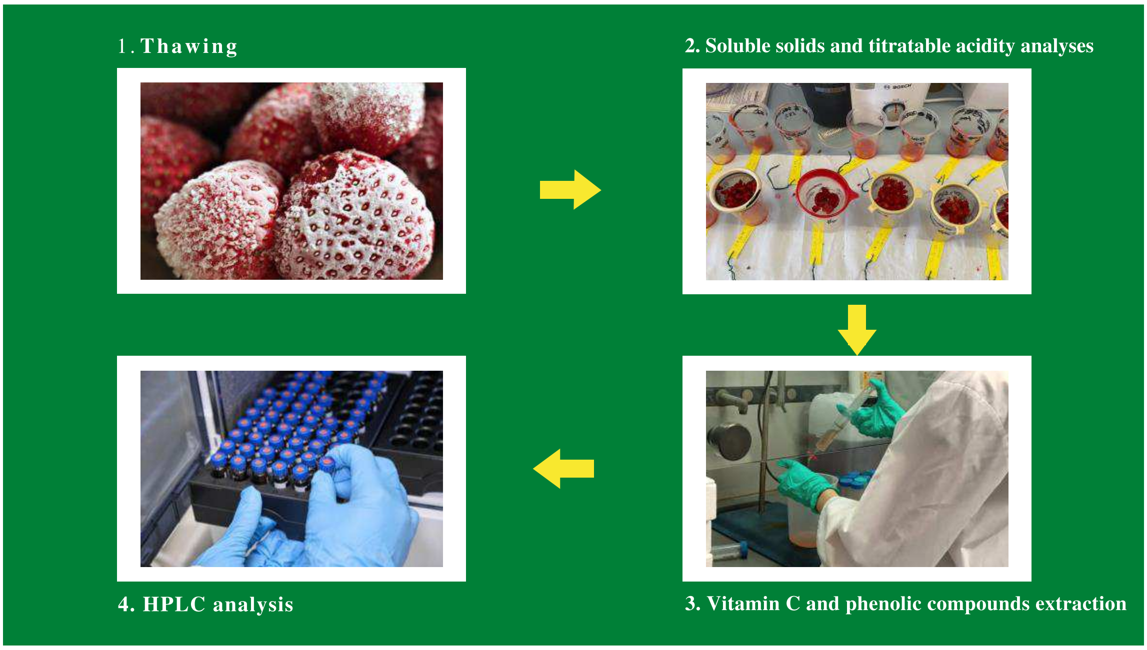


Figure 7: Stages of the thesis work

3.6.1 *Soluble solid content*

The first organoleptic parameter evaluated is the soluble solids content, which indicates the sugar concentration present in the juice of strawberry samples. The analysis was carried out using a digital palette refractometer (ATAGO™ PR-101α), ideal for measuring the Brix values of fruit juices, food and drinks, equipped with a technology and a temperature display function that allow accurate results. The instrument is based on the principle according to which the refractive index of a liquid containing sugars, or other soluble solids, is proportional to its concentration. The higher the soluble solids content in the liquid sample of the fruit, the higher the value obtained, expressed in degrees Brix (%).

The measurement requires to crush each single fruit, previously thawed, to collect the filtered juice. At each measurement the instrument is calibrated to zero, with drops of distilled water. For each fruit, an aliquot of juice is taken and placed on the surface of the refractometer slide and values in degrees Brix are directly displayed by the screen and recorded.

3.6.2 *Titratable acidity*

The second organoleptic parameter evaluated is the titratable acidity, which indicates the content of free acids present in the juice of the strawberry samples. The concentration of free acids present (predominantly malic acid, citric acid, ascorbic acid, and succinic acid), combined with the concentration of soluble solids, represent a fundamental parameter that influences the aromatic characteristics and the flavor of the final fruit product.

The analysis was carried out using an automatic titrator (HI84533, HANNA Instruments Italia Srl), equipped with a preprogrammed analysis method, optimized with an algorithm that determines the completion of the titration reaction by using a pH electrode, made of glass. The instrument presents a piston dosing pump that adjusts the dose volume according to the change in potential, which provides a very precise and quick determination of the amount of titrant used. The measurement requires to crush each single fruit, previously thawed, to collect the filtered juice. 5 ml of strawberry juice with 45 ml of distilled water are loaded into the beaker, the instrument automatically injects inside the sample small amounts of the basic solution, continuously measuring the pH, until the value of neutrality is reached. The screen directly displays the acidity of the sample, expressed as percentage of the predominant acid (% citric acid).

3.6.3 *Vitamin C extraction*

Vitamin C was determined with an ultrasound-assisted extraction and a HPLC system.
In the laboratory, the extraction is carried out with the use of a sonifier (Bioblock/ELMA 88155), an instrument that generates ultrasound waves inside a tank containing water, using high frequency electric current produced by a generator. The process is useful to speed up the dissolution of solutes in certain solvents.
The analysis requires homogenizing 1g of frozen strawberry with an aliquot of 4 ml taken from the extraction solution containing 5% metaphosphoric acid and 1 mM DTPA, followed by 5 min of sonication extraction and centrifugation at 4000 rpm for 10 min at 4°C. The supernatant obtained from each strawberry is filtered (pore size 0.45 μm) and inserted into a vial to perform analysis on an HPLC system.

3.6.4 *HPLC Determination of Vitamin C Content*

Vitamin C content was measured as described in Helsper, et al., 2003. The methanolic extract previously obtained was analyzed through HPLC-UV system consisting of a Jasco PU-2089 plus controller, and a Jasco UV-2070 plus ultraviolet (UV) detector (Jasco Easton, MD, USA) set at absorbance of 260 nm. The HPLC column used was Ascentis Express C18 150 × 4.6 mm (Sigma-Aldrich Corp., St. Louis, MO, USA). The quantification of vitamin C content was carried out through calibration curve prepared by running standard concentration of vitamin C and the results were expressed as mg vit. C per 100 g of fresh weight (mg 100 g-1 FW).

3.6.5 *Phenolic acids extraction*

Phenolic acids were determined with a double methanolic extraction and a HPLC system.

Methanolic extraction is a method used in laboratories to extract primary and secondary metabolites from the organic material of interest. The extraction is "double", or rather it is based on two extraction phases, each with a certain quantity of solvent, rather than a single extraction with the entire amount of the solvent needed. To obtain reliable results, the solvent and the organic material must remain in contact under an oscillatory motion for a certain amount of time.
The analysis requires homogenizing 5g of frozen strawberry in 10ml of extraction solution consisting of methanol, stirring for 30 minutes in the dark at room temperature (24°C), followed by centrifugation at 4000 rpm for 10 min at 4°C. The process is repeated twice and in the final step the supernatant obtained from each strawberry is filtered (pore size 0.22 µm) and inserted into a vial to perform analysis on an HPLC system.

3.6.7 *HPLC Determination of Phenolic Acids Content*

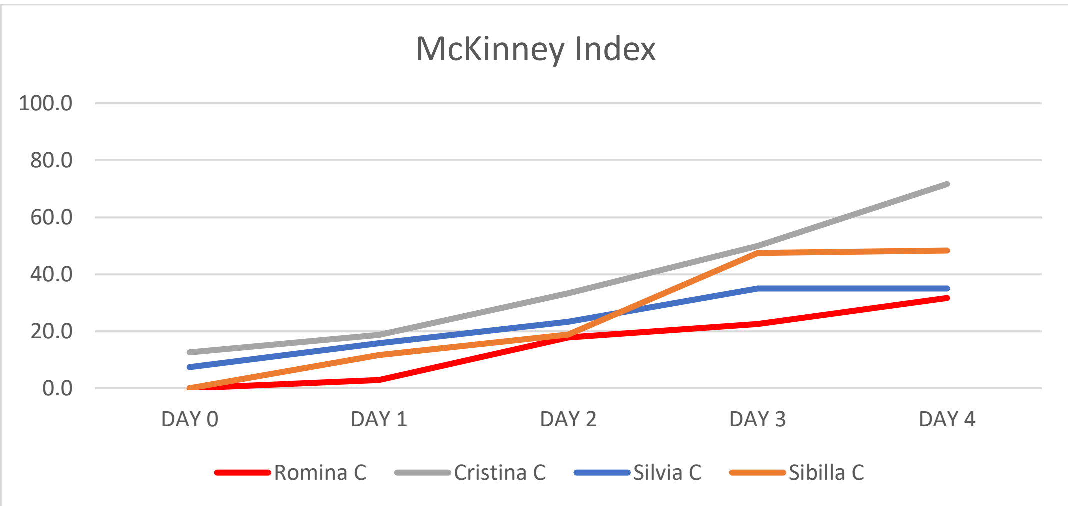
Phenolic acids were analyzed as previously described in Schieber, et al., 2001. The methanolic extract previously obtained was subjected to HPLC-UV analysis through an
HPLC system (Jasco PU-2089 plus), and a Jasco UV-2070 plus ultraviolet (UV) detector (Jasco Easton, MD, USA). The UV detector was set at 320 nm, and the column used for the separation was an Aqua Luna C18 (250×4.6 mm) reverse-phase column, with a particle size of 5 µm (Phenomenex, Lane Cove, NSW, Australia), protected by a Phenomenex 4.0×3.0 mm C18 ODS guard column. The quantification of phenolic acid content was performed using external standard of chlorogenic acid, caffeic acid, and ellagic acid (EA). Values were expressed as mg of total phenolic acids per 100 g of fresh weight (mg 100 g-1 FW).

Chapter 4
RESULTS AND DISCUSSION

4.1 **Assessment of damage from *Botrytis cinerea***

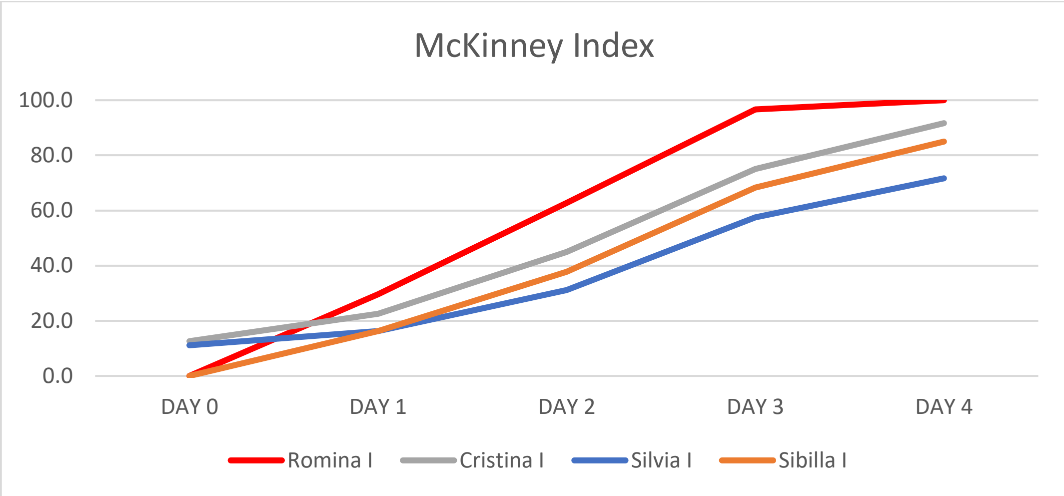
Decay severity was recorded according to an empirical scale with six degrees: 0, healthy fruit; 1, 1% to 20% fruit surface infected; 2, 21% to 40% fruit surface infected; 3, 41% to 60% fruit surface infected; 4, 61% to 80% fruit surface infected; 5, ≥81% fruit surface infected and showing sporulation (Romanazzi, et al., 2000). The infection index (or McKinney index), which incorporates both the incidence and severity of the decay, was expressed as the weighted means of the decay as percentage of the maximum possible level. This was calculated using the formula $I = \left[\sum\left(\frac{(d \times f)}{(N \times D)}\right)\right] * 100$ (Mckinney, 1923).

