Università Politecnica delle Marche
PhD School in Agricultural, Food and Environmental Sciences
Curriculum in (Agricultural, Food and Environmental Sciences)

Factors affecting strawberry quality in fresh and stored fruits

Ph.D. Dissertation of:

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Advisor:

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Abstract

The strawberry (*Fragaria x ananassa* Duch.) fruits are one of the most widely and popular consumed berries worldwide, due to the organoleptic and appreciable nutritional properties. In the past years, there has been increasing attention and growing number of scientific proofs regarding the consumption of strawberry fruit since it is beneficial to consumers. The composition of strawberry fruits varies and it depends on many pre- and postharvest factors, such as individual genotypes, environmental factors, cultivation techniques, maturity stage and postharvest practices. These considerations are also important to improve strawberry genotypes in term of nutrient and phytochemical compounds and, on the other side, to avoid processing steps and product treatments that lead to a high reduction of the quality.

This study performed the evolution of two years breeding program to increasing the nutritional quality along with near agronomic standards required by the market, also study four well-defined strawberry cultivars (Romina, Cristina, Silvia and Sibilla) were selected and tested under treatment with Botrytis cinerea to determine the susceptibility of each cultivar and its relation with fruit quality and applied different cold-storage temperatures (domestic −20 °C and industrial −80 °C) on different treatments (whole fruits and dried fruits) of three strawberry cultivars (Arianna, Francesca, and Silvia), for up to seven months, and evaluated the influence of different storage conditions and lengths on the stability of the fruits' nutritional compounds.

The results show that the high variability in bioactive compounds composition among cultivars and breeding materials, underlying the importance of including wild germplasm for increasing fruit nutritional quality through breeding programs, also highlighted important qualitative changes in the four strawberry cultivars contaminated with Botrytis cinerea, by a decrease in the soluble solids content and increase of acids, in all cultivars phenolic acids and vitamin C there was strong decreased in treated fruit compare to control. In addition, the nutritional quality of the fruits was significantly affected by storage temperature (with −80 °C storage preserving more nutritional compounds), while storage time did not greatly affect the composition of the nutritional compounds in the whole or dried fruits. Oven drying the fruits dramatically affected the nutritional compounds. In fact, results indicated the possibility to generate new cultivars producing fruit with high nutritional contents, stable at the different cultivation cycles, to be labelled with a compositional claim. Strong positive correlation was obtained from nutritional quality with resistance to decay, as high amount of nutritional content more tolerance to botrytis cinerea and it is worth considering in future studies to better plan fruit-storage conditions and time, for maintaining better fruit nutritional quality.

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Chapter 1.

Introduction

The strawberry (*Fragaria x ananassa*) are economically and commercially important and is a fruit that appreciated and widely consumed in the world all year round as fresh or in processed forms, such as jams, juices, and jellies. The annual production of strawberries exceeding 8.3 million metric t (MT) cultivated on a surface of 372,361 ha (FAO, 2019).
Strawberry is one of the important dietary sources of fiber and bioactive compounds, both micronutrients and phytochemicals. In particular Strawberries are rich of vitamin C and folate. For instance, a handful of strawberries is sufficient to cover the vitamin C recommended daily allowance (RDA) and 250–350 g of strawberries (~200 μg of folate on average) can supply 60–100% of the daily European folate intake recommendations (200–300 μg/day) (Tulipani et al., 2008). Finally, strawberry is an important source of phytochemicals such as phenolic acids, flavonoids and anthocyanins (Oszmianski et al. 2009; Giampieri et al. 2012; Ariza et al. 2016), Moreover, the total antioxidant capacity (TAC), which measures the radical scavenging activity is related to the amount of antioxidant compounds in strawberries. Several studies have demonstrated various cardiovascular, anti-proliferative, and neurologic benefits associated with the consumption of strawberries. Although most health-promoting effects were initially observed with in vitro studies, there is increasing animal and clinical research focused on translating the in vitro evidence into in vivo outcomes (Giampieri et al., 2012).

1.1. Concept of quality of strawberry

The concept of fruit quality is complex and difficult to define, as it is influenced by different factors, disparate 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 quality of fruits based on the organoleptic and nutritional properties and the possible benefits that the consumption of the fruit 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, quality of fruit 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.
So, the main descriptors of the concept of Strawberry fruit quality is mainly based on the chemical composition (sugar contents, acidity, volatile organic compounds, macro and microelements, bioactive compounds) or physical characteristics (texture, shape, size, color), or composition of these two factors. All those characteristics are highly appreciated by the consumers, but their level could change during the strawberry fruit storage, with a degradation when the storage time is prolonged (table1).

Table1. 1. Main descriptors of the concept of Strawberry fruit quality

Strawberry Plant yield Efficiency	Organoleptic Quality	Nutritional Quality
•Plant yield •Harvesting speed •Resistance of pathogens •Fruit size	•Fruit Shape •Fruit color •Fruit firmness •Fruit taste •Fruit flavor	•Fiber content •Vitamin content •Mineral content •Phenolic content •Antioxidant capacity

1.1.1. Strawberry plant yield Efficiency

For evaluation the plant yield efficiency, the first parameter that a grower considers in the decision to grow a new cultivar is Plant yield (amount of fruits with commercial value harvested for each production cycles). Furthermore, the harvesting facility is another important parameter, which directly effect on decreasing labour cost. In addition, plant adaptability to different pedo-climatic conditions and resistance to pest and pathogens are parameters are always dealing with them.
Among these, fruit size is certainly one of the most important character in the selection of new genotypes.
The fruit size is evaluated through the implementation of some different parameters:
• Fruit Weight: indicates the average weight of a representative sample of fruits and refers to the dimension of the fruit;
• Fruit Length: indicates the average size on the longitudinal region of a representative sample of fruits;
• Fruit Diameter: indicates the average size around the equatorial region of a representative sample of fruits.

1.1.1.1. 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 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 main strawberry pathogen is Botrytis cinerea, the causal agent of gray mold, followed by Rhizopus stolonifera, Mucor spp., Colletotrichum spp., and Penicillinum spp. (Feliziani and Romanazzi, 2016). Postharvest diseases caused by these pathogens are the result of latent infections that are initiated in the field during the growing season and infections from wounding during harvest and handling operations (Michailides and Manganaris 2009). Early detection of fungal infected fruit is very important both for producers and consumers. Fungal contamination is especially dangerous in packing houses during storage, transport and marketing procedures because even a very small number of infected fruit can spread the infection to adjacent healthy strawberries. The presence of fungal diseases on fruit surfaces not only causes loss of quality, but also diminishes the safety of the final product. Some fungal genera and species can produce mycotoxins, which cause infections or allergies in susceptible individuals (Gallo et al., 2015).

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.

1.1.2. Strawberry sensorial and organoleptic quality

By focusing on fruit quality, the sensorial traits are the primary guide for consumer acceptance. For evaluation of the sensorial quality, the consumers evaluate the sensory quality of the fruits by combining the appearance of the fruit (color, shape, size and firmness) with the taste of the fruit (sweetness and acidity), in order to satisfy their personal definition of the ideal fruit (Mazzoni et al., 2020). Additionally, the flavor is one of the main attributes that the consumer appreciates in berries, and that can influence the consumer's acceptance. Among the volatile organic compounds (VOCs) responsible for the aroma of berries, a subset of about 20 volatile compounds have a high impact on the human nose (Ulrich and Olbricht, 2013). For these reasons, most of the actual cultivar breeding programs include sensory quality as an important breeding objective (Ulrich et al., 1997). As a consequence, the new cultivars need to combine high yield standards to the more appreciable and appealing fruit traits.

1.1.3. Strawberry nutritional quality

The other important aspect of fruit quality is the nutritional value. In fact, a careful consumer also seeks fruits with high nutritional value. The interest raised around the nutritional quality of berries is due to the fact that bioactive compounds present in these fruits could protect the human body from the onset of several chronic diseases, such as cardiovascular events, cancer, and other age-related degenerative diseases, as well as for the general health benefits they can provide (Scalzo et al., 2005).

Strawberries contain high levels of a diverse range of phytochemicals, most of which are phenolic molecules. These phytochemicals contain a range of advantageous compounds, such as essential minerals, vitamins, fatty acids, and dietary fibers. Strawberries are an important source of provitamin A, minerals, vitamin C, and B-complex vitamins. (Giampieri et al., 2012; mazzoni et al., 2020), which acts as antioxidant molecules and helps to boost the immune systems (Pantelidis et al., 2007). Strawberry fruits contain about 15% soluble solids (mainly sugars) and their high level of fructose makes them valuable for individuals with diabetes (Nile and Park, 2014). The high dietary fiber content is important because fruit pectin acts as an intestinal regulator Ramadan et al., 2008). Some of the known chemo preventive agents present in berries include vitamins A, C, and E, and folic acid; calcium and selenium; carotene and lutein; phytosterols such as sitosterol and stigmasterol; triterpene esters; and phenolic molecules such as anthocyanins, flavonols, flavanols, proanthocyanidins, ellagitannins, and phenolic acids (Figure 1). The chemistry of berry phenolic directly influences their bioavailability, metabolism, and biological effects in vivo. In fact, the ultimate goal for the breeders is the obtainment of new cultivars with highly resilient plants, to low input cultivation systems and high adaptability to future climate scenarios, and able to bear high yields of organoleptic and nutritional quality fruits (Feliziani et al., 2016).

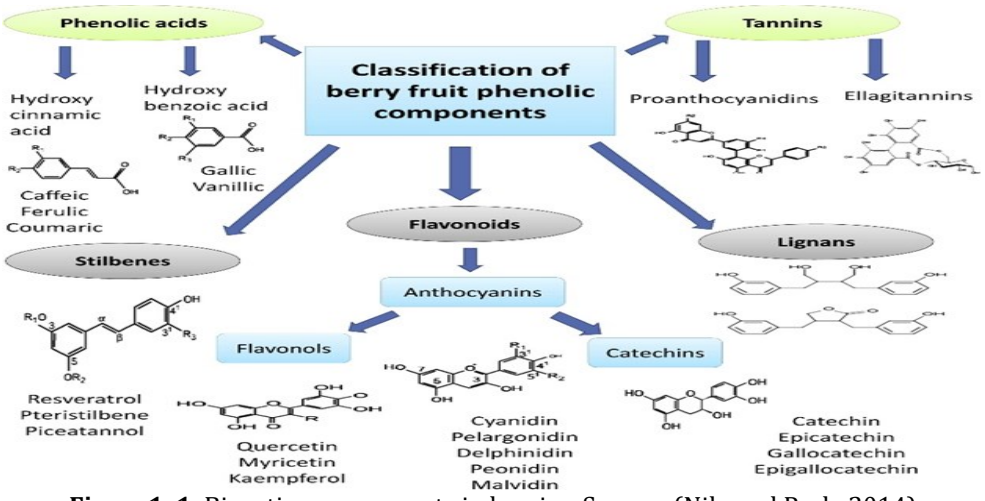

Figure1. 1. Bioactive components in berries. Source: (Nile and Park, 2014)

1.2. Factors effecting strawberry fruit quality

The huge number of strawberry cultivars available on the market are generally differing for specific yield efficiency, organoleptic and nutritional characteristics. Fruit breeding programs are mostly focus to produce new cultivars with the best compromise between these characteristics. The composition of strawberry fruits varies (Di Vittori et al., 2018) and it depends on many pre- and postharvest factors, such as individual genotypes, environmental factors, cultivation techniques, maturity stage and postharvest practices (Tulipani et al., 2011). These considerations are also important for both agronomic production and for fruit industry, on one side to improve strawberry genotypes in term of nutrient and phytochemical compounds and, on the other side, to avoid processing steps and product treatments that lead to a high reduction of the quality (Alvarez-Suarez et al., 2014).

1.2.1. Genetic and environmental Factors

Genotype factors are the main factor that effected on quality (Di vittori et., 2018). Fruit yield, organoleptic and nutritional quality are strongly controlled by the genotype (Galletta et al., 1995), and can vary with the cultivars (Prange and De Ell,1997). Therefore, from a quality standpoint, selection of the cultivar may be the most important management decision in Strawberry production. The genetic background may play a pivotal role in determining the antioxidant, micronutrient and phytochemical composition of strawberries, although few genotypes have been well characterized for these important features (Kafkas et al., 2007, Tulipani et al., 2008).
There are also variations between cultivated and wild fruit species that are very remarkable. When compared to the corresponding cultivated cultivars, the wild species have better levels of nutritional qualities, but they also have lower levels of other crucial fruit quality traits, such as, fruit size and firmness. As a result, wild germplasm plays a crucial role as a source of genes for improving the nutritional, nutraceutical, and fruit quality of crops. Volatile compounds are mainly studied for the fruit aroma but their role in fruit nutritional value has been also studied in strawberry germplasm.
Accessions of the progenitor wild species are valued by strawberry breeders as sources of novel traits, and especially for pest resistance and abiotic stress tolerance (Hancock et al., 2002). Furthermore, previous investigations (Tulipani et al., 2008; Capocasa et al., 2008; Diamanti et al., 2014; Mazzoni et al., 2020) have shown improvements in fruit nutritional quality in breeding material that originated from *Fragaria virginiana* ssp. glauca (FVG) in inter-species crosses. Improvements of these traits can also be achieved by programming F. × ananassa (Duch) intra-species crosses and producing progeny with productivity traits that are more similar to those of the commercial cultivars. The two types of combination programs (inter-species back-crosses, intra-species crosses) can be used for the improvement of strawberry nutritional quality, but the success of any such program is strictly related to the ways in which the attributes of the different parents combine.

Also, the environmental conditions to which the plants are subjected, and the annual climatic changes during the different ripening times of the fruits, may partly explain the different accumulation patterns of chemical compounds in strawberries, and may strongly influence their nutritional quality (Tulipani et al., 2011). The success of strawberry crop is deeply influenced by cultivar choice; it depends by each region weather characteristics, mainly temperature and photoperiod (Duerte Filho et al., 2007).
Some cultivars show a high productivity and good fruit quality in their origin country, but they do not exhibit the same potential in other area. Before promoting the advanced selections of a specific breeding program to commercial variety, it is essential to test their potential in private and/or farmer associations' fields. The aim is to achieve new cultivars that are the most possible adapted to different growing areas, by testing them in different climatic and environmental locations. The cultivars with best results (vegetative, productive and qualitative) can be chosen for subsequent tests in large growing area.

1.2.2. Harvest and storage

Clearly, harvest and storage conditions also influence the composition of the fresh fruit. The ripening stage of the fruit at the harvest time is an important factor, because chemical and compositional modifications occur when the fruit is attached to the mother plant (Park and Yoon, 2013). Strawberries are highly perishable. Their perishable nature can lead to the physical damage during storage and it is difficult to store them at room temperature.
Strawberries post-harvest decay can be due to physical, physiological or pathological factors that may happens in pre-harvest or in post-harvest period and their shelf life is diminished by firmness loss, fruit desiccation and the growth of spoilage micro-organisms (Bhat and Stamminger, 2018). Strawberries are highly perishable non-climacteric fruits and their perishable nature can lead to physical damage during storage and transportation. Therefore, the large market of fresh fruit relies on the capacity of fast distribution and marketing under a continuous cold storage chain. Fruit over production, especially during off-season periods, are addressed mostly to processing industry for bakery products such as jam, jellies, juice, puree, flavor additives, etc. (Giampieri et al., 2012; Bhat and Stamminger 2018), with the consequence of price reductions.
Due to high market value of strawberry fruit, post-harvest maintaining is an important research area which needs to be focused. There are many approaches pro-posed by researchers around the world for extending the shelf life of fresh strawberries, mostly addressed to increase fruit quality stability with pre-harvest (Di Vittori et al. 2018) (Table 2 and Table 3). Post-harvest treatment of strawberries could be achieved in two ways: directly expose the fruits to preservation techniques such as physical treatment (e.g., ionizing and nonionizing radiations) and use of chemicals (e.g., fumigation), or

food processing techniques (e.g., transformation in juice, purees) (Bhat and Stamminger, 2014).

1.2.2.1. Preservation methods for strawberry fruits

Strawberry fruits have chemical, physical and physiological characteristics which make them very highly perishable, with maximum 1 week of shelf life as a fresh fruit, and it is difficult to store it in consumer places (Ramirez et al., 2019).
Strawberries deterioration may be due to different factors such as fungal rots, moisture loss, softening, and mechanical damage. For this reason, many studies have been performed in the field of fresh fruit preservation techniques for safe storage as well as shelf-life extension for fresh fruit consumption by the consumer, and some methods have been successfully implemented. Today, with remarkable advancement in post-harvest technology and food engineering, many effective techniques are developed to further improve the shelf life and maintain the original quality (Bhat and Stamminger, 2014) (figure 2).
The scientific literature reports many techniques available to preserve and ex-tend the shelf life of strawberries.