Graph 1 and *graph 2* show the susceptibility index of Romina, Cristina, Silvia and Sibilla cultivars against *Botrytis cinerea* in control and treated fruits, respectively.
The data in *graph 1* shows that Romina cultivar is the most resistant in control trail, while Cristina cultivar is the most sensitive.



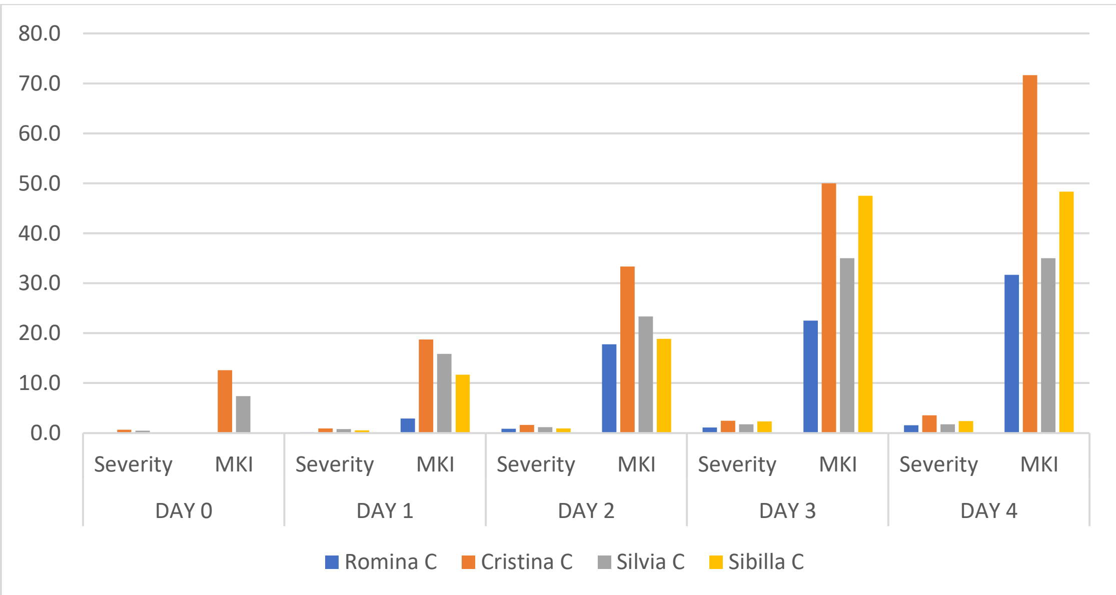
Graph 1: Susceptibility of strawberry varieties to Botrytis cinerea (control trial).

On the contrary, in treated strawberries, as shown in *graph 2*, Romina is very sensitive (almost 100%) when treated with *Botrytis cinerea*, while Silvia is the most resistant cultivar against the fungal pathogen.



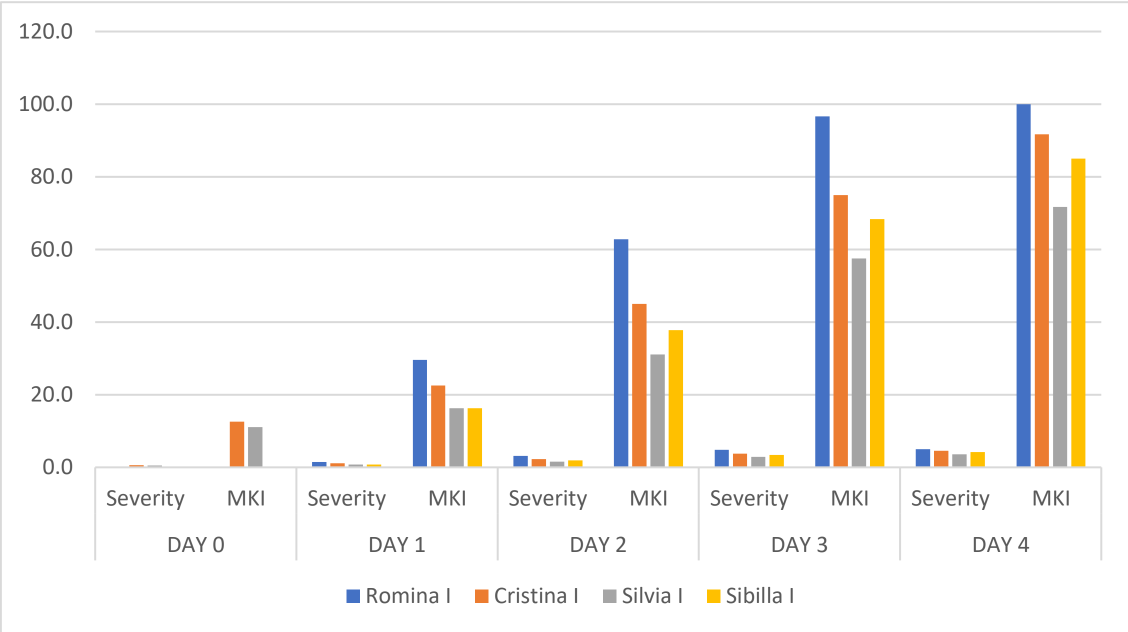
Graph 2: Susceptibility of strawberry varieties to Botrytis cinerea (treated trial).

The treatment with *Botrytis cinerea* generally increase the development of strawberry fruit decay after 4 days of shelf life. In particular, at day 4, compared to the control, for Romina, Cristina, Silvia and Sibilla cultivars, the treatment with *Botrytis cinerea* significantly increase the McKinney's index of decay by 215%, 27%, 105% and 80%, respectively (*graph 4*).



Graph 3: Decay severity and McKinney Index (MKI) of strawberries varieties (control trial).

Similarly, the severity index of strawberry fruit after 4 days of shelf life was also significantly increased by *Botrytis* treatment for Romina, Cristina, Silvia and Sibilla cultivars, by 215%, 27%, 100% and 79%, respectively (*graph 4*).



Graph 4: Decay severity and McKinney Index (MKI) of strawberry varieties (treated trial).

In general, treatment with *Botrytis cinerea* has been shown to be effective in increasing postharvest decay of strawberry cultivars. Compared to the control, the treatment significantly increase the McKinney's Index and the severity decay index.

In particular, the severity of the infection for the Romina cultivar on day 4 was assessed at grade 5 (81% to 100% fruit surface infected and showing sporulation), resulting in the cultivar most susceptible to the treatment, with a McKinney Index equal to 100%.

The severity of the infection for Cristina and Sibilla cultivars on day 4 was assessed to 4,6 and 4,3, with a McKinney Index equal to 91,7% and 85%, respectively.

Finally, the Silvia cultivar was the most resistant against *Botrytis cinerea* in treated trail, with a severity infection assessed to 3,6 (41% to 60% fruit surface infected) and a McKinney Index equal to 71,7%.

4.2 **Soluble solids content**

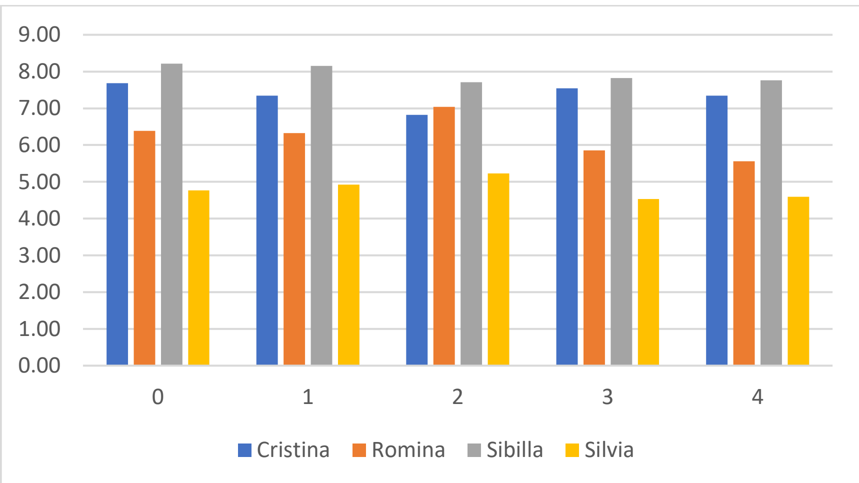
The total content of soluble solids is represented by all the substances and organic molecules dissolved in water and it is expressed in degrees Brix. Conventionally, the ° Brix is considered as the number of grams of sugar contained in a solution. Therefore, when measuring a pure sugar solution, the Brix degree corresponds exactly to the real content and can be considered an index of the sweetness of the product.

From 2020 harvest, the highest sugar content was detected by the fruits of the Sibilla cultivar with an average of 7.8 ° Brix, the lowest by the fruits of the Silvia cultivar with an average of 5.0 ° Brix. Romina and Cristina reach an average of 6.3 and 7.2 ° Brix respectively.

CULTIVAR	SOLIDI SOLUBILI (°BRIX)
CRISTINA	7,2 ± 1,1
ROMINA	6,3 ± 1,4
SIBILLA	7,8 ± 1,5
SILVIA	5,0 ± 1,0

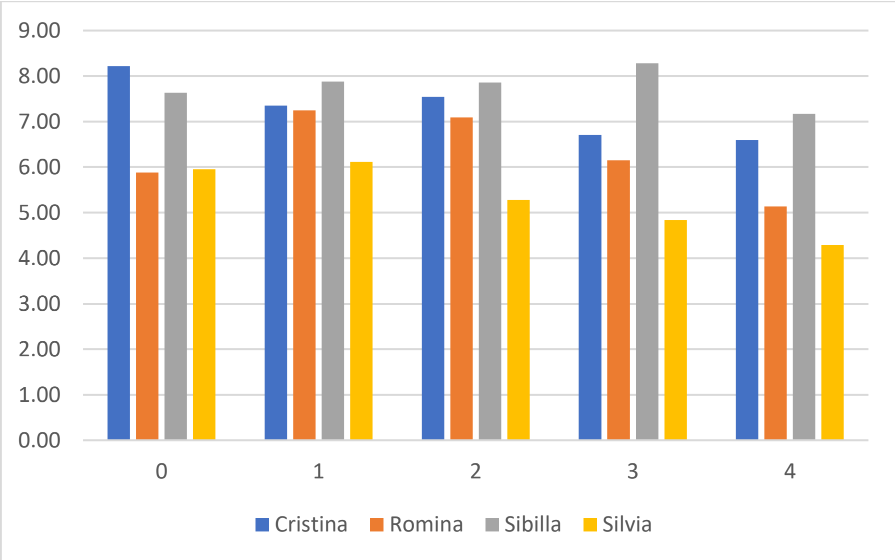
Table 2: Soluble solids content (° Brix) and relative standard deviation 2020 vintage.

In untreated fruit (control), from day zero (t0) to day four (t4), the highest sugar content was achieved by Sibilla cultivar with an average of 7.9 °Brix, the lowest by the fruits of the Silvia cultivar with an average of 4,82 °Brix. Romina and Cristina reach an average of 6.22 and 7.3 ° Brix respectively. *Graph 5* represents the soluble solids content in untreated strawberries from t0 to t4. The graph shows how the evolution of the soluble solids for the cultivars Cristina, Romina, Sibilla and Silvia has a decreasing trend variation after 4 days of shelf life of – 4,4%, - 12,8%, - 5,6% and -3,7%, respectively.



Graph 5: Soluble solids content (° Brix) in untreated strawberries (control) from t0 to t4.

In treated fruit, the highest sugar content was achieved by Sibilla cultivar with an average of 7.78 °Brix, the lowest by the fruits of the Silvia cultivar with an average of 5,22 °Brix. Romina and Cristina reach an average of 6.37 and 7.18 °Brix respectively.



Graph 6: Soluble solids content (° Brix) in treated strawberries from t0 to t4.

Graph 6 represents the soluble solids content in treated strawberries, from t0 to t4. From the graph it is possible to deduce how the Cristina and Silvia cultivars show a significant decreasing trend variation after 4 days of shelf life of -19.8% and -28%, respectively. While, in the Romina and Sibilla cultivars, the treatment with *Botrytis* did not significantly affect the soluble solids content, recording a variation similar to untreated fruit of -12.5% and -6%, respectively.

Going into detail among Romina, Cristina, Sibilla and Silvia cultivars, it is possible to identify a general decrease in the content of soluble solids in both treated and control fruits, from day 0 to day 4 (*Figure 8*).

The decrease is more marked in the fruits infected with *Botrytis cinerea*, in particular in the cultivars Cristina and Silvia. In the Cristina fruit, the value progressively decreases from 8,22° Brix on day 0 to 6.59° Brix on day 4 of treatment with *Botrytis*. In the Silvia fruit, the value progressively decreases from 5,95° Brix on day 0 to 4,28° Brix on day 4 of treatment with *Botrytis*. In the control trial, both cultivars are rather stable over time, with a slight decrease in sugars content.

In the Romina fruit the trend is decreasing, not very pronounced, and records a significant increase between day 1 and 2, both for the treated and control species. Soluble solids content progressively decreases from 5,88° Brix on day 0 to 5,14° Brix on day 4 of treatment with *Botrytis*, while in the control trial the content decreases to 5,56° Brix on day 4.

In the Sibilla fruit the trend of the treated species records a significant progressive increase until day 3, and then decreases to 7,17° Brix on day 4. In the untreated species the trend is decreasing, and then remains stable and registers a slight increase to 7,76° Brix on day 4.

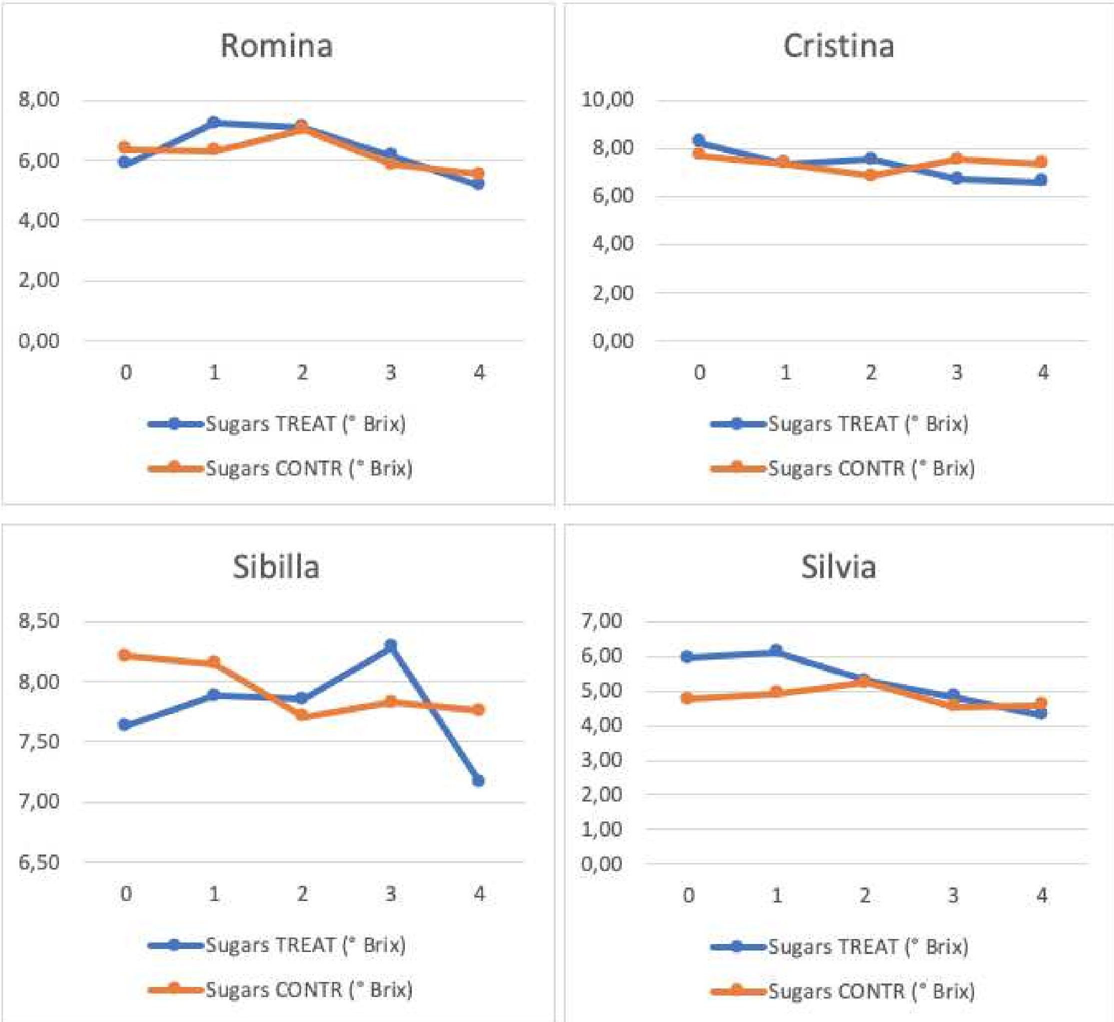


Figure 8: Soluble solids content (° Brix) from day 0 to day 4 in Romina, Cristina, Sibilla and Silvia cultivars. Differences between treat and control trials.

4.3 **Titratable acidity**

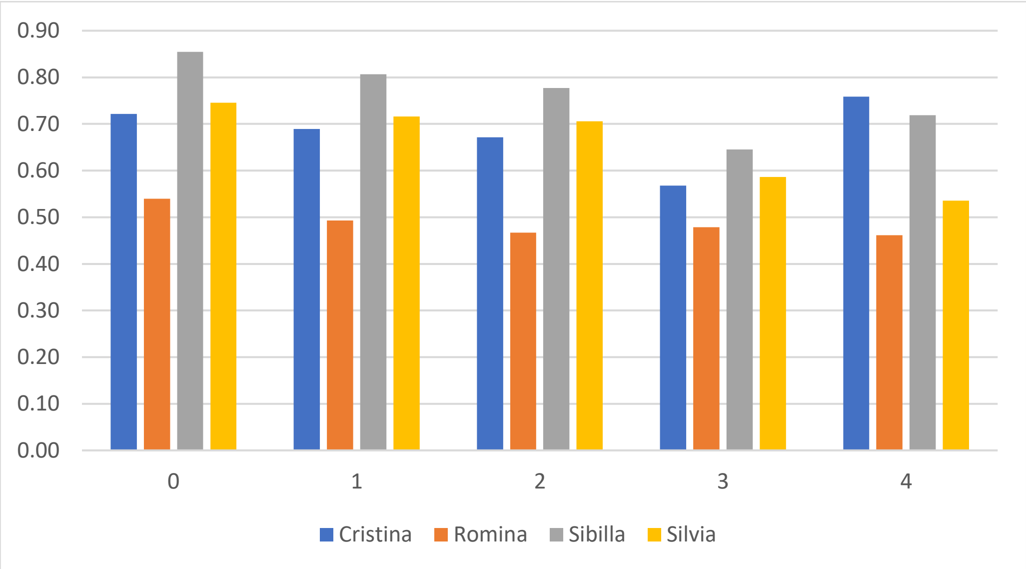
Titratable Acidity is one of the main fruit quality descriptors and a very delicate parameter, as a fruit that is too acidic is not appreciated by the average consumer.

The average values recorded in the 2020 fall within a standard range. The fruits of the Sibilla cultivar have a higher citric acid content (0.77%), while the Romina fruits are less acid with average values of 0.49%. The fruits of the Silvia and Cristina cultivars showed values of 0.66% and 0.72% respectively.

CULTIVAR	ACIDITY (% AC. CITRICO)
CRISTINA	0,72 ± 0,14
ROMINA	0,49 ± 0,11
SIBILLA	0,77 ± 0,20
SILVIA	0,66 ± 0,18

Table 3: Titratable acidity (% Citric acid) and relative standard deviation 2020 vintage.

In untreated fruit (control), from day zero (t0) to day four (t4), the highest value was achieved by Sibilla cultivar with an average of 0.75%, while the Romina fruits result less acid with average values of 0.48%. The fruits of the Silvia and Cristina cultivars showed values of 0.65% and 0.68% respectively.