Temperature management

Strawberry fruits are very sensitive to room or high temperatures; they may resist 1-2 days with marketable quality (Garcia et al., 2012). Temperature management is the key factor in minimizing the deterioration and preserving the strawberry quality. High storage temperatures increase the respiration rate and decrease the storage period, with a loss of fruit quality (Cordenunsi et al., 2005). Respiration rate increases from 28ml CO2 kg-1h-1 at 5˚C to 127ml CO2 kg-1h-1 at 20 ˚C (Robinson et al., 1975). To limit the fruit deterioration after harvesting, strawberry fruits should be pre-cooled immediately with forced air or placing in refrigerator: each hour of delay between fruit picking and precooling could decrease 1 day of shelf life (Pelletier et al., 2011). The shelf life of fresh strawberry at 0-4 ˚C is around 5 days (Vargas et al., 2006). Storing the fruit at 0-1 ˚C with 90-95% relative humidity minimizes the physiological deterioration and suppresses decay rate (Pelletier et al., 2011).
The enzymes in strawberries which are involved in antioxidant mechanisms such as glutathione reductase, glutathione peroxidase, ascorbate peroxidase, guaiacol peroxidase, catalase, superoxide dismutase, monodehydroascorbate reductase, and dehydroascorbate show high activities at high temperature (≥ 5˚C) compared to store at low temperature (0˚C). Total phenolic compounds, total anthocyanins, and ascorbic acids are higher in fruits stored at high temperature or either for longer period (Jin et al., 2013). But low temperature affects fruit firmness, titratable acidity, total soluble solids, and total terpenes in strawberry fruits (Li et al., 2015). Fruit losses of vitamin C are highly related to post-harvest conditions, including long-term storage, elevated temperatures, low relative humidity, physical damage of fruit during handling, and chilling injury (Lee and Kadeer, 2000). Vitamin C content is highly sensitive against

high temperature and influenced by storage temperature and relative humidity. Lee & Kader (2000) indicated that temperature management is the most critical tool in maintaining the nutritional quality. In fact, the loss of fruit nutritional quality has been reported after delays between harvesting and cooling. The influence of temperature on vitamin C content of strawberry fruits has been reported. Although vitamin C is also lost in refrigerated conditions, the loss is more pronounced under ambient conditions. For in-stance, strawberry fruits were stored at 1-10˚C for 8 days and 4 days at 20 ˚C; more vitamin C content was found at lower temperature (1-10°C), but there was more vitamin C content at 10 ˚C compared to 1 ˚C (Nunes et al., 1998). Cordenunsi et al. (2005) also detected an increased content of vitamin C in fruit after 6 days of storage at 16 ˚C, compared to fruit stored at 25 ˚C. Anthocyanins are quite unstable compounds. During storage, they are subjected to many chemical and enzymatic reactions that could lead to their loss or modification of their chemical form; their stability is influenced by different factors such as temperature, storage, presence of enzymes and other substances like ascorbic acid, light, oxygen and the concentration of these compound (Liu et al., 2018). To retain the initial amount of anthocyanin content during storage for fresh use, temperature management is an important factor. In this respect, is important to manage correctly the storage temperature and the speed of lowering the temperature immediately after harvest (Lee and Kadeer, 2000). Octavia & Choo (2017) reported that the anthocyanin content decreases gradually even during cold storage. Cordenunsi et al. (2005) stored the strawberries at 6, 16, 25 ˚C for 6 days, reporting that during the storage the amount of anthocyanin were increased significantly in all temperatures, and all strawberries presented lower amount of anthocyanin when stored at 6 ˚C. In another study, the stability of anthocyanin in strawberries at different pH and temperatures were studied: the results showed that there was low anthocyanin stability at 23 ˚C than at 6 ˚C, and for pH was reported that at low pH (less than 4) anthocyanins had more stability than higher pH (higher than 6) (Hernández-Herrero and Frutos, 2014). Stability of anthocyanins in cold storage can be also affected by other factors such as solvents, oxygen, light, enzymes and other ac-companying substances (Patras et al., 2010). Cooling (4°C storage) remains the most useful and effective treatment to maintain the stability of phenolic acid fruit content (Cordenunsi et al., 2005; Kadivec et al., 2013); when fruits were maintained at 0°C, a more constant value of phenolic acids, up to 13 days, was observed (Ayala-Zavala et al., 2004), while in strawberry fruit stored at 5-10°C it was observed an in-crease of phenolic acid content.

Controlled atmosphere storage

Controlled atmosphere (CA) storage aims to preserve the commercial quality of the fruit managing the composition of the atmosphere where the fruit is stored, that is different from the composition of the air (Parvez and Wani et al., 2018). Generally, CA storage is characterized by increasing the CO2 concentration and decreasing the O2 concentration in the atmosphere of the storage chamber (Berna et al., 2007). Change in the composition of the atmosphere is always along with low temperature and high humidity during storage. 15-20% CO2 concentration and 2.5- 5% O2 concentration

were suggested in many studies to create the most suitable atmosphere composition for strawberry storage (Yang et al., 2020; Li et al., 2020).
Strawberry fruits in CA storage can retain better quality even at higher temperature (10˚C) than fruits stored in air conditions. However, lower temperature (4˚C) in CA storage allowed a better fruit quality keeping than higher temperature (Nunes 2002).
Strawberry fruits stored in CA storage maintained higher firmness, titratable acidity, total soluble solids and ascorbic acid content than just cold stored fruits (Li et al., 2015). Similarly, positive results were observed regarding the weight loss, the anthocyanin content and the decay rate in strawberry fruits stored in CA storage instead than normal air storage (Yang et al., 2020).
CA storage of strawberries is not use commercially, and MAP is the backbone technology of strawberries. Therefore, the CA studies were initially performed to simulate MAP with a similar gas composition to evaluate the quality of strawberry fruit. High-CO2 leached activated MAP, selected based on the results of the CA study, is used to control the microbiology and maintain overall quality of strawberry fruit stored in a high CO2 atmosphere (Nakata et al., 2020).

Modified atmosphere packaging

Modified atmosphere packaging (MAP) is an inexpensive storage method that can be alternate to CA storage. Fruits are sealed in a package when the air inside has a high CO2 concentration. Generally, MAP provides perforated and non-perforated polymeric films which have specific permeability for CO2 and O2 (Zheng et al., 2008). Perforated film has shown positive results in maintaining the quality and extending the storability and shelf life of strawberry fruits (Van der Steen et al., 2002). The main concern with using MAP for strawberry storage is that improper designs can lead to high CO2 concentrations and reduced O2 over time when desired temperature conditions are not met during storage. This can lead to less oxygen presence in the atmosphere than necessary to maintain basic aerobic respiration. Most commercially available plastic films are unable to maintain proper O2 and CO2 permeability ratios (Mahajan and pongener, 2019).
Generally, for strawberries packaging the recommended composition of MAP is 5-10% O2, 15-20% CO2 and 70-75% N2 (Barikloo and Ahmadi, 2018). Barrios et al. (2014) studied different gaseous compositions (0–24% O2 and 0–15% CO2) and temperatures (10, 19, 23°C) and reported that low temperature is necessary for MAP storage because respiration rate is mainly affected by temperature than by gaseous concentration. In a study of Barikloo & Ahamdi (2018), strawberries were packed with MAP, and stored at 4˚C and 25˚C: the result showed that strawberries stored at 4˚C had less respiration rate than the ones stored at 25˚C.
MAP increased total soluble solids, titratable acidity, ascorbic acid, anthocyanin, reduced the decay rate, tissue softening, weight loss, browning index and cell membrane permeability (Barikloo and Ahmadi, 2018; Jouki and Khazaei, 2014; Rana et al., 2020). However, MAP alone is not able to stop the infection of fresh strawberries by Botrytis mycosis and Rhizopus. Therefore, treatment with different thermal and non-thermal techniques before packaging with MAP has been studied.

The effect of low-dose gamma irradiation (1 kGy) and active equilibrium modified atmosphere packaging (EMAP1: CO2 10%: O2 5%; N2 85% and EMAP2: CO2 5%: O2 10%; N2 85%) on quality of strawberry fruits stored at 4 °C was investigated. As a result, MAP combined with low dose gamma radiation extended the shelf life up to 14-21 days compared to only MAP that increased shelf life by 3-7 days (Jouki and Khazaei, 2014). In general, the application of modified atmosphere technologies also showed a positive effect in reducing changes in fruit chemical composition. In fact, loss of vitamin C could be reduced by storing fruits at low oxygen atmosphere (Delaporte, 1971). But losses in the fruit content of vitamin C have been detected when applying higher amounts of CO2 (10-30%) (Agar et al., 1997). Bhat & Staminger, (2015) studied the impact of modified atmosphere on strawberry at cold and room temperature storage conditions and reported that samples stored with modified atmosphere packaging had more degradation on vitamin C content during cold storage compared to fruits without modified atmosphere packaging. The same decrease of vitamin C content was detected in fruit packed with modified atmosphere during storage at 5±1˚C (Dong et al., 2014). Suitable CO2 and low O2 can slow the respiration of fruits and decrease the senescence of fruits. Thus, slow the vitamin C loss (Manurakchinakorn et al., 2004). With 60% O2 + 1.5% CO2 initial gas condition, the modified atmosphere packaging could maintain fruit firmness, and vitamin C con-tent. As the season in which strawberries are harvested is characterized by high temperature, the effect of preservation is more evident in 5±1 °C (Jing et al., 2014). Modified atmosphere packaging during cold storage had showed superior results to delay the degradation of anthocyanin content of strawberry during storage (Nunes et al., 2002; Yang et al., 2020). Pinto et al., (2020) reported that the combination of ozone and MAP led to a better preservation of the total and individual anthocyanins during cold storages. Modified atmosphere had also showed positive effects on phenolic acids content than stored on normal air storage (Yang et al., 2020).

Edible coating and films

Edible coating is a thin layer which is applied on the surface of product as protective cover (Aday and Caner, 2010). It is a nontoxic and environmentally friendly method that allows to create a modified atmosphere in the package and is used to prevent moisture loss and microbial growth. Its application delay deterioration, as well as limits O2 and CO2 exchange, maintaining post-harvest quality and increasing storage and shelf life of fruit and vegetables (Duan et al., 2011). Edible coating can be applied through immersion of the food product in the solution or by sprinkling. The structural matrix of edible coatings is composed of carbohydrates, proteins, lipids or their different combinations (Han, 2013).

Considering the economic aspect and functional advantages, edible coating also has been used for maintaining the quality of strawberry fruit during storage, and positive effects on their appearance, shelf life, and quality have been shown (Barikoloo and Ahmadi, 2018; Guerreiro et al., 215; Treviño-Garza et al., 2015).

Edible coating based on sodium alginate and pectin enriched with essential oils has been studied for the shelf-life extension of strawberries: it resulted effective in reducing

microbial decay and was reported that strawberries could be stored for 7 days maintaining good sensory properties (Guerreiro et al., 215). Fan et al. (2009) studied the effect of edible coating based on alginate combined with yeast antagonist for the quality preservation of strawberry fruits: it was reported that this edible coating exerted many bioactive and functional benefits, including less respiration rate and lower microbial infection. Chitosan, pullulan, alginate and pectin were applied in many studies to improve the strawberry quality during shelf life and was reported that polysaccharides-based coatings improve the physicochemical and sensory characteristics, delay tissue softening and decay and increase the shelf life of strawberries up to 14 days (Barikoloo and Ahmadi, 2018; Treviño-Garza et al., 2015; Wang and Gao, 2013).

Edible coating based on natural Aloe vera gel, in composition with ascorbic acid, has also been studied at different concentration during strawberry storage, and was found that treated strawberries had less weight loss, higher total soluble solids content, vitamin C concentration and titratable acidity than untreated and coating reduced the population of total aerobic mesophilic organisms, yeasts, and molds during storage (Sogvar et al., 2016). The water-solubility and thermobility of vitamin C make it very sensitive to some post-harvest treatments. Post-harvest treatments such as chitosan coating (Bal, 2019), have been reported to maintain vitamin C content in strawberry fruits. Edible coatings were also reported for reducing the rate of deterioration of anthocyanins in strawberries during storage (Khodaie et., 2021; Riaz et., 2021). The application of the chitosan coating could be favorable in maintaining phenolic acids during storage (Wang and Gao, 2013). Coated straw-berries during refrigerated storage had higher amount of phenolic acids up to 18 days (Hussain et al., 2012).

Chemical treatments

The use of chemical compounds to control the decay and delaying the senescence process for increasing shelf-life of fruits and vegetables has been widely studied. From inorganic salts to a wide variety of organic compounds have been used and have been shown to have impact on the metabolisms of plant tissues. The com-pounds used for food protection and preservation must be safe for human consumption and their concentration must not change the organoleptic properties of the treated products.

Chemical fumigants such as methyl iodide and chemical preservatives such as sodium benzoate and potassium sorbate are extremely common for post-harvest preservation in strawberries farms in United States of America (http://www.pesticideinfo.org/DS.jsp?sk=1016) (Bhat and Stamminger, 2015). Another highly studied com-pound in strawberry preservation is pure methyl jasmonate or in solution with ethanol (Ayala-Zavala et al., 2005). Different studies concluded that methyl jasmonate suppresses the fungal growth, reduces the losing of ascorbic acid and other bio active compounds, preserves the firmness and controls the decay of strawberry, also increase the volatile compounds such as methyl acetate, isoamyl acetate, ethyl hexanoate, buthyl acetate and hexyl acetate (Asghari and Hasanlooe, 2016; El-Mogy et al., 2019). Salicylic acid and gibberellic acid are also used for in-creasing shelf life of strawberries and lots of studies showed that they have ability

to control the proliferation of fungi, to delay the process of color generation in fruits, increase the activity of phenylalanine ammonia lyase (PAL) and other enzymes such as chlorophyllase which decrease the speed of fruit ripening and increase the shelf life period (El-Mogy et al., 2019; Asghari and Hasanlooe, 2015).

Application of synthetic chemicals for the control of diseases is a standard commercial practice worldwide; however, interest is increasing in the physical or biological methods to maintain the post-harvest quality and control disease in fruit because of increasing the awareness of chemical compounds that are harmful to human health and the environment. In recent years, essential oils (EOs) have gained particular interest to control post-harvest disease due to their volatility, relatively safe status, wide acceptance by consumers, and eco-friendly and biodegradable proper-ties (Tzortzakis and Economakis, 2007). Use of essential oils in preventing microbial contamination and their positive effects on quality parameters such as firmness, color and quantity of polyphenols of strawberries have been reported in many research (Bhat and Stamminger, 2015; Ramirez et al., 2019). Essential oils are mixture of volatile terpenoids (monoterpenes, sesquiterpenes) and other compounds such as aldehydes, ketones, esters. The amount and presence of those compounds in an essential oil depends on the sources from which it is extracted and on the extrac-tion methodology (Ramirez et al., 2019). In the case of strawberries, tea tree oil (Melaleuca alternifolia), cassia oil (Cinnamomum Cassia), ajwain oil (Trachyspermum ammi L.), and cinna-mon oil (Cinnamomum zeylanicum) are reported in different studies to significantly control the microbial contaminations, as well as quality parameters such as color total soluble solids, pH, total acidity and antioxidants (Shao et al., 2013; Wei et al., 2018).

Calcium Chloride ($CaCl_2$) is one of the most common used compounds in post-harvest treatment. Positive results were obtained in maintaining the quality parameters of strawberries in different studies (Nguyen et al., 2020; Shahzad et al.,2020). Another inorganic compound is Hydrogen sulfide (H_2S), which play a key role in the metabolism of the maturation in fruits. This compound prolongs the useful life of the product and its effect depends on the dosage (Ramirez et al., 2019). In different studies, positive effects of H_2S on post-harvest shelf life and antioxidant metabolism in strawberry fruits was investigated, and strawberry fruits treated with different concentrations of H_2S had a significantly lower decay index, higher fruit firmness, and lower respiration intensity than the controls (Bhat and Stamminger, 2015; Kuchi and Sharavani, 2019).

Acidic electrolyzed oxidized water (EOW) is produced by electrolyzing the water containing dissolved sodium chloride (NaCl) and has been considered as a safe and active antimicrobial agent by world health organization (WHO). In several studies has been reported that EOW have bactericidal effect in strawberry fruits (Guentzel et al., 2011; Ding et al., 2015).

Ozone is a strong oxidant, is commonly applied in gaseous or aqueous form and the ozone disintegrates to O_2. It is recognized as a GRAS (Generally Regarded As a Safe) by the US Food and Drug Administration and has come out as one of the most potential chemical methods for the preservation of strawberries (Tzortzakis and Chrysargyris, 2017; Lafarga et al., 2019). The use of ozone in fruits and vegetables is mainly focused on its effects on microbial population and its efficiency depends on the contact conditions (exposure time, con-centration, pH, etc.) and surface characteristics of fruits

and vegetables (Barman et al., 2018). The best concentration of ozone for strawberry treatment is 4-5 ppm gas and could be good candidate for maintaining the quality of strawberry and to provide longer storage and shelf life (Zhang et al., 2011; Chen et al., 2019).

The main advantage of the chemical treatments is that they could be used alone or in combination with physical treatments such as ultrasounds (US) or water-assisted ultraviolent (UV) irradiation (Lafarga et al., 2019). However, these applications need to be further studied for strawberry post-harvest applications, and treatment combinations as physical and chemical strategies could also result in antagonistic effects.

Heat treatment

Heat treatment is the most common and widely employed method for preserving and extending the shelf-life of fruits and their products. Basic purpose of heat treatment is to reduce microbial and enzymatic activity to maintain the standard quality during storage.

Over last decades' different technologies with shorter start-up, faster heating, greater energy efficiency and improved nutritional quality at the final product, have been developed and the most common of them are microwave heating (Sisquella et al., 2014), hot water dip, hot water rinsing and brushing, hot air and curing (Huan et al., 2017) and ohmic heating (Lafarga et al., 2019).