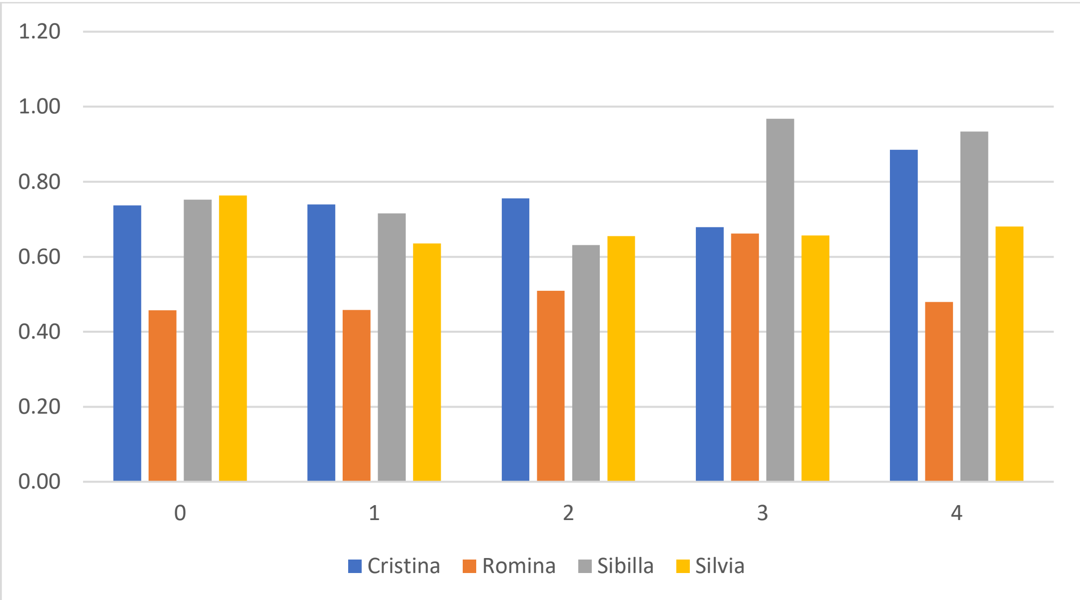
Graph 7: Titratable acidity (% Citric acid) in untreated strawberries (control) from t0 to t4.

Values and differences in titratable acidity in untreated strawberries from day 0 to day 4 of treatment are shown in *graph 7*. The graph shows how the evolution of the titratable acidity for the control cultivar Cristina has an increasing trend after 4 days of shelf life, with a variation of 5,5%. While, Romina, Sibilla and Silvia control cultivars show a significant decrease up to -14,8%, -15,2% and -28%, respectively.

In treated fruit, from t0 to t4, the highest value was achieved by Sibilla cultivar with an average of 0.79%, while the Romina fruits result less acid with average values of 0.51%. The fruits of the Silvia and Cristina cultivars showed values of 0.67% and 0.76% respectively.

Values and differences in titratable acidity (% citric acid) in treated strawberries from day 0 to day 4 of treatment are shown in *graph 8*.

The evolution of titratable acidity for the fruit infected with *Botrytis cinerea*, varied depending to the cultivar: Romina, Cristina and Sibilla, had an inverse variation compared to that observed for sugars from day 0 to day 4, registering an increase of titratable acidity over 4%, 20.3% and 24%, respectively, while the Silvia cultivar recorded an average decrease in values of -10.5%.



Graph 8: Titratable acidity (% Citric acid) in treated strawberries from t0 to t4.

Figure 9 shows the different average trend of titratable acidity of the fruits of the 4 cultivars, between the control and the infected fruits.

In Romina fruit, the value progressively increases from 0.46% on day 0 to 0.66% on day 3 of treatment with *Botrytis*, and then decreases on day 4 to 0.48%. A different trend is followed in control trial, in which the value progressively decreases until day 4.

In Cristina fruit, the value decreases from 0.74% on day 0 to 0.68% on day 3 of treatment with *Botrytis*, and then increases on day 4 to 0.89%. A similar trend is followed in control trial, in which the value progressively decreases until day 3, and then increases on day 4 to 0,76%.

In Sibilla fruit, the value decreases from 0.75% on day 0 to 0.63% on day 2 of treatment with *Botrytis*, and then increases on day 4 to 0.93%. A similar trend is followed in control trial, in which the value progressively decreases until day 3, and then increases on day 4 to 0,72%.
In Silvia fruit, the value initially decreases on day 1, and then gradually increases up to day 4 at 0.68%, of treatment with *Botrytis*. A different trend is followed in the control test, in which the value progressively decreases from 0,75% on day 0 to 0,54% on day 3.

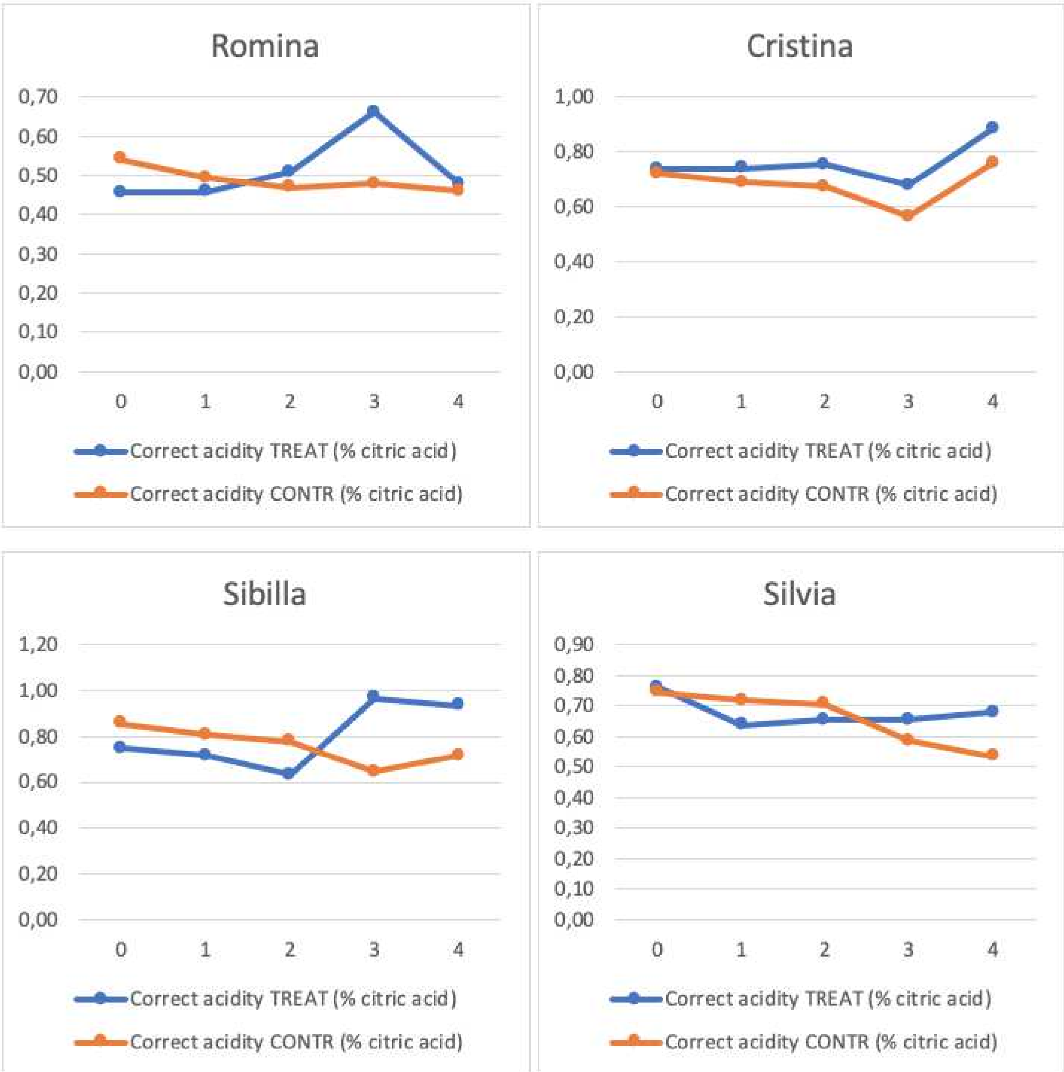


Figure 9: : Titratable acidity (% citric acid) from day 0 to day 4 in Romina, Cristina, Sibilla and Silvia cultivars. Differences between treat and control trials.

4.4 **Vitamin C content**

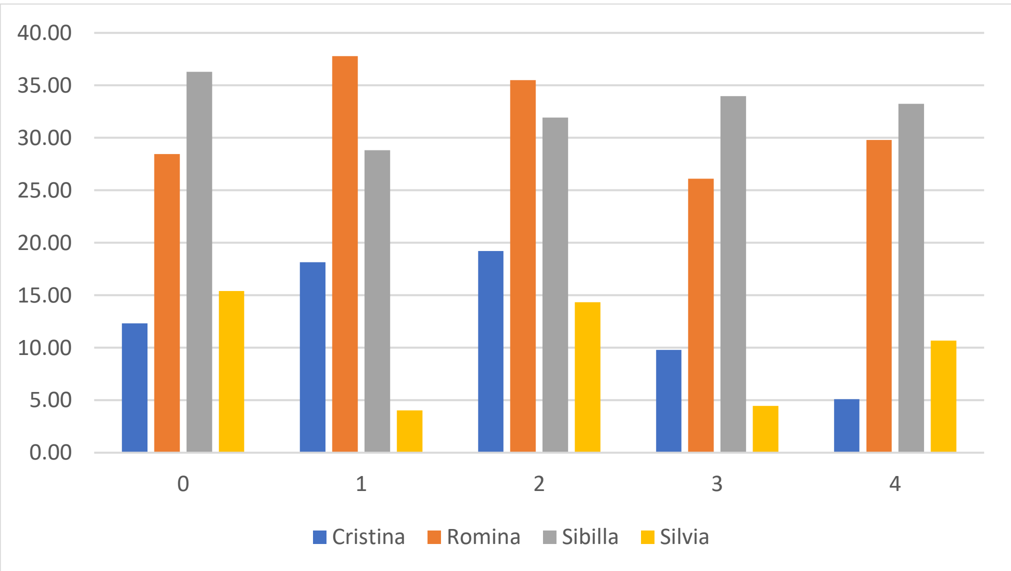
Vitamin C is one of the nutritional components of strawberries, essential for its high antioxidant power and the various biological activities it exerts in the human body. Therefore, it is important to measure the vitamin C content in strawberries, to verify its activity.

In the 2020, the highest vitamin C content was detected in fruits of the Sibilla cultivar with an average of 28,3 mg/100g, the lowest by the fruits of the Silvia cultivar with an average of 6,7 mg/100g. Romina and Cristina reach an average of 25,6 and 12,1 mg/100g, respectively.

CULTIVAR	VITAMIN C (mg/100g)
CRISTINA	12,1 ± 10,8
ROMINA	25,6 ± 32,6
SIBILLA	28,3 ± 21,8
SILVIA	6,7 ± 9,1

Table 4: Vitamin C content and relative standard deviation.

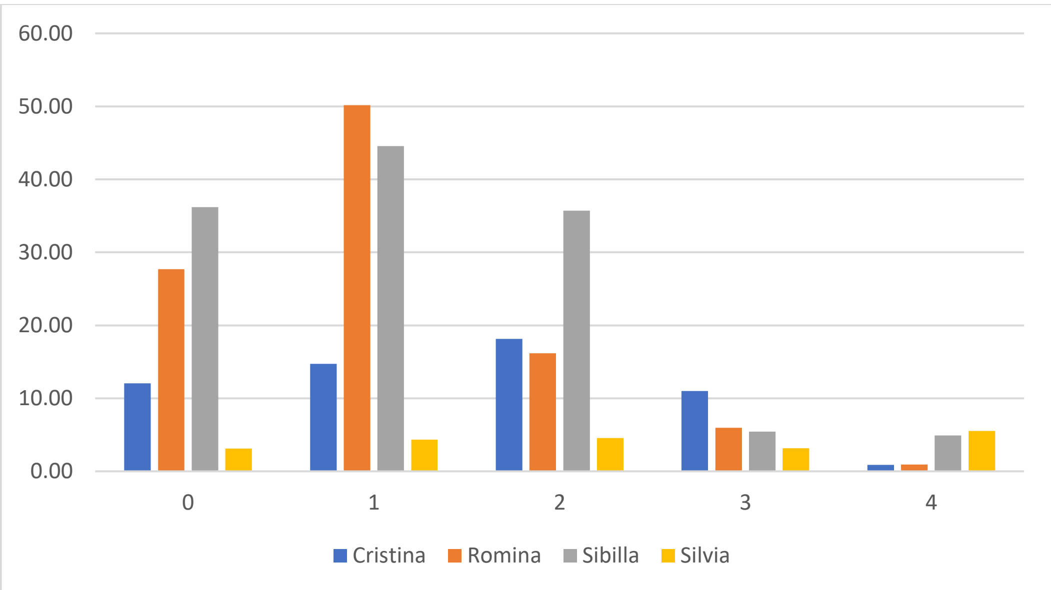
Graph 9 represents the vitamin C content in untreated strawberries from t0 to t4: the highest vitamin C content was achieved by Sibilla fruits with an average of 32,47 mg/100g, the lowest by the fruits of the Silvia cultivar with an average of 9,13 mg/100g. Romina and Cristina reach an average of 31,87 and 13 mg/100g, respectively.



Graph 9: Vitamin C content in control strawberries from t0 to t4 (control trial).

Generally, fruits and vegetables show a gradual decrease in vitamin C content as the storage temperature or duration increases (Adisa, 1986). In this study, in untreated fruits (control trial), for the 4 cultivars there is no clear or general vitamin C content trend of increase or decrease, during the 5 days of shelf life. Each cultivar seems to respond differently: in Romina fruit, the vitamin C content reaches a maximum peak on day 1. The value progressively decreases to 26,1 mg/100g on day 3, recording a slight increase on day 4.
In Cristina fruit, the vitamin C content increases progressively, reaching a maximum peak on day 2 of 19.21 mg/100g, and then decreases until day 4.
In Sibilla fruit, the vitamin C content reaches a maximum peak on day 0 of 36,3 mg/100g. After a decrease to 28.8 mg/100g on day 1, the value progressively increases until day 4.
In Sibilla fruit, the vitamin C content shows an oscillating trend, consisting of decreases and reductions that follow one another from day 0 to day 4, recording a maximum peak on day 0 of 15,4 mg/100g.

In treated fruit, the highest vitamin C content was detected in Sibilla fruit, with an average of 24,16 mg/100g, the lowest in the fruits of the Silvia cultivar with an average of 4,26 mg/100g. Romina and Cristina reach an average of 19,35 and 11,22 mg/100g, respectively.



Graph 10: Vitamin C content in treated strawberries from t0 to t4.

Graph 10 represents the vitamin C content in treated strawberries from t0 to t4. From the graph it is possible to deduce how the Cristina, Romina and Sibilla cultivars show a marked decreasing trend variation after 4 days of shelf life. The treatment with *Botrytis cinerea* significantly affects the vitamin C content, recording a variation for Cristina, Romina and Sibilla of – 92,7%, – 96,6% and – 86,4%, from t0 to t4, respectively. The Silvia cultivar shows a low vitamin C content since day 0 (3,12 mg/100g), registering a slight increase on day 4 (5,53 mg/100g). However, the reduced concentration of vitamin C does not allow to ascertain the effect of the *Botrytis* infection over time.

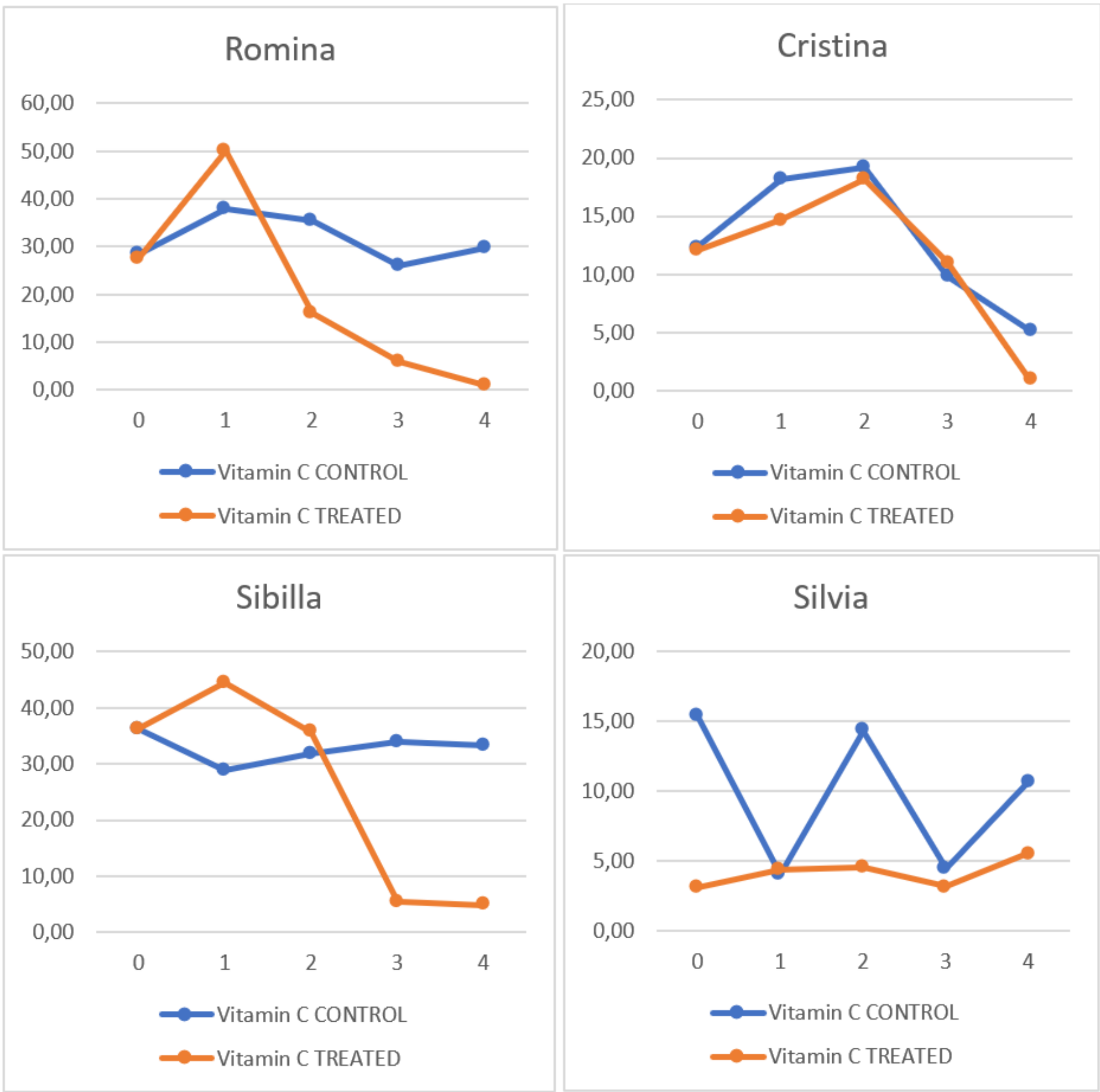


Figure 10: Vitamin C trend from t0 to t4 in Cristina, Silvia, Romina and Sibilla cultivars. Differences between Treated and Control trials.

The results showed that the vitamin C content of the inoculated fruits decreased significantly compared with those of the non-inoculated fruits. The decrease in vitamin C content can be associated with the degree of pathogen severity in the fruits, as the parameters are inversely proportional. As the decay severity index and the McKinney index increase up to day 4, reaching maximum levels of 100% of severity, the vitamin C content decreases.

Figure 10 shows the different average trend of vitamin C of the Romina, Cristina, Sibilla and Silvia cultivars, between the control and the treatment trials.

In fruit of Romina cultivar, the value progressively decreases from 50,1 mg/100g on day 1 to 0,95 mg/100g on day 4 of treatment with *Botrytis*. A different trend is followed in control trial, in which the value progressively decreases from day 1 to day 3, and then increases on day 4 to 29,8 mg/100g.

In Cristina fruit, the value progressively increase from 12 mg/100g on day 0 to 18,1 mg/100g on day 2 of treatment with *Botrytis*, and then decreases until day 4 to 0,88 mg/100g. A similar trend is followed in control trial, in which the value progressively increases until day 2, and then decreases until day 4 to 5,1 mg/100g.

In Sibilla fruit, the value progressively decreases from 44,5 mg/100g on day 1 to 4,9 mg/100g on day 4 of treatment with *Botrytis*. In the control trial, the value is mantained within the range, recording a slight decrease in vitamin C content on day 1, and then a progressively increase on day 4 to 33,2 mg/100g.

At day 0, Silvia cultivar shows an extremely low vitamin C content in the fruits of the treatment trial. The value gradually increases up to day 4 at 5,53 mg/100g, of treatment with *Botrytis*. A different trend is followed in the control test, in which the vitamin C values show an oscillating trend, until day 4 to 10,7 mg/100g.