Caleb et al. (2016) tested the heat treatment with two different temperatures (35 and 45 ˚C) for 5 and 10 minutes by hot water dipping then stored them in open packaging trays at 4 ˚C for 9 days and then transferred to 16 ˚C for 3 days. It was re-ported that strawberry fruits dipped at 45 ˚C for 5 min had the highest total soluble solids, antioxidant capacity, texture profile and less weight loss than control; how-ever, the color lightness of strawberries was decreased. In another study the effect of hot air (45 ˚C for 3.5h) treatment on reducing gray mold and possible mechanisms in strawberry fruits was investigated. The result showed that the treatment significantly reduced lesion diameter, increasing activities of chitinase and phenylalanine amomonialyase (PAL) in strawberry fruits. Higher values of total phenolic content and the activities of antioxidant enzymes such as SOD, CAT and APX were detected in treated fruits. In addition, hot air was inhibited the spore germination and tube growth of B. cinerea (Jin et al., 2016).

Other studies also investigated the effect of heat treatment and composite film on strawberry storage, the result showed that 40 ˚C hot water treatment for 5 min, followed by a second microwave treatment and 1% of a composite coating mem-brane treatment, is the best combining method to prolong the shelf life, and reported that treated strawberries can keep good freshness for 5 to 7 days at room temperature (Fang et al., 2013).

Ultraviolet (UV) radiation

UV radiation is wide band of wavelengths, comprised of short-wave UV-C (200-280nm), medium-wave UV-B (280-320), and long-wave UV-A (320-400nm). Application of UV is

a non-thermal technology, and it is used to decontaminate, to re-duce decay, to increase the shelf life and minimize the quality loss (in terms of nutritional values) of fresh and minimally processed fruits and vegetables. The UV treatment can induce resistance mechanisms against pathogens or directly damage the bacterial DNA (Ramos et al., 2013). UV-C radiation, since it has more energy, can also damage the cytoplasmic membrane integrity and cellular enzyme activity (Gómez et al., 2011). Phenylalanine ammonia-lyase (PAL) activity increase after UV-C irradiation (Nigro et al., 2000), and this enzyme plays a key role in biosynthesis of phenolic compounds which many of these com-pounds have antifungal activity.

Fruit softening is one of the main factors of fruit post-harvest life, and the expo-sure to UV-C are reported to delay the fruit softening (Barka et al., 2000); the reduction of strawberry fruit softening by UV-C application has been also reported (Pan et al., 2004). Pombo et al. (2009) treated the strawberry fruits with UV-C (at 4.1 KJ/m2) and found that UV-C treatment changes the enzymatic activities and decrease the transcription of genes involved in cell wall degradation and attributed to delay fruit softening.

UV-C irradiation for strawberry quality maintenance during storage were also studied and it was detected a positive effect of UV-C to reduce decay, weight loss, fruit softening and calyx browning (Araque et al., 2018) and in maintaining the fruit pH (Aday et al., 2013). Furthermore, UV-C radiation had the ability to decrease the biological load without affecting the sensory quality (color, firmness, texture) of strawberries among others (Nigro et al., 2000; Xie et al., 2015).

UV-C treatment of strawberries is not only effective in extending shelf life and improving the organoleptic qualities, but also increases the fruit content in phytochemicals. Different studies on the impact of UV-C radiation on phytochemicals have shown that it has impact on the synthetic route of phenylpropanoids and PAL, which it effects on the production of secondary metabolites such as polyphenols and antioxidants (Cai et al., 2015; di Oliveira et al., 2016). The increase of phenolic acids in samples treated with irradiation alone and in combination with coating were also observed (Štajner et al., 2007).

Table1. 2. Nutritional compounds stability in different storage conditions/treatments. CA: Controlled Atmosphere; MAP: Modified Atmosphere Packaging

COMPOUND	STORAGE CONDITION/TREATMENT	EFFECT	REFERENCE
Vitamin C	Temperature management	Less degraded in low temperature	Cordenunsi et al., 2005
		Decreased the vitamin C content stored at 0 ˚C	koyuncu and Dilmaçünal, 2010
		Decreased vitamin C stored at 1 ˚C	Nunes et al., 1998
		High vitamin C detected at higher temperature (≥ 5˚C)	Jin et al., 2011
	CA	Reduce the loss of vitamin C	Delaporte, 1971
		Maintain vitamin C	Agar et al., 1997; Li et al., 2015
	MAP	Maintain the vitamin C content (60% O_2 + 1.5% CO_2)	Jing et al., 2014
		High degradation during cold storage (1.2-4.2% CO_2, 3-7% O_2)	Bhat and Stamminger, 2015
		Decreased the vitamin C (11-14% O_2+ 9-12% CO_2)	Yun et al., 2017
	Edible coating	Chitosan coating maintain vitamin C	Wang and Gao, 2013

		Increased vitamin C coated with chitosan	Petriccione et al., 2015
		Chitooligosaccharide coating maintain vitamin C during cold storage	Kerch et al., 2011
	Ultraviolet irradiation	Decreased the vitamin C treated with γ-irradiation	Maraei and Elsawy, 2015
		Decreased the vitamin C, treated with UV-C	Li et al., 2019; Bal., 2019
	Heat treatment	-	-
Anthocyanins	Temperature management	Decreased during cold storage	Cordenunsi et al., 2005;Octavia et al., 2017; Hernández-Herrero and Frurus, 2014
	CA	Increased	Zheng et al., 2007 ; Yang et al., 2020
		Not significant difference between air storage and control atmosphere	Holcroft and Kader, 1999
	MAP	Effectively delayed the decrease of anthocyanin	Xiao et al., 2004
	Edible coating	Chitosan based coating maintained higher anthocyanin content	Wang and Gao, 2013

		Methylcellulose-based edible coating not significant difference on anthocyanin	Nadim et al., 2015
		Chitosan- based apple peel polyphenols edible coating, maintained higher amount of anthocyanin	Riaz et al., 2021
	Ultraviolet irradiation	Increased the anthocyanin content	Pan et al., 2004 ; Erkan et al., 2008 ; Li et al., 1997
		Maintained the anthocyanin	Bal, 2019
	Heat treatment	Delayed the anthocyanin accumulation	Pan et al., 2004 ; Civello et al., 1997
Phenolic acids	Temperature management	Maintain the phenolic acids during cold storage (4˚C)	Kadevicet al., 2013 ; Nicoletto et al., 2014
		Maintain longer during storage at 0 ˚C	Shin et al., 2007
	CA	Maintained	Yang et al., 2020

	MAP	Maintained the phenolic acids during storage	Bhat and Stamminger, 2015
	Edible coating	Chitosan based coating maintain the phenolic acids during storage	Wang and Gao, 2013
		carboxymethyl cellulose based edible coating maintained phenolic acids during storage	Hussain et al., 2013
		Chitosan based apple peel polyphenols edible coating delay the degradation phenolic acids	Riaz et al., 2021
	Ultraviolet irradiation	UV-C treatment increase the phenolic acids	Breitfellner et al., 2002; Maraei and Elsawy, 2017
		Gamma radiation increase the phenolic acids	Barkaoui et al., 2021
	Heat treatment	Hot water treatment decreases the phenolic acids	Maghoumi et al., 2013

Table1. 3. Physical methods used for the preservation of minimally processed Strawberry fruits.

Method	Advantages	Disadvantages	REFERENCE
Temperature management	• Reduction of respiration • Reduction of the growth of mold • Maintain the nutritional quality	• Temperature control is difficult and costly	Cordenunsi et al., 2005; Li et al., 2015 ; kadivec et al., 2013 ; Massoud et al., 2021
Controlled Atmosphere	• Reduction of respiration • Reduction of the growth of mold	• Off-flavor compounds due to anaerobic conditions	Massoud et al., 2021 ; Thompson, 2010 ; Shewfelt et al., 2014

Modified atmosphere Packaging	• Extend storage life • Reduced economic losses • Provides a high quality product • Odorless and convenient packages • Delay of ripening	• Often produces high level of CO2 • consequent development of off-flavors and potential stimulation of pathogens growth • Temperature control necessary • Different gas formulations for target microorganisms • Plastic films maybe environmentally undesirable	Oliveira et al., 1998; Cui et al., 2011; Graça et al., 2011; Ramos et al., 2013; Yang et al., 2020 ; Zhang et al., 2022

Edible Coating and films	• Edible characteristics and biodegradability • Protect microbial growth on the surface • Retain volatile flavor compounds, natural color pigments and nutrients • Restrict insect infection • Low cost	• Modification of internal atmospheres by the use of edible coatings can increase disorders associated with high CO_2 or low O_2 concentration • Coating formulations that provide adequate gas exchange are often not good barriers to water vapour • Lack of knowledge about biopolymer and permeant properties	Andrade et al., 2012; Ramos et al., 2-13; Treviño-Garza et al 2015; Barikloo and Ahmadi, 2018; Zhang et al., 2022

		the formation of an internal modified atmosphere is not enough to provide the desired preservating effects, and therefore an additional package is needed.	
Heat Treatment	• Alternative of fungicide treatment • Control the growth of Botrytis cinerea • Disinfect from insects • Delays ripening • Low cost	• Color changes and the brightness of the fruit • Skin browing • Flesh darkening • Water loss • negatively affect physical and nutritional qualities	Pan et al., 2004 ; Fallik, 2010; Massoud et al., 2021

Ultraviolet irradiation	• Can be performed at room temperature • Reduce deterioration • Extends shelf-life • Absences of residual toxicity • Exposure to UV also induces the synthesis of health-promoting compounds such as anthocyanins • Low cost	• Acceptance of irradiation by consumers • Produce quality may be affected specially at high doses • Pre-treatment necessary • Increase stress and respiration rate • Can cause off-flavors and color changes • Fruit softening	Alexandre et al., 2012; Neves et al., 2012; Ramos et al., 2013; Du et al., 2014; Severo et al., 2015; Syamaladevi et al., 2015; Massoud et al., 2021; Fan et al., 2022

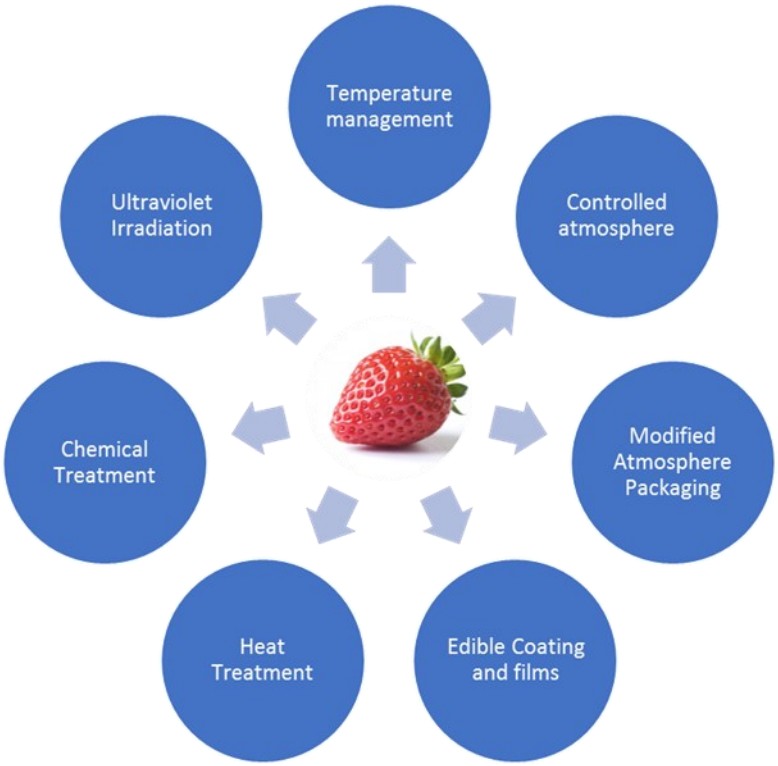

Figure1. 2. Main methods to maintain fresh strawberry quality during storage

The aim of the thesis

The main aim of the thesis was the evaluation of different pre and post-harvest factors, that can affect the commercial, sensorial and nutritional quality of the fresh and stored fruit quality.

In particular, the main pre-harvest factor considered was the genetic factors (Wild and cultivated germplasm), while the main post-harvest factors were storage time, temperature and fruit treatment. In the first study, the aim was to improve the sensory and nutritional quality of strawberry fruit. The study provided the implementation of an intensive crossing program (inter specific crossings) and the selection of different progenies in order to identify strawberry genotypes suitable for being registered as commercial varieties or to be used as a parental in subsequent crossing programs.

In the second study, the aim was to compare different strawberry cultivars and evaluate the most tolerant to *Botrytis cinerea,* analysing the nutritional and qualitative changes in response to the disease caused by *Botrytis cinerea*, evaluating the content of soluble solids, titratable acidity, the content of vitamin C, anthocyanins, and phenolic acids. And in the last study, the effect of fruit treatment on whole fruits and dried fruits (WF and DF) at different storage times (from 0 to 7 months) and temperatures (−20 and −80 °C) on the nutritional quality (vitamin C, anthocyanins, phenolic acids, and folate) of three strawberry cultivars was studied, with aim of determining the best combination of fruit treatment and storage condition to better retain the nutritional quality after long storage.

Chapter 2.

Effect of wild genotype on strawberry fruit quality in UNIVPM breeding program

Abstract

In recent years the lifestyle modification in modern society has put remarkable view in nutritional fruits. Among them strawberries are a very rich source of bioactive compounds (vitamin C and B9) and phenolic compounds (phenolic acids, flavonoids and anthocyanins). The nutritional properties of strawberries depend on the amount and characteristics of the bioactive and antioxidant compounds (such as polyphenols and vitamins).

Strawberry (*Fragaria × ananassa*) breeding programs, including that from Università Politecnica delle Marche (UNIVPM), focus on increasing plant resilience and fruit nutritional quality. Wild octoploid strawberry (*F. virginiana*) are very important sources of genes for increasing disease resistance, fruit quality, and bioactive compounds, in particular vitamins and polyphenols. These compounds contribute to the strawberry fruit antioxidant capacity and, consequently, for the many health benefits of strawberry consumption. In this study, the genetic variability that originated from five types of interspecific cross-combination (F1, BC1 back cross, BC2 back cross, BC3 back cross, and BC4 back cross of *Fragaria x ananassa* x *Fragaria virginiana glauca* at UNIVPM in the 2020 and 2021 harvest season was analysed, together with some commercial varieties, with the aim of identifying the minimum number of generations needed to produce new pre-breeding materials combining interesting traits to obtain high nutritional and sensorial quality strawberries with optimum commercial agronomic level. Results demonstrate the high variability in bioactive compounds composition among cultivars and breeding materials, underlying the importance of including wild germplasm for increasing fruit nutritional quality through breeding programs. In fact, results indicated the possibility to generate new cultivars producing fruit with high contents of polyphenols and vitamins, stable at the different cultivation cycles, to be labelled with a compositional claim.

2.1. Introduction

In the recent years, interest has been paid by consumers to the health and nutritional quality. Berries, specially strawberries play an important and common role in the human diet due to their composition aspects, which are naturally enriched with many

nutritional and bio active compounds. Such as minerals, vitamins, dietary fibers, and especially polyphenolic phytochemicals (flavonoids, phenolic acids, lignans and tannins) (Mazzoni et al., 2015; Mezzetti et al, 2016), which exhibit strong antioxidant and anti-inflammatory activities that may reduce sensitivity to oxidative stress. Therefore, berries may have a role in the prevention of degenerative pathologies, stimulating researchers to implement new biotechnological approaches for the improvement of bioactive contents.

In the previous researchers were focused on the improvement of agronomical or qualitive traits, like size, number, yield, ripening, softening and firmness of fruit (Qin et al., 2008). Recently efforts have focused on improving nutritional bioactive components, combined with a high standard of sensorial and agronomic fruit quality through breeding and/or biotechnology to maintain a higher antioxidant intake, particularly when fruit consumption is low (Diamanti et al., 2012). The breeding process of more nutritious, better-tasting cultivars can be successful if the variability and heritability of bioactive compounds, which is defined as the total antioxidant capacity (TAC), is assured for the progenies derived from parental fruits. The biotechnological approach is a methodology that can provide a genetic improvement through modification of specific biosynthetic pathways (Diamanti et al., 2014).

Since the biotechnological approaches are still highly limited in commercial exploitation of new products by public concern and biosafety rules, a deep knowledge of both cultivated and wild genetic resources, which may be used for genetic and genomic studies, is important for obtaining new cultivars of high interest (Capocasa et al., 2008). Recently the possible way to improve the fruit content of such phytochemicals is traditional breeding program and for this purpose, it is important to accurately describe the genetic resources used in cross combination of a component. Inclusion in the breeding programs of wild species with a genetic background able to produce progeny that have increased phytochemicals is also important (Diamanti et al., 2012).

In this study, the genetic variability that originated from an interspecific cross-combination (F1) *Fragaria x ananassa* x *Fragaria virginiana* glauca, followed by 4 backcross generations (BC1, BC2, BC3, and BC4), was analyzed on fruit of the different selections harvested in 2020 and 2021, at UNIVPM strawberry breeding field, in comparison with fruit of some commercial varieties, with the aim of identifying the minimum number of generations needed to produce new pre-breeding materials combining interesting traits to obtain high nutritional and sensorial quality strawberries with optimum commercial agronomic level.