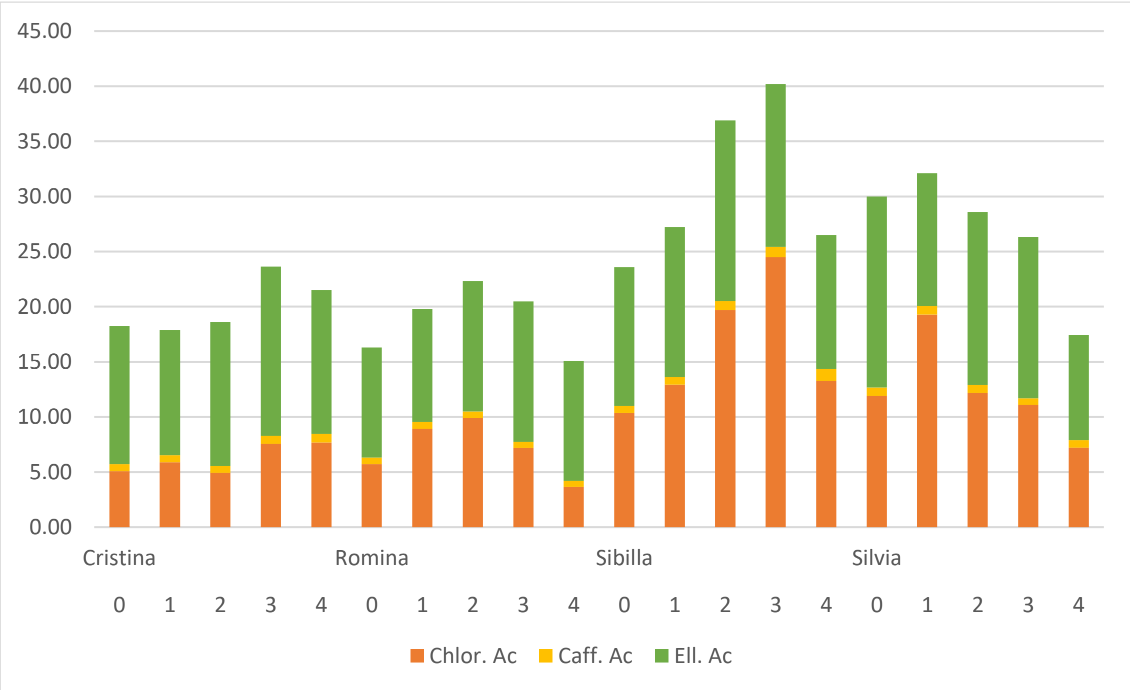
4.5 **Phenolic acids content**

Phenolic acids are important secondary plant metabolites that have attracted considerable interest in the past few years due to their many potential health benefits, as they are powerful antioxidants. Therefore, it is important to verify their activity.

CULTIVAR	CHLOROGENIC ACID (mg/100g FW)	CAFFEIC ACID (mg/100g FW)	ELLAGIC ACID (mg/100g FW)
CRISTINA	6,80	0,68	13,71
ROMINA	7,56	0,60	11,91
SIBILLA	16,42	0,78	14,78
SILVIA	13,36	0,77	14,3

Table 5: Phenolic acid content in 2020 harvest season.

In the 2020, the highest Chlorogenic acid content was detected in fruits of the Sibilla cultivar with an average of 16,42 mg/100g, the lowest by the fruits of the Cristina cultivar with an average of 6,8 mg/100g. Romina and Silvia reach an average of 7,56 and 13,36 mg/100g, respectively.
The highest caffeic acid content was detected in fruits of the Sibilla cultivar with an average of 0,78 mg/100g, the lowest by the fruits of the Romina cultivar with an average of 0,60 mg/100g. Silvia and Cristina reach an average of 0,77 and 0,68 mg/100g, respectively.
The highest ellagic acid content was detected in fruits of the Sibilla cultivar with an average of 14,78 mg/100g, the lowest by the fruits of the Romina cultivar with an average of 11,91 mg/100g. Silvia and Cristina reach an average of 14,3 and 13,71 mg/100g, respectively.

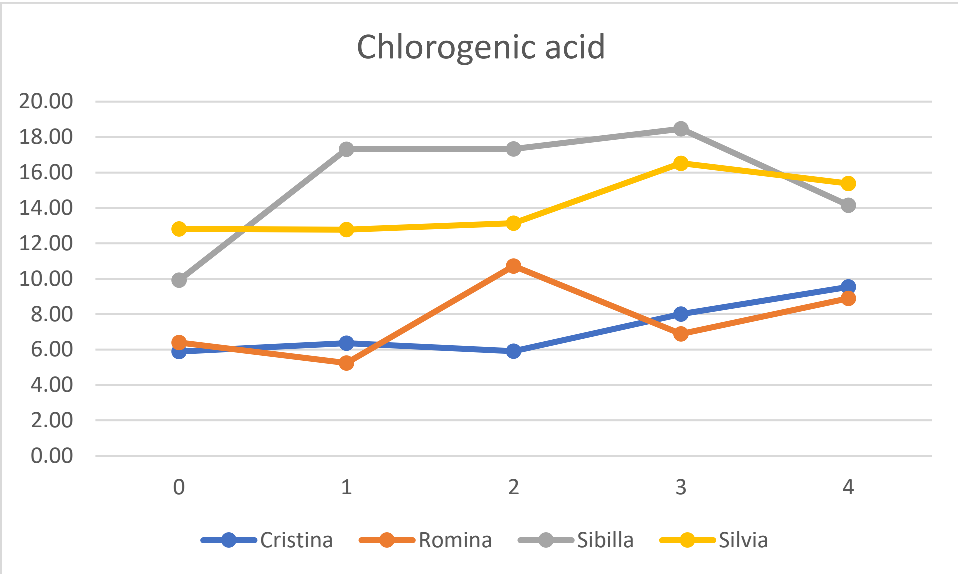


Graph 11: Phenolic acid content in control strawberries from t0 to t4 (control trial).

Graph 11 represents the phenolic acid (chlorogenic acid, caffeic acid and ellagic acid) content in untreated strawberries from t0 to t4: the highest chlorogenic and ellagic acid content was achieved by Sibilla fruits with an average of 16,05 and 15,51 mg/100g, respectively, while the highest caffeic acid content was achieved by Silvia fruits with an average of 0,84 mg/100g. The lowest caffeic acid and ellagic acid content was achieved by Romina cultivar, with an average of 0,60 and 12,41 mg/100g, respectively, while the lowest chlorogenic acid content was achieved by Cristina fruits with an average of 7,27 mg/100g.

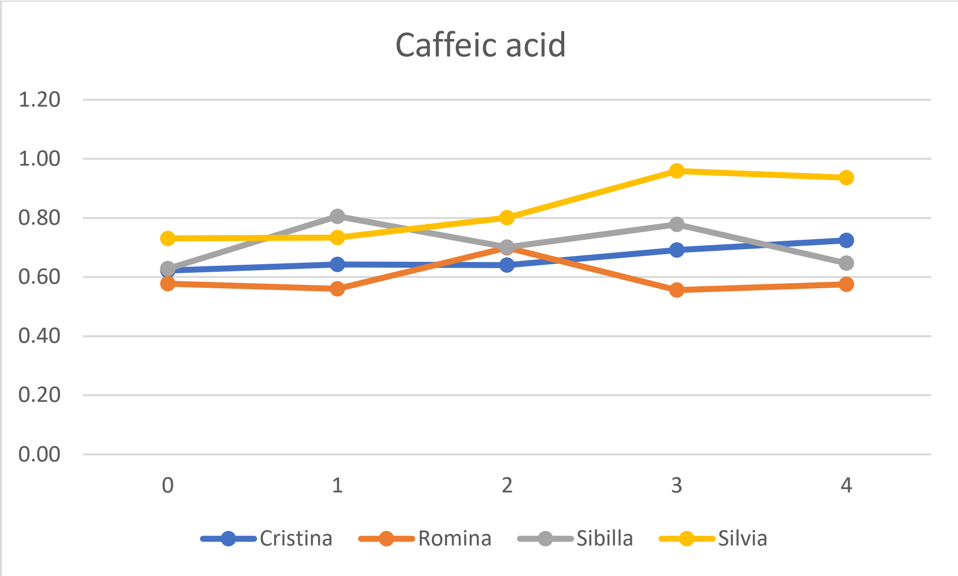
From the results it is possible to identify an increasing trend of the phenolic acid content in the Cristina, Romina and Sibilla cultivars, from day 0 to day 2 and 3, and then a decrease on day 4. In the Silvia cultivar there is an early increase from day 0 to day 1, then decreasing linearly until day 4.

Going into detail, in the Cristina fruit the chlorogenic acid content shows a linear increase from 6,35 mg/100g on day 1 to 9,54 mg/100g day 4. In the Silvia fruit, the value progressively increases to 16,52 mg/100g on day 3, and then decrease on day 4. In the Romina fruit the value records a significant increase to 10,72 mg/100g on day 2, to then decrease to 6,89 mg/100g on day 3, and then increases again on day 4. In the Sibilla fruit the trend records a significant progressive increase until day 3, and then decreases to 14,15 mg/100g on day 4 (*graph 12*).



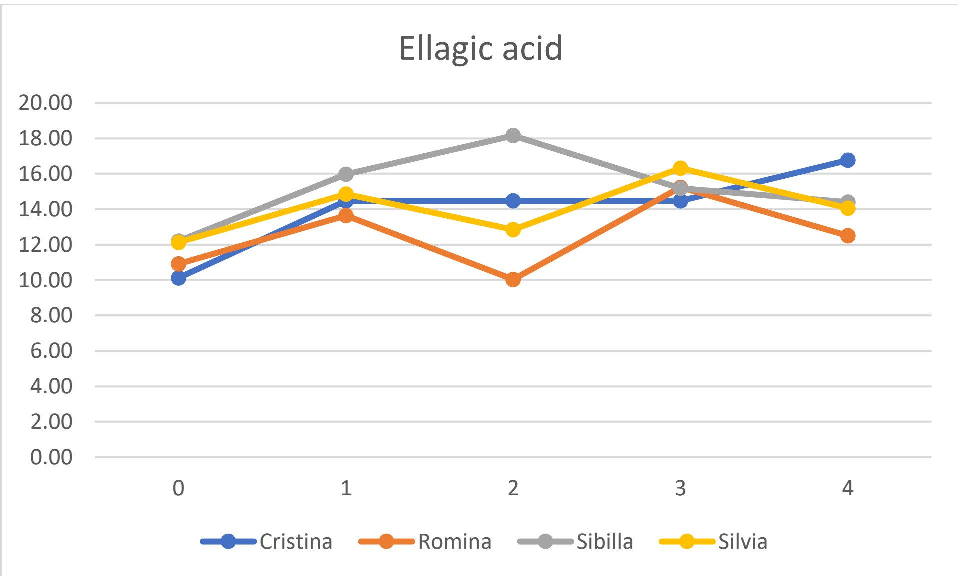
Graph 12: Chlorogenic acid content in Cristina, Romina, Sibilla and Silvia cultivars (control trial).

As *graph 13* shows, in the Cristina fruit the caffeic acid content shows a linear increase until 0,72 mg/100g day 4. In the Silvia fruit, the value progressively increases to 0,96 mg/100g on day 3, and then slightly decrease on day 4. In the Romina fruit the value records a significant increase to 0,70 mg/100g on day 2, to then decrease to 0,56 mg/100g on day 3. The Sibilla cultivar shows an oscillating trend from day 0 to day 4, in which it records a content equal to 0,65 mg/100g.



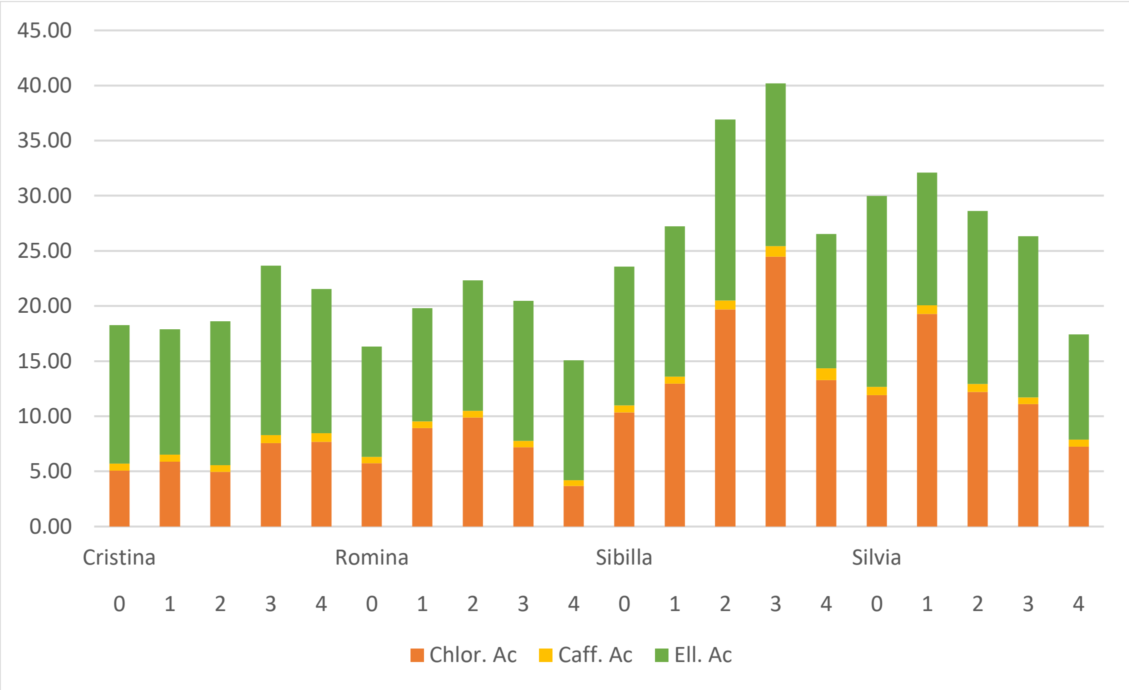
Graph 13: Caffeic acid content in Cristina, Romina, Sibilla and Silvia cultivars (control trial).

The ellagic acid content, as shown in *graph 14*, in the Silvia and Romina cultivar shows an oscillatory trend, from day 0 to day 4, reaching a maximum value on day 3 of 16,31 and 15,24 mg/100g, respectively. In the Cristina fruits the ellagic acid content shows a linear increase until 16,76 mg/100g day 4. In the Sibilla fruit the trend records a significant progressive increase until day 2, and then decreases to 14,39 mg/100g on day 4.



Graph 14: Ellagic acid content in Cristina, Romina, Sibilla and Silvia cultivars (control trial).

Graph 15 represents the phenolic acid (chlorogenic acid, caffeic acid and ellagic acid) content in strawberries contaminated with *Botrytis cinerea* from t0 to t4: the highest chlorogenic and caffeic acid content was achieved by Sibilla fruits with an average of 16,80 and 0,84 mg/100g, respectively, while the highest ellagic acid content was achieved by Silvia fruits with an average of 14,36 mg/100g. The lowest caffeic acid and ellagic acid content was achieved by Romina cultivar, with an average of 0,60 and 11,29 mg/100g, respectively, while the lowest chlorogenic acid content was achieved by Cristina fruits with an average of 6,34 mg/100g.

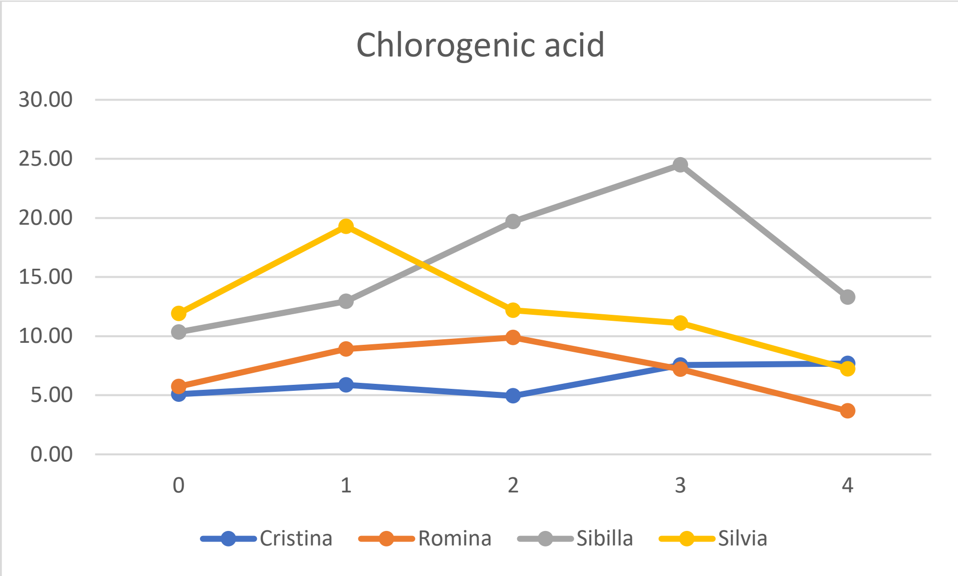


Graph 15: Phenolic acid content in control strawberries from t0 to t4 (treated trial).

Going into detail, it is possible to identify an increasing and linear trend of the Sibilla cultivar, with a chlorogenic acid content that increases from day 0 to 24,49 mg/100g on day 3, and then drastically decreases to 13,29 mg/100g on day 4. In the Cristina fruit the chlorogenic acid content shows a slight linear increase from 4,94 mg/100g on day 2 to 7,68 mg/100g day 4.

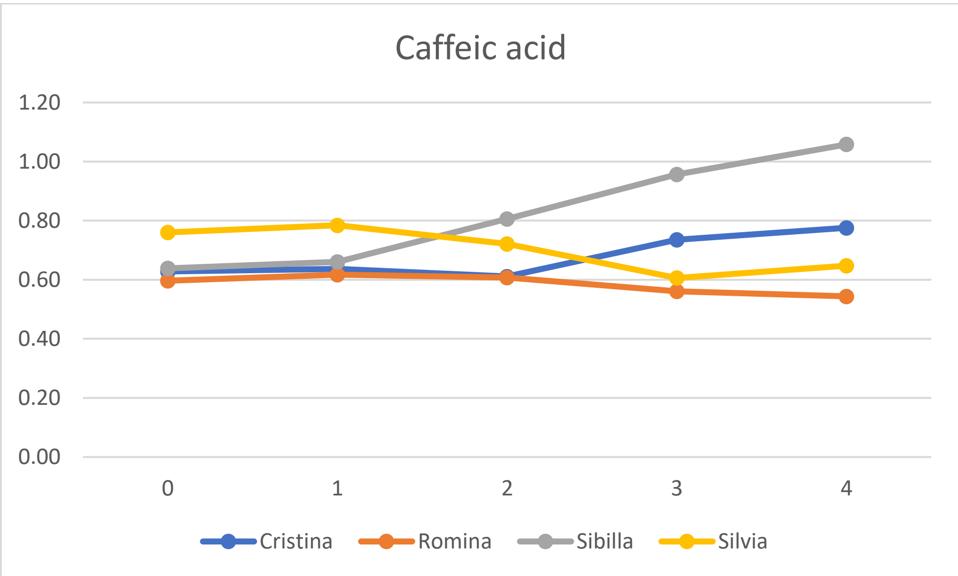
In the Silvia cultivar the content increases prematurely on day 1 to 19,29 mg/100g, and then decreases linearly to7,23 mg/100g until day four.

In the Romina fruit the value records an increase to 9,89 mg/100g on day 2, to then decrease to 3,67 mg/100g on day 4 (*graph 16*).



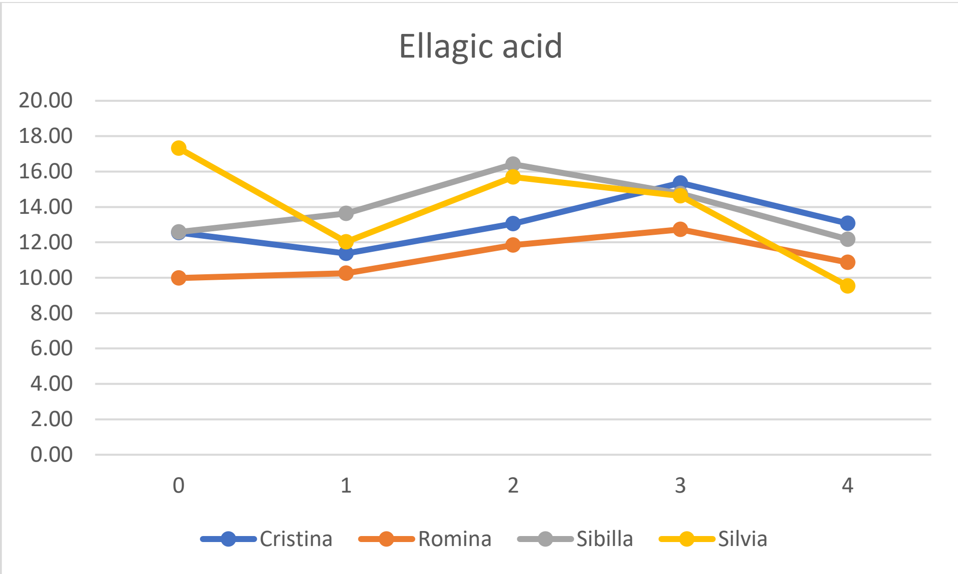
Graph 16: Chlorogenic acid content in Cristina, Romina, Sibilla and Silvia cultivars (treated trial).