2.2. Material and Methods

2.2.1. Plant Material

Breeding selections generated from *F.* × *ananassa* and *F. virginiana* subsp. glauca F1 and 4 subsequent backcross generations (bc1-bc4), were evaluated at the "P. Rosati" Experimental Farm (43°31'N; 13°36'E, 46 m altitude) Agugliano (Ancona, Italy), on non-fumigated soil, having the following main characteristics: pH 7.9, active calcium 9%

and texture composed at 40% clay, 25% sand and 35% silt, following the procedure described in Capocasa et al. (2016). Data reported are referred to two cultivation cycle with fruit harvested in May 2020and May 2021. For each of the following generation: F1, backcross 1, backcross 2, backcross 3, backcross 4 (F1-bc1-4), were evaluated different selections (Table 2.1), grown in a single plot of 8 plants each. The selections were chosen after a two-years evaluation, according to the best morphological and agronomic characteristics, resistance to pathogens, and then for the productive characteristics and sensorial and nutritional fruit quality. Different f×a genotypes were used as parents for each crossing generation, according to the characteristics of interest to transmit to the progeny. Furthermore, 4 wild accessions (fvg), used for generating the F1, and 7 different commercial cultivars (f×a), representative of well-adapted commercial cultivars, were evaluated to be compared with the backcrossing selections.

Table2. 1. Number, names (or codes) and parents of genotypes per crossing type.

Crossing type	n.o genotype	Crossing number/genotype	origin
fvg	4	FVG	*Fragaria virginiana glauca (*fvg*)*
f×a	7	CRISTINA L4	*Fragaria x ananassa* (f×a)
		DINA	
		FRANCESCA	
		LAURETTA	
		ROMINA	
		SIBILLA	
		SILVIA	
f1	8	AN12,48,54	Cristina x fvg
		AN12,49,53	Monterey x fvg
		AN12,49,65	
		AN12,50,52	Portola x fvg
		AN12,51,56	Romina x fvg
		AN13,15,57	AN06,164,52 x fvg
		AN13,20,52	AN08,113,53 x fvg

		AN13,20,58	
bc1	1	AN00,239,55	AN94,414,52 x 91,143,5
bc2	5	AN11,05,53	AN07,04,51 x Monterey
		AN11,05,58	
		AN15,04,54	AN07,03,57 x AN06,221,57
		AN16,04,52	AN07,03,57 x Dely
		AN16,04,53	
bc3	11	AN16,27,52	AN11,04,51 x AN07,105,53
		AN16,27,53	
		AN16,27,54	
		AN16,32,51	AN11,12,55 x AN10,04,51
		AN16,32,53	
		AN16,32,55	
		AN17,07,51	AN11,09,54 x AN13,16,57
		AN17,07,52	
		AN17,07,53	
		AN17,07,54	
		AN17,07,55	
bc4	18	AN16,37,51	AN11,16,51 x Monterey
		AN16,37,52	
		AN16,37,53	
		AN16,37,57	
		AN16,37,58	
		AN16,37,60	
		AN17,12,51	AN11,13,56 x AN13,16,57
		AN17,12,52	

AN17,12,53	
AN17,12,54	
AN17,12,55	
AN17,19,51	AN11,24,63 x Sibilla
AN17,19,52	
AN17,19,53	
AN17,19,54	
AN17,19,55	
AN17,19,56	
AN17,19,57	

2.2.2. Productive Parameters

Strawberry plant production was evaluated in the single plot of each of the crossing types (F1-bc4), cultivated (f×a) and wild (fvg) genotypes at each harvest, by evaluating the following parameters:

a) *Total fruit production (g plant^{-1});*

b) *Commercial production*: total weight (g plant^{-1}) of ripe fruit with a commercial diameter (Ø≤22 mm) and without injuries;

c) Average fruit weight: average weight (g) of 20 commercial fruits from each harvest;

d) *Second category fruits*: total weight (g plant^{-1}) of ripe small fruits without injuries and misshapen fruits;

e) *Waste fruits*: total weight (g plant^{-1}) of rotten fruits.

2.2.3. Sensorial quality

Commercial strawberry fruits of all selections from the different crossing generations (F1, bc1, bc2, bc3, bc4) and from cultivated (f×a) and wild (fvg) genotypes, were sampled at the 2nd, 3rd, 4th harvest of the season and analysed fruit sensorial quality, by taking in account the following parameters:

a) Solid Soluble (SS): determined using a hand-held refractometer (ATAGO), results are expressed as °Brix.

b) Titratable Acidity (TA): determined from 10 mL of juice diluted with distilled water (1/2 v/v) and titrated with 0,1N NaOH solution, until pH8.2, and expressed as mEQ of NaOH per 100g Fresh Weight (FW).

c) Fruit Color: determined as Chroma index by a Minolta Chromameter CR 400, for two side of 10 ripe, undamaged and uniform fruits. The instruments measured three parameters: L* (luminescence), a* (red tone), b* (yellow tone). Chroma index is

evaluated from a and b value [(a*2 +b*2)1/2]; higher Chroma value represents pale fruits and low Chroma index represents dark fruits.
d) Fruit Firmness: measured by a penetrometer 327 (Effegì, Ravenna, IT), results are expressed as grams.

2.2.4. Nutritional Analysis

2.2.4.1. Methanolic Extraction

Fruit extracts were prepared as described by Diamanti et al. 2012. First, 10 g of frozen fruit were homogenized with Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Denmark) in 20 mL of methanol and agitated for 30 min in the dark. The suspension was centrifuged at 4500 rpm for 10 min at 4 °C, the supernatant was collected, and the pellet of the fruits was extracted for a second time by adding another 20 mL of methanol and repeating the procedure. The second supernatant was added to the first one and one part of extracted solution was taken for Spectrophotometer analysis and the other was filtered (pore size 0.22 μm) then stored at -20 ˚C until injecting to HPLC.

2.2.4.2. Vitamin C Extraction

Vitamin C of fruits was extracted with an ultrasound-assisted extraction protocol, as described by Tulipani et al., 2008. The extraction was carried out with the use of an ultrasound bath (Bioblock/ELMA 88155, Stuttgart, Germany), 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 1 g of frozen strawberry with an aliquot of 4 mL taken from the extraction buffer solution, containing 5% metaphosphoric acid and 1 mM DTPA, followed by 5 min of sonication and centrifugation at 4000 rpm for 10 min at 4 °C. The supernatants obtained from each sample were filtered (filter pore size 0.45 μm) and inserted into a vial to perform analysis on an HPLC system.

2.2.4.3. Folate content Extraction

Following the method described by Mezzetti et al., 2016, with slight modifications, 8 mL of the extracting solution (0.1 mol/L sodium acetate containing 10% (w/v) sodium chloride, 1% (w/v) ascorbic acid, and 0.1% 2-mercaptoethanol) were pipetted in 2 g of the frozen strawberry, then homogenized with Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Denmark). The falcon tube was loosely capped, boiled in water for 12 min, and rapidly cooled in the freezer for 10 min. Hog kidney folate conjugate enzyme was prepared, and about 1.5 mL of the enzyme was added to the cooled solution and incubated in a shaking oven at 37 °C for 3 h. Afterwards, the enzyme was inactivated by boiling in water for 5 min followed by cooling for 10 min in the freezer. The samples were then centrifuged at 4500 rpm for 30 min at 4 °C, and the supernatant was transferred into a new labelled falcon tube. Moreover, another 8 mL

of the extracting solution was added to the resulting pellet and centrifuged again for 30 min. The second supernatant was added to the first one, then extracting solution was added to top up the supernatants to 25 mL. The final supernatant of 25 mL was then filtered using 0.45 µm filter pore size, 25 mm inner diameter, nylon disposable syringe filters, and the filtrates were purified through solid-phase extraction on strong anion-exchange isolate cartridges, as described by Iniesta et al., 2009.

2.2.4.4. Extraction for primary metabolites

Extraction for primary metabolites was performed at Fruit Genomic and Biotech Lab of University of Malaga (Spain) under supervision of prof Sonia Osorio. Relative levels of primary metabolites were determined from frozen samples following the protocol established by Osorio et al. (2012). 120mg of frozen powder fruit was vortexed with a solution of 1200µl (100% methanol+ 48µl Ribitol). The mixture was incubated for 15 min at 70°C, added 600µl of water and centrifuged at 4000rpm for 15 min; from the upper phase (Polar phase) 60µl was taken and the water/methanol supernatant was reduced to dryness under vaccum. Samples were stored at -80°C until GC-MS analysis. The dried extract was re-dissolved and derivatised for 120 min at 37 ºC in 60 µl (30 mg/ml methoxyamine hydrochloride in pyridine), followed by a 30 min treatment at 37 ºC with a mixture of 100 µl of N-Methyl-N-[trimethylsilyl] trifluoroacetamide and 20 µl of retention time standard mixture composed by 0.4 ml·ml-1 of the 13 fatty acid methyl esters (FAMEs). Sample volumes of 1 µl were then injected in the GC-MS using a splitless or split mode and a hot needle technique.

2.2.4.5. Spectrophotometer Analysis

a) Total Antioxidant Capacity (TAC)

TAC was evaluated by the ABTS assay, according to a previously validated procedure (Miller et al., 1993). ABTS, a chromogen and colorless substance, is changed into its colored monocationic radical form (ABTS•+) by an oxidative agent. Addition of antioxidants reduces ABTS•+ into its colorless form. The extent of decolorization as percentage of inhibition of $ABTS^{\bullet+}$ is determined as a function of concentration and calculated relative to the reactivity of Trolox, a water-soluble vitamin E analogue.

The day before analysis, the stock solution of $ABTS^{\bullet+}$ was generated by oxidation of ABTS

with potassium persulfate ($K_2S_2O_8$) overnight. Phosphate buffered saline (PBS) at pH 7.2-7.4 and a concentration of 5 mM was prepared with dipotassium hydrogen phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4).

For analysis of the samples, 100 µl of strawberry methanol extract were mixed with 1900 µl of PBS in an Eppendorf tube. Again 100 µl of this solution were mixed with 1900 µl of $ABTS^{\bullet+}$ working solution (stock solution diluted with PBS 1:50 to 1:70, absorbance of 0.7-0.8) and vortexed. After 6 min in the dark the absorbance was measured with the UV spectrophotometer UV-1800 (Shimadzu Corp., Kyoto, JP) at a wave length of 734 nm. Every sample was analysed three times. The standard was prepared by diluting the Trolox stock solution (2.5 mM) with PBS so that the final concentrations range from 0.025 to 0.45 mmol L^{-1}. The absorbance was measured in the same way as the samples.

In the following the transformation of the measured absorbance into Trolox equivalent is explained:

Calculation of the percentage of inhibition (= ΔA) of $ABTS^{\bullet+}$:

$$\Delta A = \frac{Ab\ black - Abs\ ssample/standarad}{Abs\ blank} \times 100\%$$

By linear regression a standard calibration curve for each Trolox concentration (mean of absorbance for three measurements) was calculated (ΔA=ac+b).

For calculating the TAC of the strawberries the following formula was applied:

$$TAC\ (\frac{mmol\ TE}{kg\ FW} = \frac{(\Delta A - b) \times F}{E \times a} \times 1000$$

- ΔA = % inhibition (sample)
- a= slope (calibration line)
- b= intercept (calibration line)
- c= Trolox concentration [mmol]
- F= dilution factor (20)
- E= sample weight [g/L extracting agent]

Antioxidant activity was expressed as µg of Trolox equivalent per g of fresh pulp weight. The calibration was calculated as linear regression from the dose response Trolox Standard. The results are expressed as mM of Trolox equivalent per kg of Fresh fruit.

b) Total Phenol Content (TPH)

TPH was evaluated by the Folin-Ciocaltou's reagent method (Slinkard and Singleton 1977)

using gallic acid as standard for the calibration curve. Results were calculated and expressed as mg of gallic acid per kilogram of fresh fruit. Range value of standards were from 10 to 50 mg gallic acid/l. Briefly, a test tube (glass) was filled with 7.0 ml of water. Afterwards, 1 ml of the diluted sample (1:20) was added, followed by the addition of 500 µL Folin-Ciocalteu-Reagent and vortexed. After 3 minutes, 1.5 mL sodium carbonate (0.53 mol/l) was added and the tube was mixed one more time and then stored in the dark for 60 minutes. The absorbance of the sample was measured after exactly 60 minutes at 760 nm with a spectrophotometer.

c) Total Anthocyanin Content (ACY)

ACY assay was performed by the pH differential shift method (Giusti and Wrolstad, 2001),

using the anthocyanins' characteristic to change intensity of hue depending on pH shifting.

Briefly, the samples were diluted to a ratio of 1:10 with potassium-chloride (pH 1) and with sodium acetate (pH 4.5) and the corresponding maximum absorbance for both solutions was measured (respectively at λ = 500 nm, and λ = 700 nm).

The results were expressed as mg of pelargonidin-3-glucoside (the compounds more representative for anthocyanins in strawberry) per kilogram of fresh weight (mg of Pel-3-

Glu/Kg FW).

2.2.4.6. HPLC-UV determination

a) Phenolic acids

Phenolic acids were analyzed as previously described in Schieber et al., 2001 and Fredericks et al., 2013. The HPLC system comprised a Jasco PU-2089 plus controller (Jasco, Easton, MD, USA), a Jasco UV-2070 plus ultraviolet (UV) detector (Jasco Easton, MD, USA), and an autosampler Jasco AS-4050 (Jasco, Easton, MD, USA). The HPLC UV detector was set at 320 nm and the column used was an Aqua Luna C18 250 × 4.6 mm (Phenomenex, Torrance, CA, USA) protected by a Phenomenex 4.0 × 3.0 mm C18 ODS guard column (Phenomenex, Torrance, CA, USA). The gradient program consisted of two mobile phases: A (2% Acetic acid) and B (acetic acid, acetonitrile and H2O 1:50:49). It started with 55% A and 45% B for 50 min, followed by 10 min 100% of B, and then decreased to 10 % B until the end.

For the quantification of phenolic acid content, external chlorogenic acid (CHA), caffeic acid (CA), and ellagic acid (EA) calibration curves were used. Values were expressed as mg corresponding to phenolic acid per kilogram of fresh weight of strawberries (mg/kg FW).

b) Total anthocyanins

Anthocyanin content was analyzed following the method of Fredericks et al., 2013. The HPLC system comprised a Jasco PU-2089 plus controller (Jasco, Easton, MD, USA), a Jasco UV-2070 plus ultraviolet (UV) detector (Jasco Easton, MD, USA), and an autosampler Jasco AS-4050 (Jasco, Easton, MD, USA). The compounds were separated on an Aqua Luna C18 (2) (250 × 4.6 mm) reverse-phase column with a particle size of 5 µm (Phenomenex, Torrance, CA, USA) protected by a Phenomenex 4.0 × 3.0 mm C18 ODS guard column (Phenomenex, Torrance, CA, USA), and monitoring was performed at 520 nm. The gradient program consisted of two mobile phases: A (formic acid, acetonitrile, and H2O 10:3:87) and B (formic acid, acetonitrile, and H2O 10:50:40). It started with 75% A for 10 min, decreased to 69% A for 5 min, decreased again to 60% A for 5 min, and later continued 50% A for 10 min, followed by 90% A for 16 min.

Anthocyanins were quantified using calibration curves made with external standards of cyanidin-3-glucoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside, and were calculated as mg per 1 kg of fresh weight of strawberries (mg/kg FW).

c) Vitamin C

Vitamin C content was measured as described in Helsper et al., 2003. Extracts were subjected to HPLC analysis after the extraction procedure. The HPLC system comprised a Jasco PU-2089 plus controller (Jasco, Easton, MD, USA), a Jasco UV-2070 plus ultraviolet (UV) detector (Jasco Easton, MD, USA) set at an absorbance of 260 nm, and an autosampler Jasco AS-4050 (Jasco, Easton, MD, USA). The HPLC column used was Ascentis Express C18 150 × 4.6 mm (Supelco, Bellefonte, PA, USA), protected by a Phenomenex 4.0 × 3.0 mm C18 ODS guard column (Phenomenex, Torrance, CA, USA). The gradient program consisted of two mobile phases: A (50mM phosphate buffer with pH 3.2) and B (Acetonitrile), which started with 100% of A until 6 min, then decreased to 50% for 2 min, and again increased to 100% until the end.

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 1 kg fresh weight of strawberries (mg/kg FW).

2.2.4.7. HPLC-FLD determination

Folate

Folate was quantified using the HPLC as cited by Stralsjo et al., 2003. The HPLC system comprised a pump model Jasco PU- 2089 (Jasco, Easton, MD, USA), a fluorescence detector (FLD) Jasco FP-2020 Plus (Jasco, Easton, MD, USA) set at wavelengths of 290 nm excitation and 360 nm emission, and an autosampler Jasco AS-4050 (Jasco, Easton, MD, USA). The analytical column was a Luna C18, 250 × 4.6, 5 µm (Phenomenex, Torrance, CA, USA), protected by a Phenomenex 4.0 × 3.0 mm C18 ODS guard column (Phenomenex, Torrance, CA, USA). Quantification of folate content was determined through a calibration curve prepared by running standard concentrations of 5-methyl-tetrahydrofolic acid (5-CH3-H4 folate). The gradient program consisted of two mobile phases: A (30 mM phosphate buffer with 2.3 pH) and B (Acetonitrile). It started with 94%A for 8 min, then decreased to 75% A for 27 min, and increased again to 94% A for 15 min.

Results are expressed as µg 5-CH3-H4 folate per 1 kg of fresh weight of strawberries (µg 5-CH3-H4folate/kg FW).

2.2.4.9. Gas chromatographer (GC-TOF–MS) Analysis

Gas chromatographer (GC-TOF–MS) Analysis was performed at Fruit Genomic and Biotech Lab of University of Malaga (Spain) under supervision of prof Sonia Osorio. The GC-TOF–MS system was composed of a GC 6890 N gas chromatographer (Agilent Technologies, Böblingen, Germany), and a Pegasus III time-of-flight mass spectrometer (LECO Instruments, St. Joseph, MI, USA), provided with an Electron Impact ionization source. GC was performed on a MDN-35 capillary column, 30 m in length, and 0.32 mm in inner diameter, 0.25 mm in film thickness (Macherey–Nagel). The injection temperature was set at 230°C, the interface at 250°C, and the ion source adjusted to 200°C. Helium 5.0 was used as the carrier gas at a flow rate of 2 ml/min. The analysis was performed under the following temperature program: 2 min of isothermal heating at 80°C, followed by a 15°C per min ramp to 330°C, and holding at this temperature for 6 min. Mass spectra were recorded at 20 scans/s with a scanning range of 70 to 600 m/z.

chromatograms and mass spectra were evaluated using ChromaTOF software, version 3.00 (LECO Instruments, St. Joseph, MI, USA), and.peg files were exported to cdf using a baseline off set of 1 ('just above the noise'), an average of 5 points for smoothing, a peak width of 10 and a signal to noise ratio of 10. Identification and semi-quantitation of the compounds detected in the GC-TOF–MS metabolite profiling experiment were performed with TagFinder software (Luedemann et al., 2008). Metabolites were identified by comparison to database entries of authentic standards and spectral data

from the public library GMD@CSB.DB (The Golm Metabolome Database; Kopka et al. (2005).

2.2.5. Data Analyses

The results are presented as the values ± standard error and were subjected to one-way analysis of variance (ANOVA), at a confidence level of 95%. Significant differences were calculated according to Tukey's tests, and differences at $p < 0.05$ were considered to be significant. Statistical analyses were performed by using Statistica 7 software (StatSoft, TIBCO Software, Palo Alto, CA, USA). The metabolomic data were analysed by R-environment (R version 4.1.1 (2021-08-10) -- "Kick Things" Copyright (C) 2021 The R Foundation for Statistical Computing Platform: x86_64-apple darwin17.0 (64-bit). https://CRAN.R-project.org), and the clustering was made according to Langfelder and Horvath (2012).

2.3. Results and Discussion

2.3.1. Productive parameters

Plant yield of fvg, f×a and different crosses (f1-bc4) that originated from fvg and f×a were measured in two cultivation cycles (2020 and 2021). The result was expressed that f×a had the highest amount of production by 695.3g/plant and fvg had the lowest production (13.2g/plant), while by crossing f×a with the wild genotype, productivity has been clearly
lost. However, by increasing each crossing generation the trend of production had increased and in bc4, the total production reached the average production of the commercial strawberry with 644.8 g/plant (Figure 2.1). In addition, the second category production and waste production of commercial cultivars were higher than fvg, and, the values on the production of second category (small and misshapen fruits) and waste fruit (rotten) also increased when passing from F1 to bc4, so as to confirm the contribution of the cultivated species in increasing the susceptibility of the fruits (Figure 2.1).

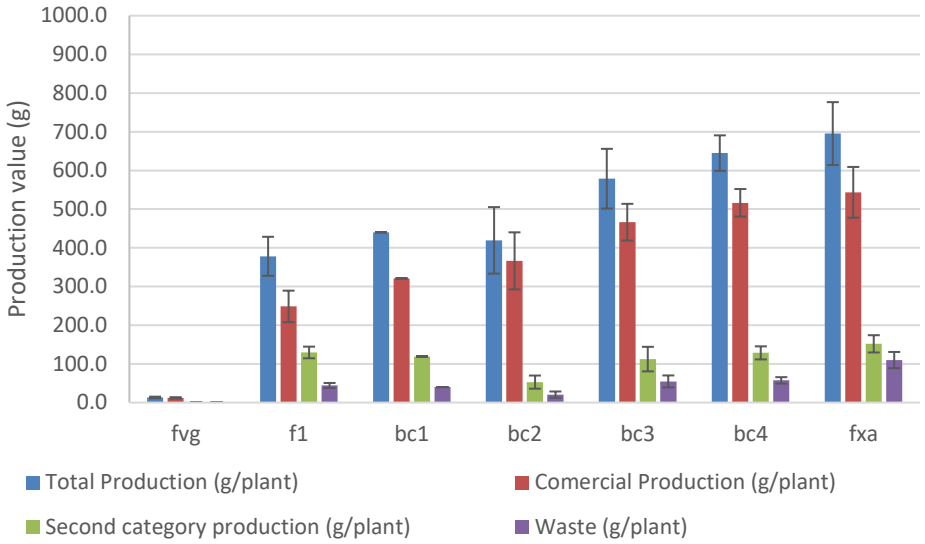

Figure2. 1. Productive parameters of the different types of crossing in 2020 harvest, the data are expressed as mean ± standard error

Regarding the 2021 harvest cycle, similarly, to 2020 harvest season, wild clone had very low yield (6.64g/plant) and the commercial genotypes had the highest production with amount 919.4 g/plant), and by each crossing generation, the total production had increased by increasing commercial genome. In bc4 the production improved by arriving 462.57 g/plant, but compared to commercial genotypes, the total production of bc4 is half of the average commercial cultivars production (figure 2.2).
As a comparison, the production of f×a in 2021 increased compared to 2020 harvest. But the production of back crosses was lower than 2020 harvest. In addition, in 2021 the values reached in bc4 seem to be lower compared to the average observed for the cultivars analyzed both years, but the production of second category and waste fruit in 2021 was less compared to 2020 but the values reached in bc4 seem to be lower compared to the average observed for the cultivars analyzed both years.

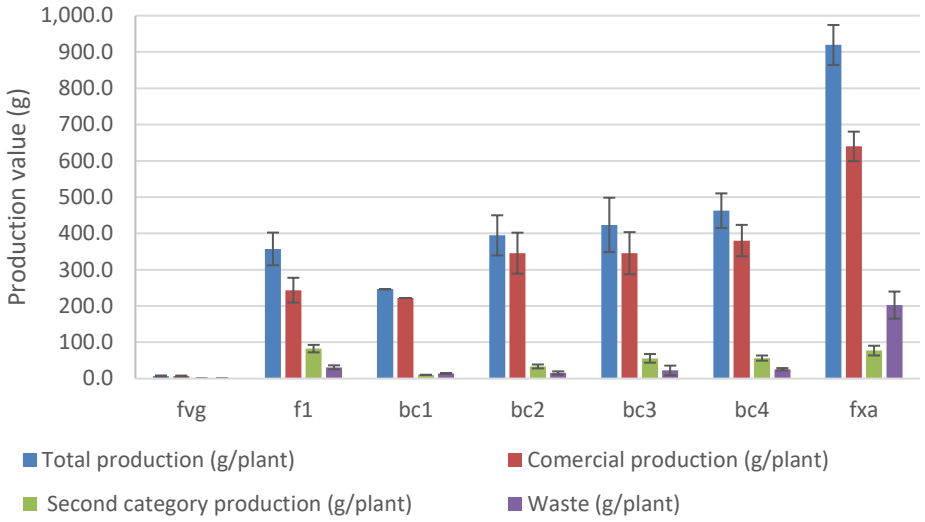

Figure2. 2. Productive parameters of the different types of crossing in 2021harvest, the data are expressed as mean ±standard error

2.3.2. Sensorial quality

a) Total soluble solids and Titratable Acidity

The result from 2020 shows that total soluble solids of wild genotype (fvg) (12°Brix) was much higher than the commercial genotype (fxa) (8.8°Brix), while by increasing each crossing generation the trend of total soluble solids had decreased but there was no significant difference in each crossing type. Furthermore, in bc4 fruit soluble solids content mean values (8.9°Brix) were higher in comparison to what was detected in fxa, On the contrary, the highest mean values of Titratable Acidity (TA) obtained in bc1 fruits were statistically similar to those obtained in f1 fruit (Figure 2.2). There was reduced TA from fruit of subsequent backcrossing generation (bc2-bc4), in bc4 the TA (11.1 mEq NaOH/100g FW) were statistically similar to f×a (10.7 mEq NaOH/100g FW).

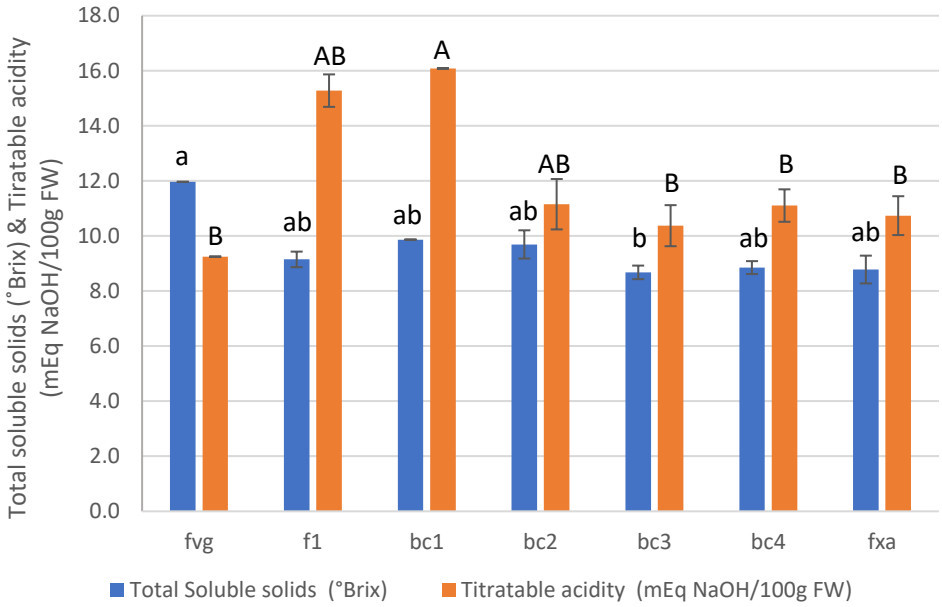

Figure2. 3. Total soluble solids and titratable acidity of the different types of crossing in 2020, the data are expressed as mean ±standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type for total soluble solids and uppercase letters indicate significant differences for $p \leq 0.05$ in each crossing types for titratable acidity.

Regarding 2021 harvest cycle, the highest soluble solids content was detected in bc1, however, the soluble solids content of fvg (8.6 °Brix) were significantly similar to the following back crosses and higher than f×a (8.49 °Brix) (Figure 2.3). Similar to 2020 harvest, in 2021 the highest TA content obtained in f1 and bc1 fruits (Figure 2.3). There was reduced TA from fruit of subsequent backcrossing generation (bc2-bc4), in bc4 the TA (11.31 mEq NaOH/100g FW) were statistically similar to f×a (11.5 mEq NaOH/100g FW).

In comparison of 2020 and 2021 fruits, fvg fruits in 2020 had more soluble solids content compare to 2021, but high fruit soluble solids content was detected in bc4 selections of 2021 compare to 2020. While the fruit TA content of 2021 bc4 selections were higher than 2020. The result demonstrates the potential of wild genotypes to improve fruit sweetness and balancing the sugar acids ratio of commercial cultivars. However, consumers generally prefer balanced SS and TA values (Capocasa et al., 2016).

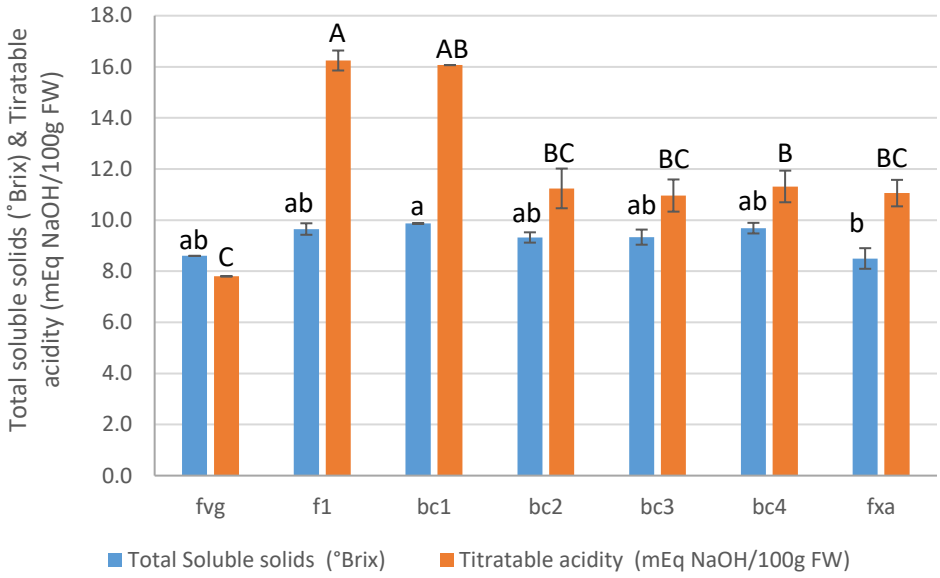

Figure2. 4. Total soluble solids and titratable acidity of the different types of crossing in 2021 harvest, the data are expressed as mean ±standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type for total soluble solids and uppercase letters indicate significant differences for $p \leq 0.05$ in each crossing types for titratable acidity

b) Fruit color

With regard to fruit color, L* (a measure of the brightness of the color), and Chroma index (intensity of fruit color) were evaluated for all genotypes for two cultivation cycle. In 2020 harvest, the highest Chroma was obtained from f×a and was significant similar with all back cross genotypes. In addition, the highest L* were obtained from f×a and the lowest L* value was obtained from f1, while by increasing back cross generation L* increased and from bc1-bc4 the L* was significantly similar to commercial cultivars. It means that from f1 till bc4 the fruits become lighter with a final value similar to commercial strawberry (Figure 2.5).

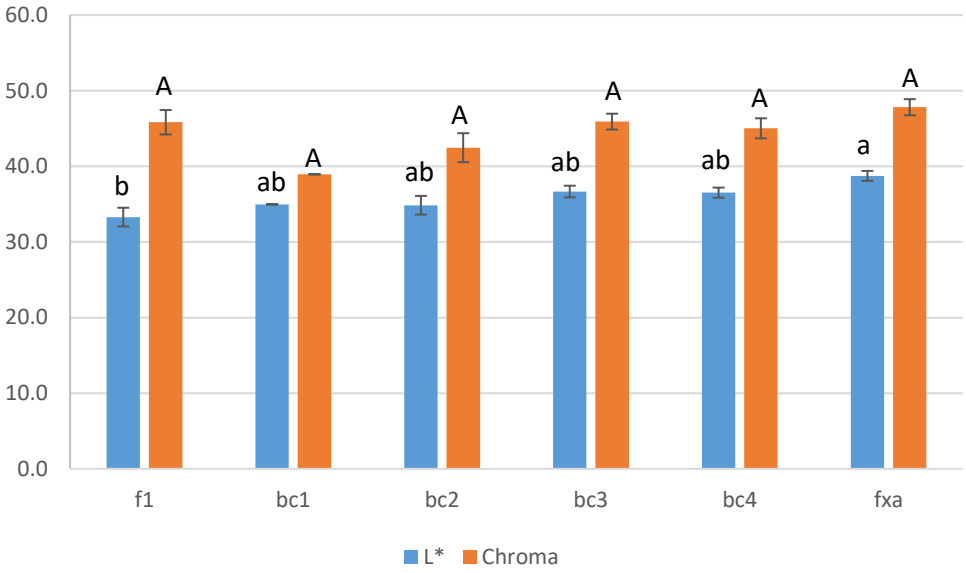

Figure2. 5. Fruit color of the different types of crossing in 2020 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type for L* and uppercase letters indicate significant differences for $p \leq 0.05$ in each crossing types for Chroma

In 2021, the highest Chroma was detected in F1 and the lowest were obtained in bc2, Furthermore, the Chroma of bc3 and bc4 were significantly similar to fxa cultivars. In addition, fxa cultivars had the highest L* and f1 had the lowest L*, While by increasing crossing generation similar to 2020 harvest fruits L* were increased and fruits become lighter.

As comparison, between two harvest cycles, in both years fxa had highest L* and by increasing back cross generation the fruits become significantly similar to commercial cultivars. The fruit color is a primary trait, which is fundamental to attract the interest of the Consumer, and determined by the genotype but also highly influenced by the environmental conditions and maturation stage (Mazzoni et al., 2020).