As *graph 17* shows, in the Sibilla fruit the caffeic acid content shows a linear increase from day 1 to 1,06 mg/100g on day 4. In the Silvia fruit, the value progressively decreases to 0,61 mg/100g on day 3, and then slightly decrease on day 4. In the Romina fruit the value records a slight decreasing trend variation until day 4 to 0,54 mg/100g. The Cristina cultivar shows an increasing trend from day 2 to day 4, in which it records a content equal to 0,78 mg/100g.



Graph 17: Caffeic acid content in Cristina, Romina, Sibilla and Silvia cultivars (treated trial).

The ellagic acid content, as shown in *graph 18*, in the Cristina and Romina cultivar shows an increasing trend, from day 1 to day 3, reaching a maximum value on day 3 of 15,36 and 12,73 mg/100g, respectively. In the Silvia fruits the ellagic acid content decreases drastically to 12,03 on day 1, and then increases on day 2 to decrease again until day 4 to 9,54 mg/100g. The Sibilla cultivar reaches a maximum values on day 2 of 16,41 mg/100g, and then decreases to 12,18 on day 4.



Graph 18: Ellagic acid content in Cristina, Romina, Sibilla and Silvia cultivars (treated trial).

Figure 11 shows the different average trends of the phenolic acids content (chlorogenic, caffeic and ellagic acids) of the 4 cultivars, between the control and the infected fruits. From the comparative graphs it is possible to identify minimal but fundamental differences between the inoculated and the non-inoculated fruits.

Going into details, from the control trial it is possible to deduce how in the cultivars Cristina, Romina, Sibilla and Silvia, the content of phenolic acids tends to an increase, even if with varying trends among the four cultivars.

On the contrary, in the treatment trial, for the Cristina, Romina, Sibilla and Silvia cultivars, it is possible to identify a slight decrease in the content of chlorogenic and ellagic acid, with a decreasing trend at day 4 of shelf life. Caffeic acid represents the exception: while for the Romina and Silvia cultivars, at day 4 of shelf life, the caffeic acid content records a decreasing variation, the Cristina and Sibilla varieties show an increasing trend from day 2 to day 4, recording an increase in the caffeic acid content on day 4, compared to the control trial, of the 8,3% and 63,1%, respectively.

In conclusion, compared to the control trial, the contamination with *Botrytis cinerea* of the Cristina, Romina, Sibilla and Silvia cultivars records a slightly decreasing trend in the content of phenolic acids, albeit with some exceptions.

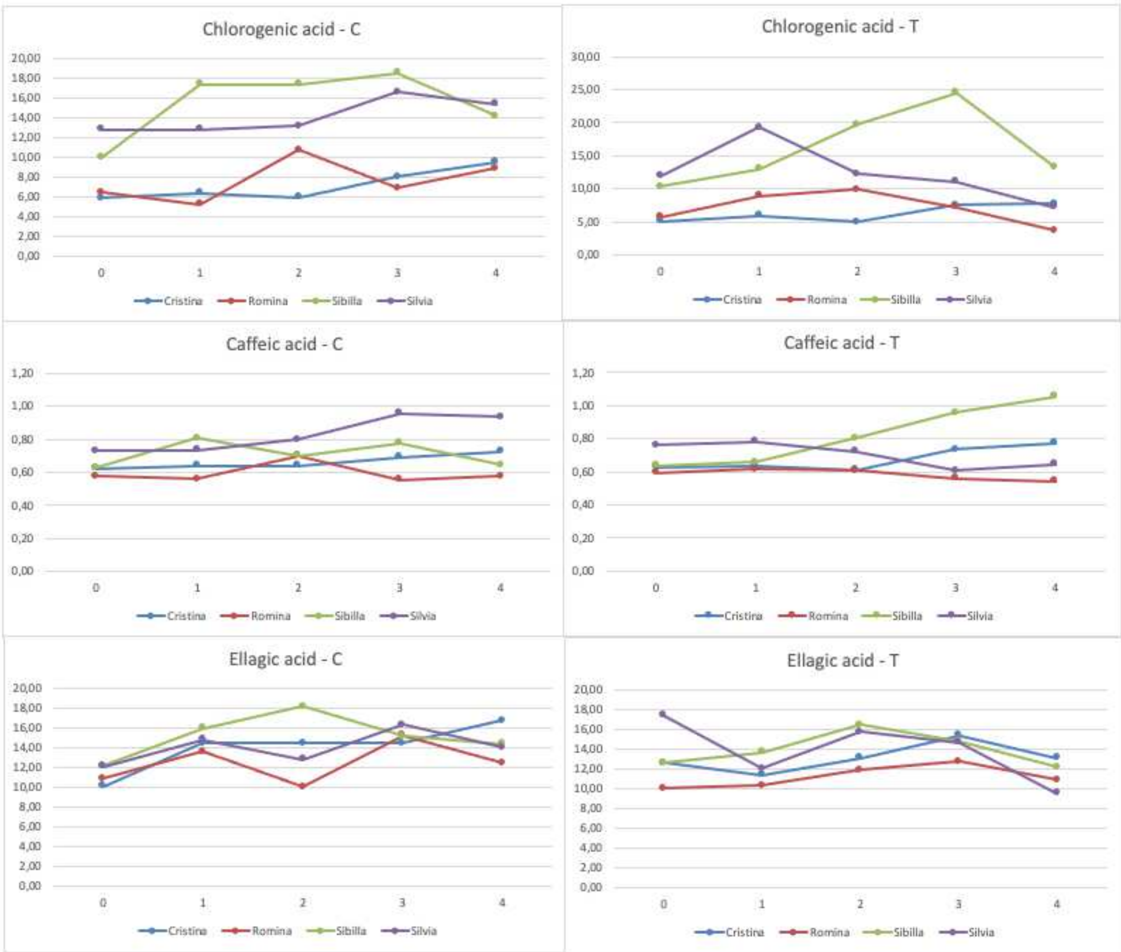


Figure 11: Phenolic acids trend from t0 to t4 in Cristina, Silvia, Romina and Sibilla cultivars. Differences between Treated and Control trials.

Chapter 5
CONCLUSION

For the present study, four well-defined strawberry cultivars (Romina, Cristina, Silvia and Sibilla) were selected in the 2020 harvest season and tested under treatment with *Botrytis cinerea*, the fungus that causes gray mold. The thesis work had two main objectives: determine the nutritional and qualitative changes, evaluating the content of soluble solids, titratable acidity, vitamin C and phenolic acids; and to evaluate the response and susceptibility of each cultivar, in response to the disease caused by the fungal pathogen.

The analyses carried out allowed to reach the following conclusions:

- The contamination with *Botrytis cinerea* has been shown to be effective in increasing the postharvest decay of strawberry cultivars after 5 days of shelf life. The severity index and McKinney's index of strawberry fruit were significantly increased by *Botrytis* treatment for Romina, Cristina, Silvia and Sibilla cultivars.

  Among the strawberry cultivars analyzed, the Silvia cultivar is the one that demonstrated a higher level of resistance to *Botrytis* infection during the treatment trial, compared to the Romina, Cristina and Sibilla cultivars, with a severity infection assessed to 3,6 (41% to 60% fruit surface infected) and a McKinney Index equal to 71,7%. The Romina cultivar on day 4 was assessed at grade 5 (81% to 100% fruit surface infected and showing sporulation), resulting in the cultivar most susceptible to the treatment, with a McKinney Index equal to 100%.

  This finding may be useful as a starting point for a more in-depth study of cultivars resistant to *Botrytis* infection.
- The results of the study also highlighted important qualitative changes in the four strawberry cultivars contaminated with *Botrytis cinerea*.

  It is possible to identify a general decrease in the content of soluble solids in both treated and control fruits, from day 0 to day 4. However, on day 4, compared to the control trial, the cultivars Romina, Cristina, Silvia and Sibilla contaminated with *Botrytis cinerea* recorded a decrease in the soluble solids content of -7.5%, -10.2%, -6.7% and -7.6%, respectively.

The contamination with *Botrytis cinerea* has been shown to be effective also in increasing the content of free acids of strawberry cultivars after 5 days of shelf life, compared to the control trial. The evolution of titratable acidity for the fruit infected with *Botrytis cinerea*, varied depending to the cultivar: at day 4, compared to the control trial, it is possible to identify an increase in the free acid content for the Sibilla, Silvia, Cristina, and Romina cultivars of 29%, 26%, 17.1% and 4.3%, respectively.

The results show how the soluble solids content present in strawberry cultivars can serve as nutrient for *Botrytis cinerea*, which can reduce their concentration, as the fungus assimilates simple sugars as a carbon source. Titratable acidity shows an opposite and increasing trend, compared to that of the control trial. In general, in strawberries, the total acidity increases to a maximum in mature green fruits, and then declines in the later stages of maturation. In this study, the contamination with *Botrytis cinerea* led to an increase in titratable acidity in the 5 days of shelf life of strawberry cultivars.

- The results of the study also highlighted important nutritional changes in the four strawberry cultivars contaminated with *Botrytis cinerea*. The contamination showed that the vitamin C content of the inoculated fruits decreased significantly compared with those of the non-inoculated fruits. The evolution of vitamin C for the fruit infected with *Botrytis cinerea*, varied depending to the cultivar: Cristina, Romina and Sibilla cultivars registered a decreased variation of – 92,7%, – 96,6% and – 86,4%, from t0 to t4, respectively; while, since day 0, the Silvia cultivar has shown a low content of vitamin C, such that it does not allow to ascertain the effect of *Botrytis* infection over time.

  The decrease in vitamin C content can be associated with the degree of pathogen severity in the fruits, as the parameters are linked by a negative correlation. As the decay severity index and the McKinney index increase up to day 4, reaching maximum levels of 100% of severity, the vitamin C content decreases.

- The contamination with *Botrytis cinerea* of the Cristina, Romina, Sibilla and Silvia cultivars records a slightly decreasing trend in the content of phenolic acids, albeit with some exceptions. Compared with the control trial, for the Cristina, Romina, Sibilla and Silvia cultivars, it is possible to identify a slight decrease in the content of chlorogenic and ellagic acid, with a decreasing trend at day 4 of shelf life. Caffeic acid represents the exception: while for the Romina and Silvia cultivars, at day 4 of shelf life, the caffeic acid content records a decreasing variation, the Cristina and Sibilla varieties show an increasing trend from day 2 to day 4, recording an increase in the caffeic acid content on day 4, compared to the control trial, of the 8,3% and 63,1%, respectively.

In conclusion, the purpose of the thesis has been largely achieved. Based on these considerations, *Botrytis cinerea* can alter the sugar content, the sugar-acid balance and can decrease the nutritional content of the strawberry fruits, in particular by drastically reducing the vitamin C content, an essential compound in the antioxidant action of the fruit and which makes the strawberry an important source for human nutrition.

Many details of the *Botrytis cinerea*-strawberry relationship are still not fully understood, and more research will be needed to understand the interaction between qualitative and nutritional content and the action of the pathogenic fungus.

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