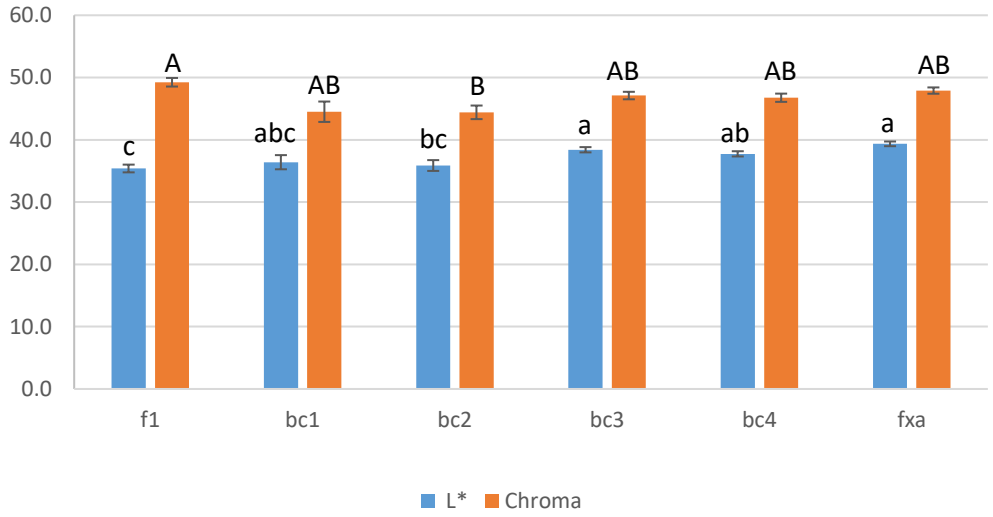

Figure2. 6. Fruit color of the different types of crossing in 2021 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type for L* and uppercase letters indicate significant differences for $p \leq 0.05$ in each crossing types for Chroma

c) Fruit firmness

Fruit firmness of f×a and different crosses (f1-bc4) that originated from fvg and f×a were measured in two cultivation cycles (2020 and 2021). The result was expressed that in 2020 harvest, the fxa had the highest fruit firmness while the wild genotypes produced very small fruits and was not possible to measure their firmness. The lower value of firmness was registered for (fvg) but increased in every following cross generation, reaching a value of firmness significantly similar to the commercial cultivars at (bc4) (Figure 2.7).

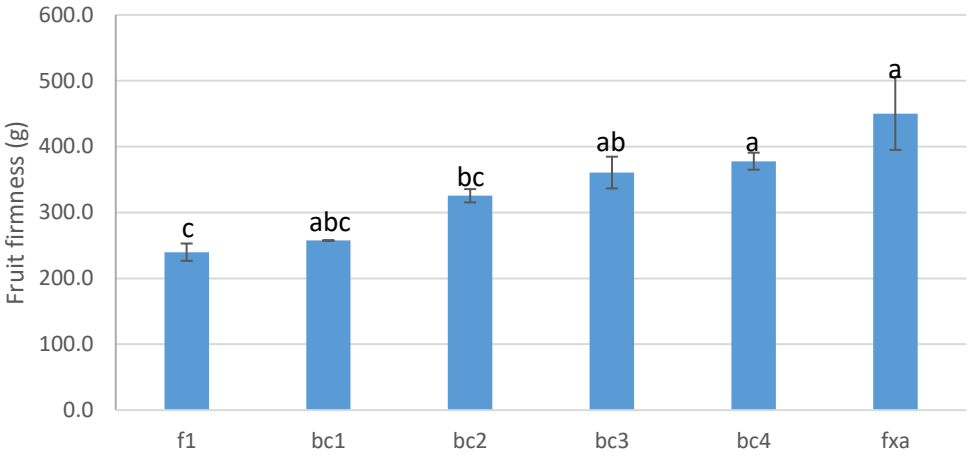

Figure2. 7. Fruit firmness of the different types of crossing in 2020 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for p ≤ 0.05 in each crossing type

Regrading to 2021 harvest, similar to 2020, fvg produced very small fruits and was not possible to measure their firmness. The firmness of the fruits had increased in every cross generation and were obtained more fruit firm in bc4 fruits compare to commercial genotypes. This result is in agreement with the study of Diamanti et al. (2012), where similar fruit firmness was found in their backcrossing types. This result also demonstrates a good progress in the breeding program for this parameter.

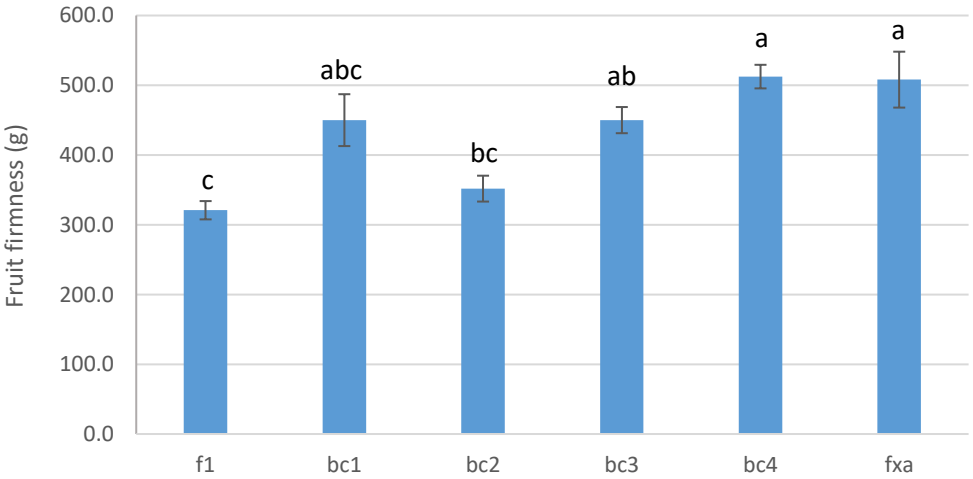

Figure2. 8. Fruit firmness of the different types of crossing in 2021 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for p ≤ 0.05 in each crossing type

2.3.3. Nutritional Analysis

To the consumer, high consumption of fruit is due to the quality acceptance. For that matter, there is an increase in knowledge and awareness of the relationship between the health of consumers and the diet which has resulted in the increased demand for highly acceptable and quality fruit. As a consequence, the release of new cultivars having an improved fruit nutritional quality is strictly linked to the health of the consumer (Capocasa et al., 2008). Furthermore, to identify wild genotype with high quality to produce new pre-breeding materials that are requested for high commercial value is needed.

2.3.3.1. Spectrophotometer Analysis

Regarding the fruit nutritional parameters, the fruit total antioxidant capacity (TAC) of fvg, f×a and different back crosses (f1-bc4) that originated from fvg and f×a were analysed in two cultivation cycles (2020 and 2021). In 2020 harvest, fvg had significantly higher amount (31.24 mM Trolox eq/kg FW) than fxa (17.24 Trolox eq/kg FW). The antioxidant capacity in back crosses from wild to commercial are decreasing because the commercial genome is increasing. While fruit Total Antioxidant Capacity (TAC) detected for BC4 fruit was interesting as it had the high mean value (19.30 mM Trolox eq/kg FW), even if it was not statistically different from value obtained from fxa (Figure 2.9)

In 2021 harvest, similar to 2020 harvest the fvg had the highest mean (19.18 mM Trolox eq/kg FW) but not statistically different from fxa (17.24 mM Trolox eq/kg FW). and all crossing selection had also showed not significantly difference to fvg and fxa (figure 2.10). Comparison of two years, shows that fvg had lower mean in 2021 compare to 2020 but fxa had higher mean in 2021 compare to 2020. Usually, the wild germplasm is believed to possess strong antioxidant activity, higher than the cultivated germplasm (Scalzo et al., 2005; Halvorsen et al., 2002) as happens in this study. Furthermore, the trend in back crosses were also different as in 2020 harvest, fruit TAC were decreasing by increasing commercial genome, but in 2021 harvest all back cross selections had almost same mean.

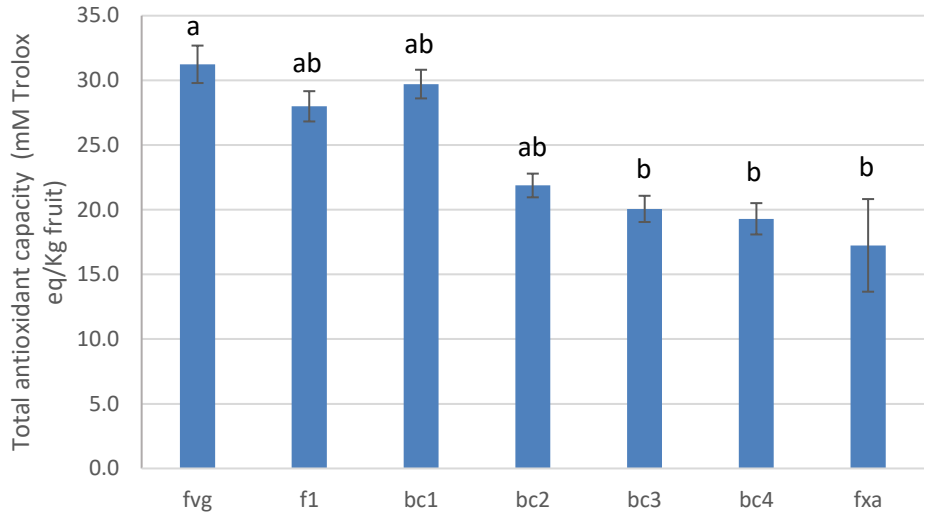

Figure2. 9. Total antioxidant capacity of the different types of crossing in 2020 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type

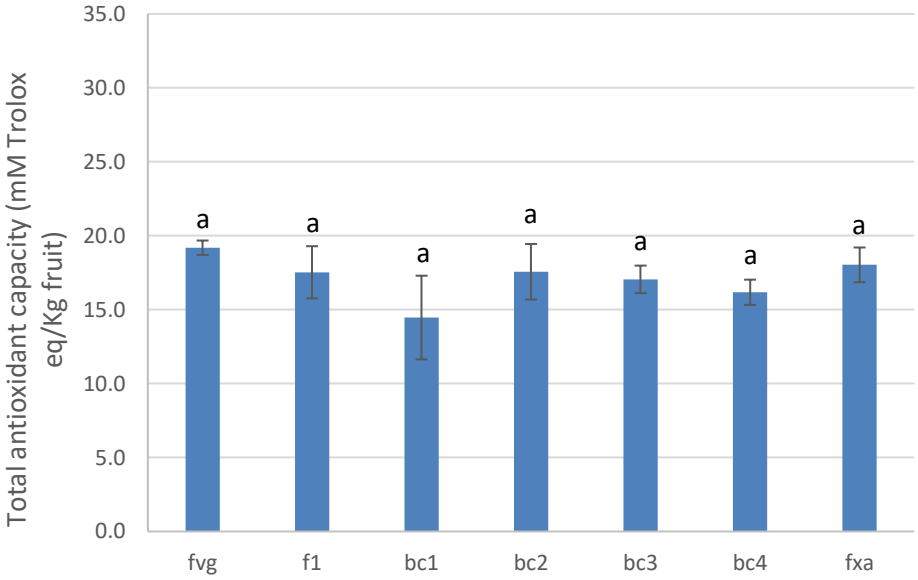

Figure2. 10. Total antioxidant capacity of the different types of crossing in 2021 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type

For total phenolic content, in analyses of 2020 harvested fruit, Wild germplasm (fvg) confirms to present highest mean (6336.86 mg GA/kg FW) and fxa had the lowest mean (1508.94 mg GA/kg FW). In every further step of crossing, the total phenolic compounds are significantly decreasing, while in bc4 generation the total phenolic content was detected 2584.37 mg GA/kg FW, had much improved compare to fxa, even if it was not statistically different (Figure 2.11).
A similar behaviour was observed in the results obtained on fruit Total Phenolic Content of 2021 harvested fruits; fvg confirms to had the highest mean (4728.66 mg GA/kg FW) and fxa had the lowest (1471.31 mg GA/kg FW) total phenolic content. In addition, the trend from f1 to bc4 were significantly decreased as wild genome were decreasing. In bc4, the total phenolic content was detected 1484.98 mg/kg FW, and was higher than fxa but not significantly difference (Figure 2.12). In comparison between two years, in 2020 harvested fruits all selection presented higher mean than 2021 harvested fruits.

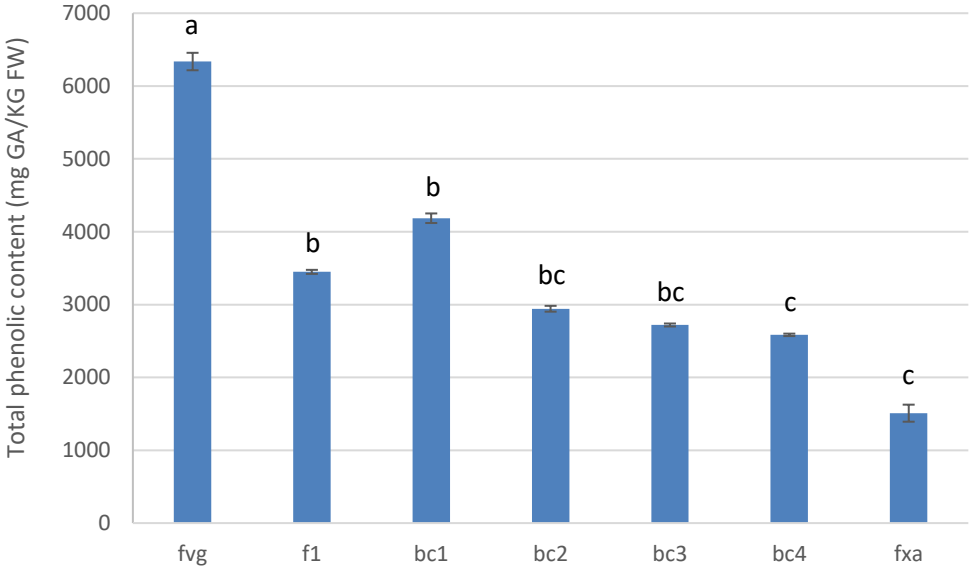

Figure2. 11. Total phenolic content of the different types of crossing in 2020 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type

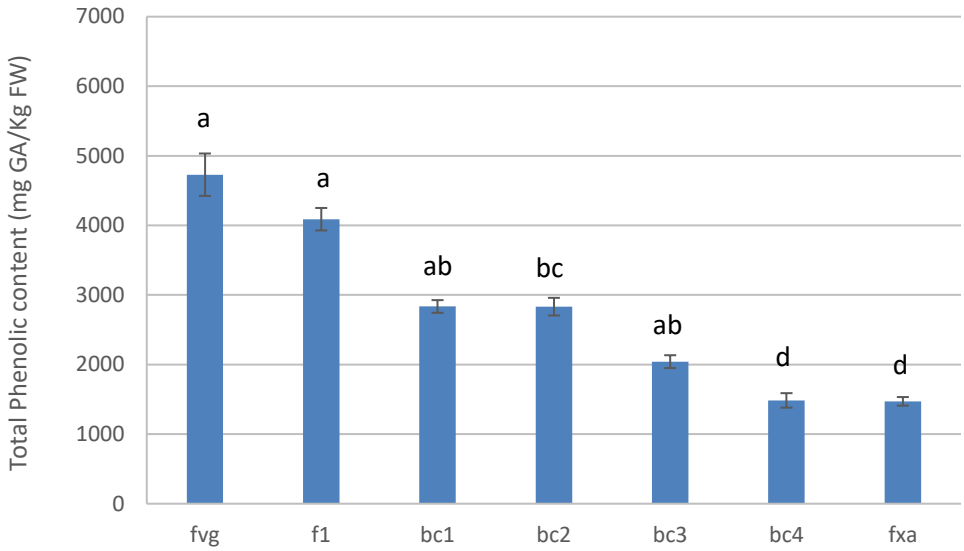

Figure2. 12. Total phenolic content of the different types of crossing in 2021 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type

The total anthocyanin contents of each crossing selections in 2020 and 2021 harvested fruits are showed in Figure 2.13 and Figure 2.14. In 2020 harvested fruits, the analysis of total anthocyanin contents, indicates that the wild genotype had a higher total anthocyanin content (328.82mg PEL-3-GLU/kg FW) than the commercial (289.38 mg PEL-3-GLU/kg FW); regarding the back crosses, the highest anthocyanin content were detected in bc1 (334.2282 mg PEL-3-GLU/kg FW); However, in the following back crosses total anthocyanin content had significantly decreased and in bc4 the amount of total anthocyanin content was detected 237.91 mg PEL-3-GLU/kg FW (Figure 2.13). Regarding, the analysis of total anthocyanin contents of the wild, commercial and back crosses of 2021 harvested fruits indicate that the wild genotype (195.7 mg PEL-3-GLU/kg FW) had a lower total anthocyanin content than the commercial (379.58 mg PEL-3-GLU/kg FW), and trends in back crosses are increasing when the commercial genome is increasing (Figure 2.14). In 2021, the total anthocyanin content of the fxa was much higher than 2020 fxa, and fvg of the 2021 was lower compared to 2020 fvg fruits. Increasing the amount of anthocyanin concentration, despite the benefit for the health, will also affect the color of strawberry fruit: an excess of anthocyanins amount will generate darker fruits, and their market value could decrease if the color turns excessively toward black shade (Diamanti et al., 2016).

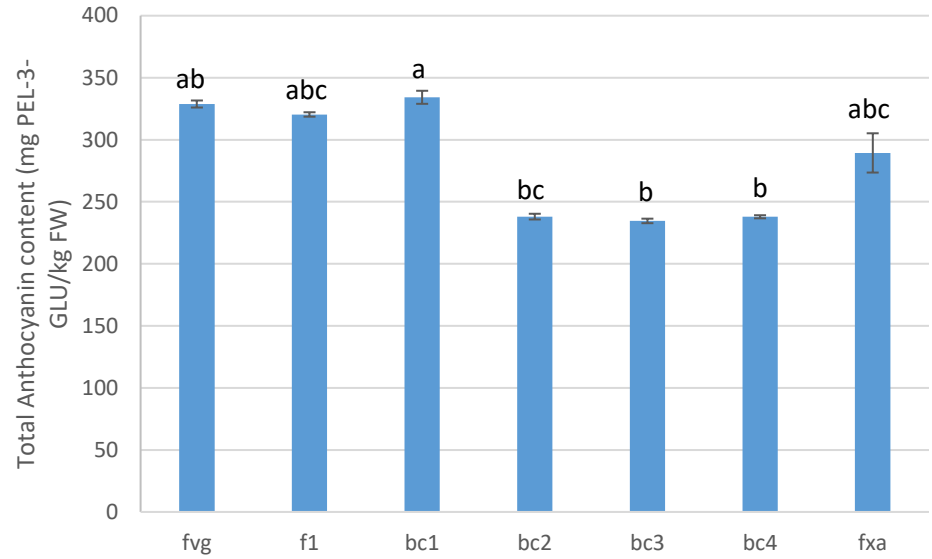

Figure2. 13. Total Anthocyanin content of the different types of crossing in 2020 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type

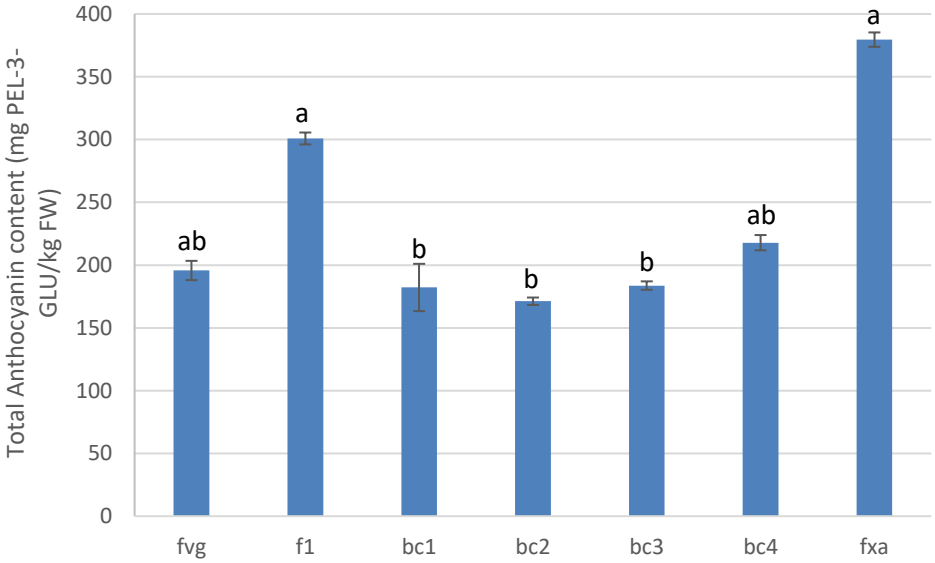

Figure2. 14. Total Anthocyanin content of the different types of crossing in 2021 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type

2.3.3.2. HPLC-UV Analysis

The fruit content of vitamin C of fvg, fxa and each backcross genotypes that originated from fvg and fxa was analysed for two cultivation cycles. The results from 2020 harvested fruits, demonstrates that the fvg is the main sources of vitamin c content with the highest mean (71.3 mg/100g FW) and fxa presented the lowest mean (30.47 mg/100g FW). values in f1 and bc1 significantly similar to fvg and from bc2-bc4 values significantly decreased; while in bc4 selections the vitamin C content mean were 37.28 mg/100g FW, which is higher than commercial cultivars (Figure 2.15). Similarly, to 2020 fruits, in 2021 fruits wild genotypes presented high content of vitamin c, with the peak in f1 (65.91 mg/100g FW) and in the following cross generation the vitamin C content had decreased even if it was not statistically different. In bc4 selection the vitamin C content was observed 58.47 mg/100g FW (Figure 16). As comparison the two years' data, fvg fruits in 2020 presented higher amount of vitamin C content compare to 2021 and fxa fruits in 2020 had less vitamin C content compare to 2021. Generally, all back cross selections presented more vitamin C content in 2021 cultivation cycle compare to 2020. Similar results were obtained in the previous study of breeding program of UNIVPM by Mazzoni et al. (2021). In another study, Zhong et al. (2017) evaluated amount of vitamin C in different strawberry genotypes and found values in the range of 38-52.5 mg/100gr FW.

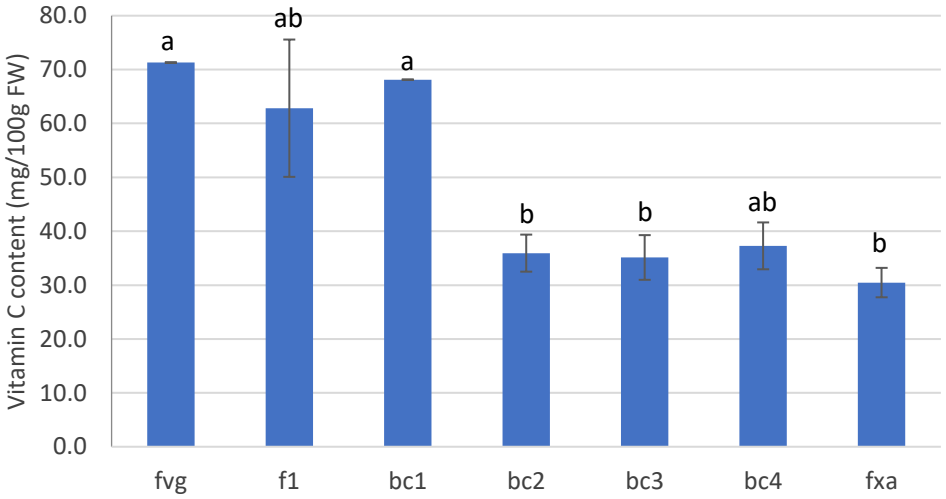

Figure2. 15. Vitamin C content of the different types of crossing in 2020 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type

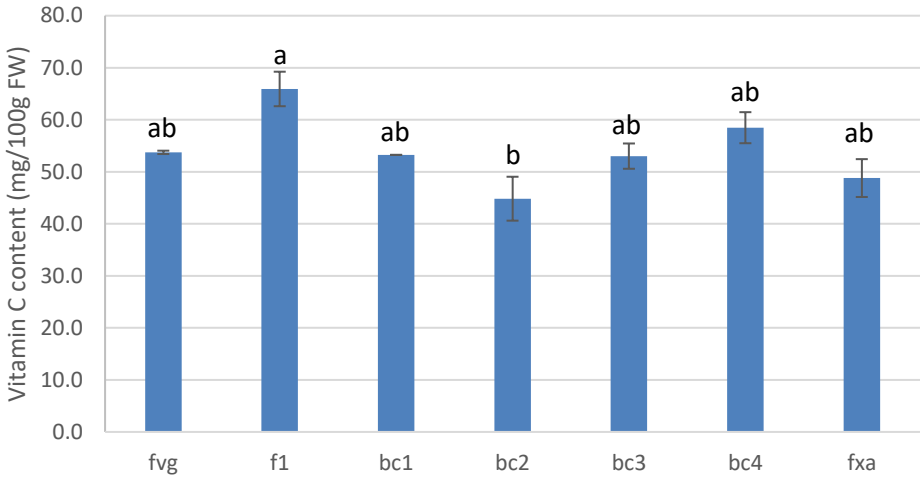

Figure2. 16. Vitamin C content of the different types of crossing in 2021 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type

Phenolic acids of fvg, fxa and each back crosses genotypes of 2020 and 2021 harvested fruits were analysed by HPLC and the results for 2020 harvested fruits showed that the wild genotype had much higher phenolic acids, with 88 mg/100g FW. The most important phenolic acids of strawberries are Chlorogenic acid, Caffeic acid and Ellagic acid. Chlorogenic acid was the predominant phenolic acid among all the genotypes. The trend of phenolic acid is strongly decreasing when the genome of commercial strawberry is introduced in the crossing, with all the bc generations having strongly lower levels of phenolic acids than fvg and f1; in bc4 the phenolic acid content was reached to 26.1 mg/100g FW, which is significantly lower than fxa (Figure 2.17).
Regards on 2021, the commercial cultivars had the highest mean value (46.95 mg/100 g FW) but significantly similar to fvg and all back crosses selections. Furthermore, in bc4 phenolic acid content were 43.30 mg/100g FW (Figure 2.18). As comparison the two years' data, fvg had much higher fruit phenolic acid content in 2020 cultivation cycle compare 2021, but fxa cultivar showed same mean in both years, while backcrosses also showed lower amount of phenolic acids content in 2021 compare to 2020 fruits. In addition, compared to previous study of breeding program UNIVPM from Mazzoni et al. (2021), genotypes of the present study have much higher content of phenolic acids.

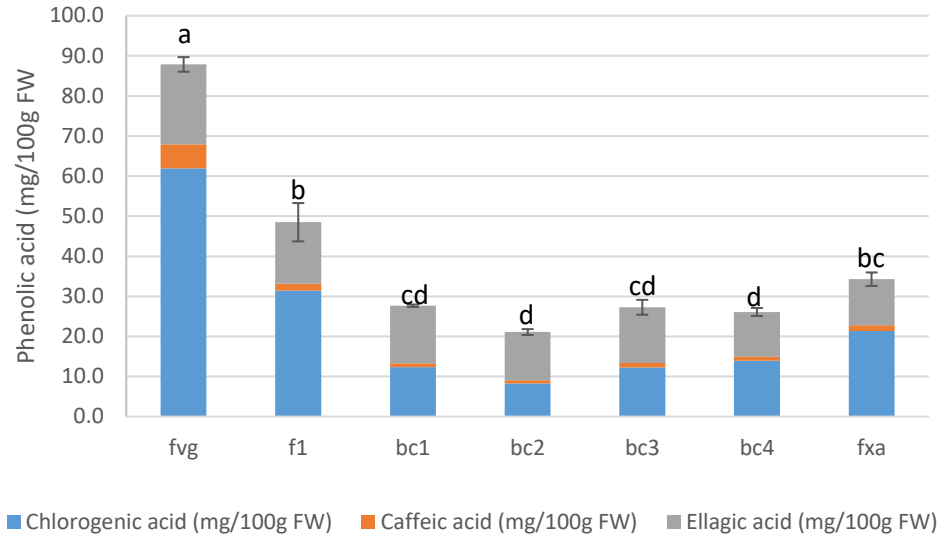

Figure2. 17. Phenolic acids contents of the different types of crossing in 2020 harvest, the data are expressed as mean ±standard error. Different lowercase letters indicate significant differences for p ≤ 0.05 in each crossing type

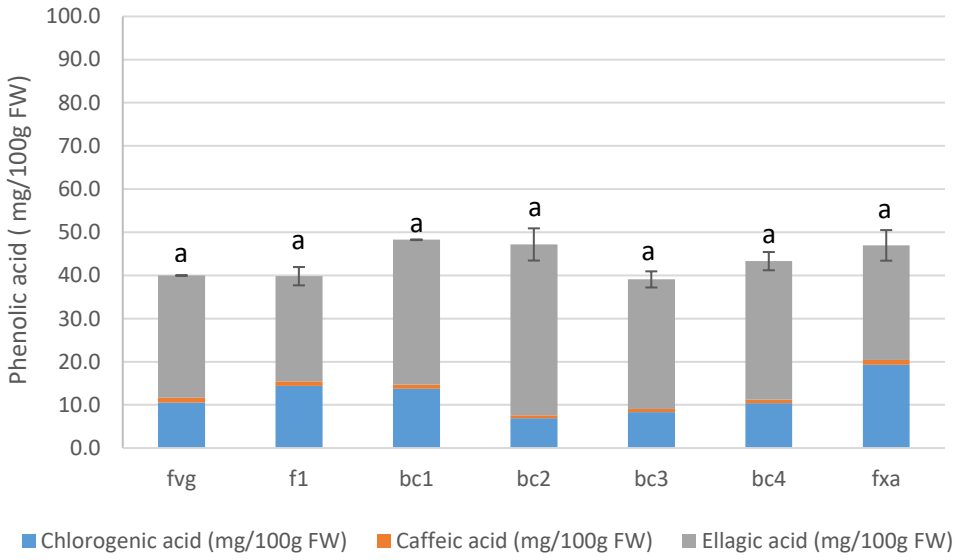

Figure2. 18. Phenolic acids contents of the different types of crossing in 2021 harvest, the data are expressed as mean ±standard error. Different lowercase letters indicate significant differences for p ≤ 0.05 in each crossing type

The most important anthocyanins of strawberries are cyanidin-3-glucoside, pelargonidin-3-glucoside and pelargonidin-3-rutinoside. Evolution of the color is strongly correlated with anthocyanin concentration across various fruit and vegetables (Gonçalves et al., 2007; Borochov-Neori et al., 2011). Increasing the amount of anthocyanin concentration affect the color of strawberry fruit. Excess of anthocyanins amount will generate darker fruits. Total anthocyanin content of each backcross genotypes of two years' cultivation cycles (2020-2021) was analysed by HPLC; and results for 2020 demonstrate that fruit content of the major anthocyanins resulted significantly similar among all back crosses and to both wild and cultivated germplasm. The highest mean value of total anthocyanins was found in f1 (26.30 mg/100g FW). Regards on 2021, fvg had significant highest mean value of total anthocyanins (64.31mg/100g FW) and the lowest mean were obtained bc1 (26.47 mg/100g FW) followed by fxa with (35.94 mg/100g FW). In bc4 higher anthocyanin content (36.51mg/100f FW) were obtained than fxa but not signifantly difference. Comparison of two years, shows that higher anthocyanin content was detected in all 2021 crossing selections fruits compare to 2020 fruits.

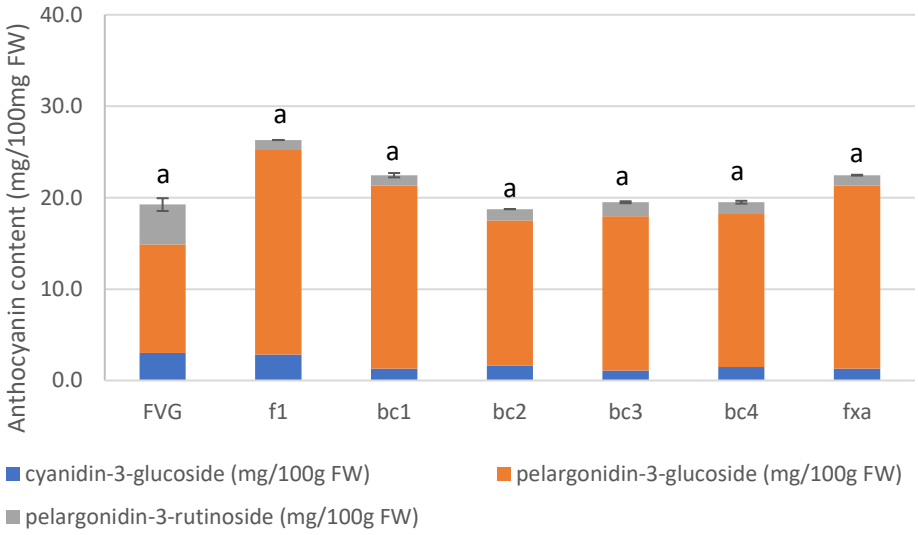

Figure2. 19. Anthocyanin content of the different types of crossing in 2020 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type

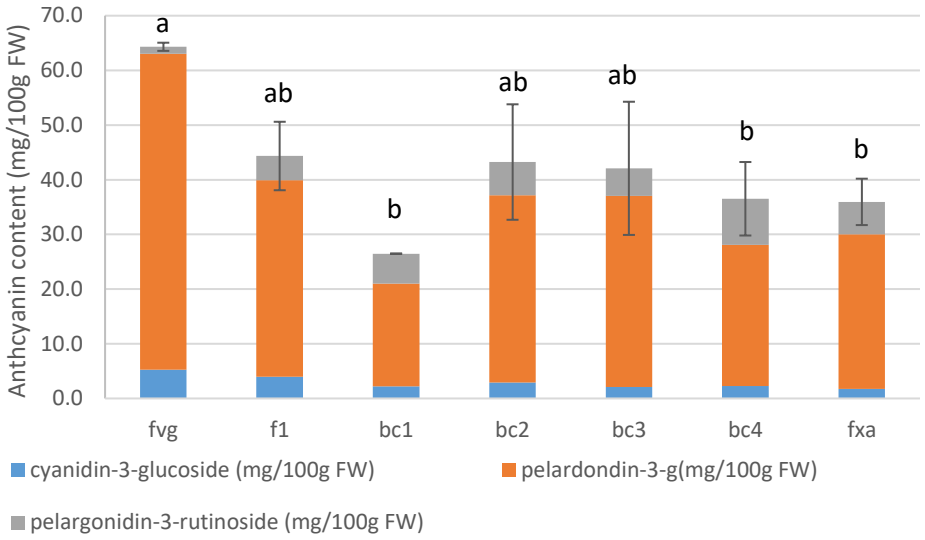

Figure2. 20. Anthocyanin content of the different types of crossing in 2021 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each crossing type

2.3.3.3. HPLC-FLD Analysis

The folate content of fvg, fxa and back crosses selections during two years of study are analysed, and the results indicates that: In 2020, Fruits of the wild genotype (fvg) had significantly highest folate content (19.55 ug 5methyltetrahydrofolic acid/100g FW), whereas the commercial was lower (12.06 ug 5methyltetrahydrofolic acid/100g FW), and the trend through back crosses is slightly decreasing due to increasing the commercial genome. In bc4 selections the folate content was detected (11.86 ug 5methyltetrahydrofolic acid/100g FW) significantly similar to commercial cultivars (Figure 2.21).

Regards on 2021, the highest Folate content were obtained in bc4 with mean of 79.97 ug 5methyltetrahydrofolic acid/100g FW, followed by 58.45 ug 5methyltetrahydrofolic acid/100g FW, where significantly similar to wild genotype (Figure 2.22). as comparison of two years, it shows that all genotypes had higher folate content in 2021 compared to 2020. In previous study of breeding program UNIVPM from Mezzetti et al. (2016), the range of folate in different genotypes were 5-7ug 5methyltetrahydrofolic acid/100g FW and compared to this, genotypes of the present study had much more folate content. Folate contents shown in this work are in keeping with values reported in European food data tables, where folate in strawberries is indicated to range from 0.2 to 0.99 µg/g of FW (Strålsjo et al., 2003).

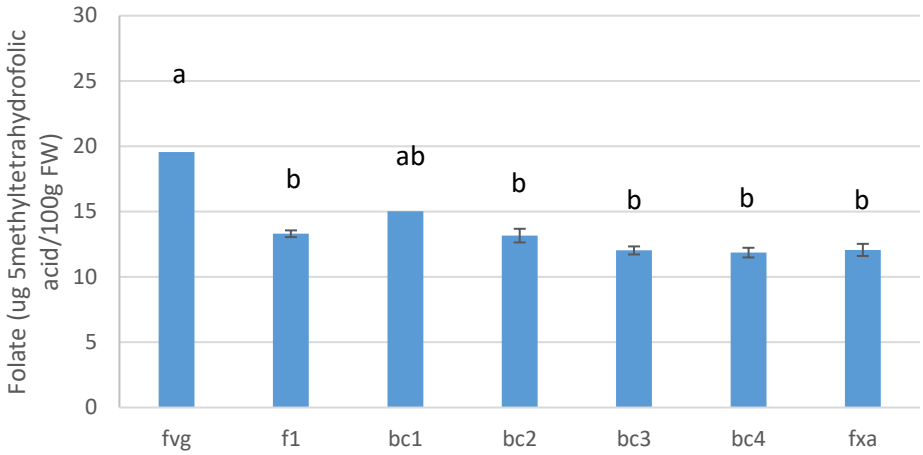

Figure2. 21. Folate content of the different types of crossing in 2020 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for p ≤ 0.05 in each crossing type

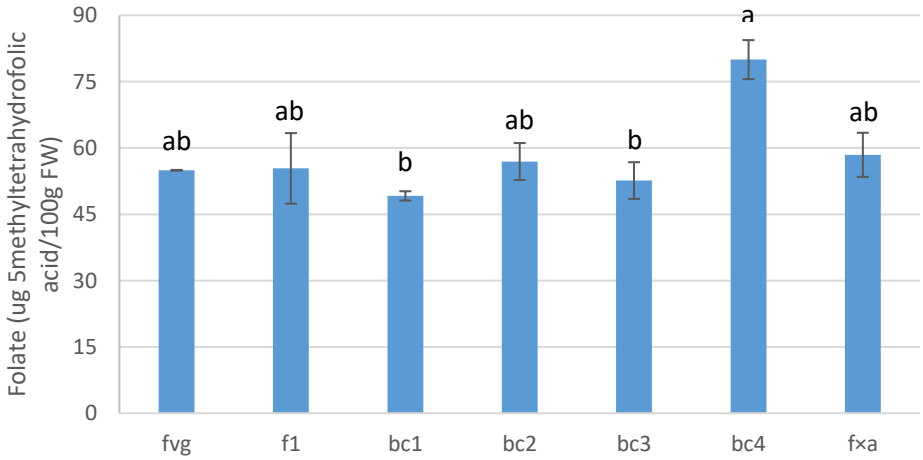

Figure2. 22. Folate content of the different types of crossing in 2021 harvest, the data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for p ≤ 0.05 in each crossing type

2.3.3.4. Gas chromatographer (GC-TOF–MS) Analysis

The analyses of primary metabolites by GC-MS-TOF produced many interesting data for the different genotypes analyzed in this study. A first general view of all the detected metabolites (Figure X) revealed a high rate for many Aminoacids and sugars (except sucrose, raffinose and xylose) in the FVG group, which represent one of the parental

line. Contrarily, organic acids were generally low in FVG. Interestingly, an opposite behaviour was registered for FxA group, which represent the other parental line. In this group, in fact, Organic acids were particularly high (with some exceptions), contrarily to sugars (excluding glucopyranoside 1-O-Methyl-Alpha-D and raffinose, which were particularly present). Regarding the other crossing groups, they presented intermediate characteristics between the the parental groups. It is particularly interesting to note that in all the generations, from F1 to BC4, the amount of aminoacids and sugars has been gradually lost in respect to FVG. An exception is partially represented by the BC3 and BC4 groups, in which some sugars (glucose and fructose in particular) and some aminoacids (tryptophan, glutamine and threonine among others) increased in a positive manner, being the highest in all the study. F1 group showed intermediate characteristics between the wild parent (FVG) and the commercial parent (FxA). BC1 was the generation which presented the worst results, presenting quite low amount for all the groups of compounds, except for some sugars (xylose and raffinose) and citric acid. From BC2 onward, the organic acid profile became more similar to the commercial parent, until BC4, which showed both good aminoacid (from FVG), organic acids and sugars (xylose, sucrose) profile.

Grouping genotypes for crossing type (genotypes with the same parentals), or leaving single genotypes when they have no "brothers", it can give us further information regarding the characteristics of the analyzed genotypes, underlying the high variability that occurrs in rimary metabolites composition even in the same crossing type (Figure Y). For example, in the different f1 lines, the AN12,50,52 seemd to be particularly interesting and oustanding in respect to the other F1 selections, having increased aminoacid and organic acids profile than the other F1 lines, keeping other parameters similar. In BC2, the line AN15,04,54 is slightly different from the other two lines, in particular standing out for higher amount of many organic acids and some other compounds (galactinol and putrescine). All the BC3 lines presented good sugar profile, but the line AN16,32 presented also improved aminoacid profile in respect to other BC3 groups. All the BC4 groups presented a balanced profile, with good results for aminoacids and sugars profile, and presenting lower organic acids. Only the AN17,19 line presented some more organic acids. Finally, regarding FxA genotypes, it is possible to recognize a quite substantial difference among the analyzed cultivars. Silvia presented under-average values for all the analyzed primary metabolites, while Dina standed out for aminoacid and organic acids amount. Sibilla showed the best profile for the sugar composition, with higher amount than the other FxA genotypes of sucrose, raffinose, glucose, fructose, raffinose, and glucopyranoside 1-O-Methyl-Alpha-D.

The analysis of primary metabolites represented a higly useful tool for the aim of this study: first of all, it provides a complete and exhaustive description of the main primary metabolites found in the analyzed genotypes. Furthermore, it allows to cluster crossing types and genotypes, detecting the similar genotypes for some specific groups of compounds, and facilitating the selection of the most interesting genotypes. This tool, applied to the breeding program, will help to evaluate the success of the new selections in improving the required characters, and will help to select the most interesting to continue the breeding program.

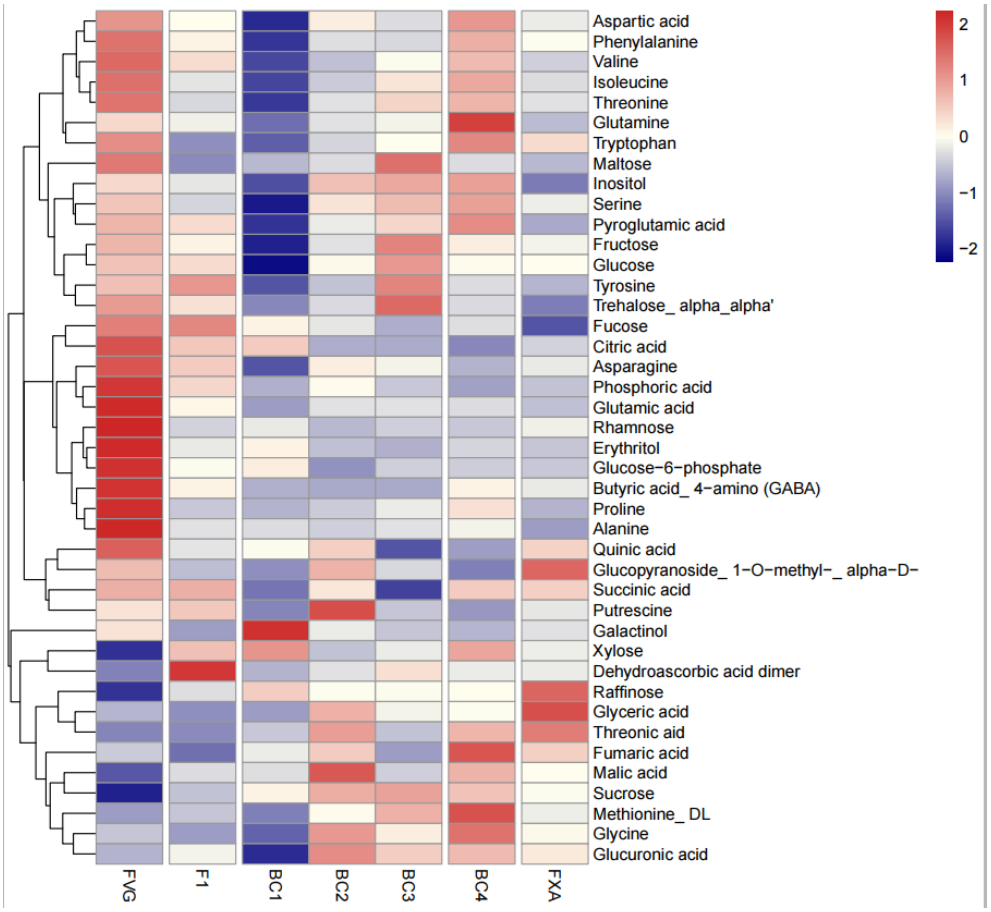

Figure2. 23. Heatmap of primary metabolites of crossing types analyzed in the study. This graph was made by using pheatmap package (Kolde, 2019).

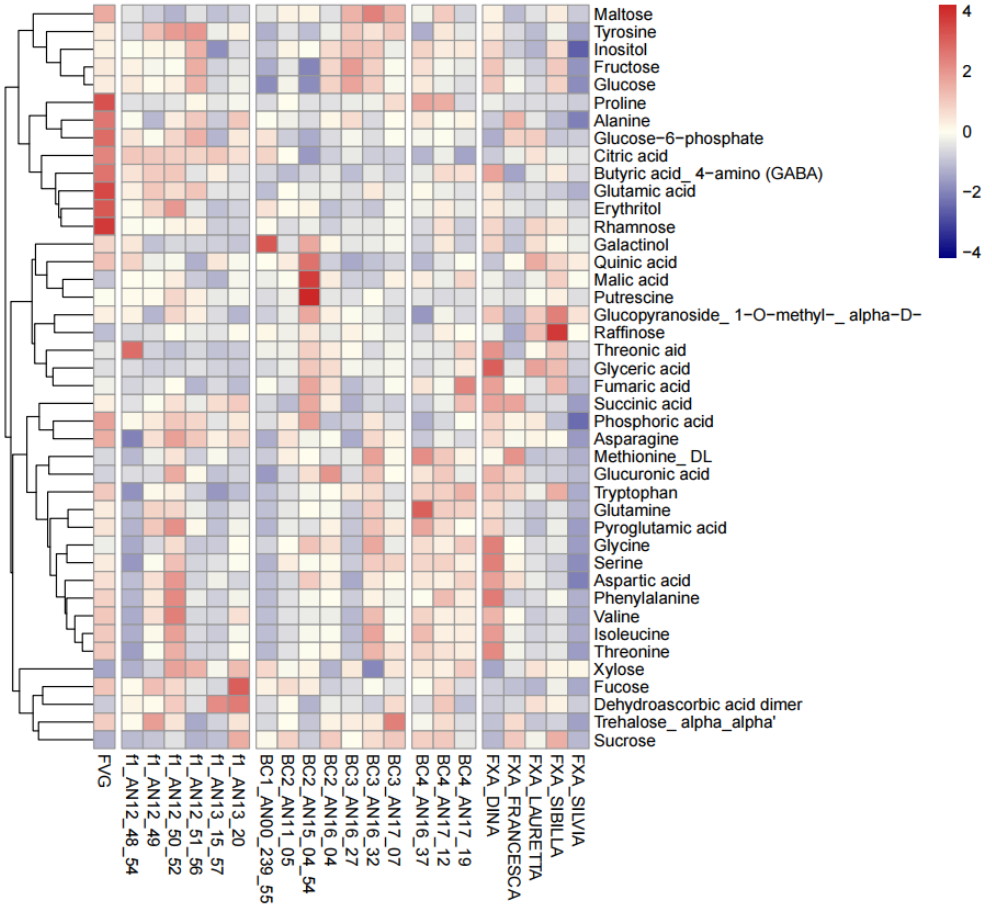

Figure2. 24. Heatmap of primary metabolites of crossing types analyzed in the study, grouped for crossing lines or for single genotype. This graph was made by using pheatmap package (Kolde, 2019).

2.3.4. General Remarks

For most fruit breeding programmes, fruit quality components have increased in importance as targets for new cultivars in recent years, and now they should be considered together with longer-standing agronomic objectives, such as yield and pathogen resistance. The ultimate goal for breeders and commercial growers is a plant variety that combines both agronomic and quality traits, and that can be grown sustainably within under the future climate scenarios. Breeding for the enhancement of one or more beneficial phytochemicals in strawberry fruit is likely to be achievable by using selected germplasm as source of enounced metabolisms and specific breeding programs able to combine them with the commercial traits. This approach has been already adopted to improve many different fruit, including strawberries (Capocasa et

al., 2008; Diamanti et al., 2012). Furthermore, there is an increasing awareness on the need to study the multiple genetic factors and their interaction with the environment and cultivation techniques in order to increase the production and fruit accumulation of bioactive compounds with health benefits for the consumer (Davik et al., 2006; Kruger et al., 2012).

In this study parallel to the aim of study commercial cultivars increased the yield, along with decreasing the waste fruits as wild germplasm is well known for pathogen resistance germ. In addition, as expected in bc4 to reach at almost same production at commercial level; in 2020 cultivation cycle, bc4 selections had significantly reached to the expected target. While in 2021, didn't reached on the aim for productions, that might be due to environmental factors (Tulipani et al., 2008; Diamanti et al., 2012), that for some genotypes could be strong limiting factors (Di vitorri et al., 2018).

For total soluble solids and titratable acidity, in bc4 selections more soluble solids content, with more balanced SS and TA were obtained, that demonstrate positive potential of wild genotypes in order of sweetness. Regards on color, as it is fundamental attract of consumers; Similar to expectations bc4 selections significantly reached at same level of commercial fruit brightness. Furthermore, commercial cultivars positively increased the fruit firmness in back cross generation.

Regarding on Nutritional quality parameters, as wild genotype is known for its high nutritional value (Diamanti et al., 2012). Wild germplasm is an interesting source of genes for improving fruit nutritional quality of new cultivars, but long-term backcross programs are needed to produce new pre breeding materials that can facilitate the release of new cultivars with fruits of superior quality, and also in this study wild genotype recorded as high nutritional germ. In back crosses generation Significantly increased, the total antioxidant capacity, total phenolic content, Vitamin C content, folate content.

These results demonstrated that a good progress in the breeding program. To obtain high nutritional and sensorial quality strawberries with optimum commercial agronomic level.

2.4. Conclusions

The evolution of the backcrossing originated by the breeding program developed at UNIVPM-D3A, which included *F. virginiana subsp. glauca* and *F. × ananassa*, showed that the bc4 generation, which are actually the most recent generation of this program, possessed many improved characters in respect to the fxa genotype and the fvg. The bc4 generation improved productive and sensorial parameters that is not far from commercial requirements. However, it is possible to conclude that with four backcrossing generations it is possible to reach the productive and sensorial levels of the commercial genotypes. Regarding the nutritional parameters, bc4 showed higher total antioxidant capacity values and, consequently, of the higher values of total phenolic content, demonstrating the efficiency of the breeding program to increase the nutritional value of strawberries in bc4. The total anthocyanin content level was also very interesting, but far from the Fxa. This was expected, given that Fxa genotypes were elected for their intense red coloration. Finally, HPLC data confirmed the excellent

nutritional value of bc4 generation, presenting high amount of Anthocyanins, vitamin C content and Folate content. Regarding phenolic acids, the value was statistically similar to the commercial Fxa genotypes in 2021 cultivation cycles.
Based on bc4 selections some selections (Table 2.2) performed well in most Agronomic, sensorial and nutritional parameters, and could be used as parents for further breeding programs to increase the nutritional values. In addition, these selections could be a potential genotype for a new cultivar. The release of a new cultivar is not only an ending point, it is also a starting point for further breeding programs for increasing the nutritional quality, and it is noted that the breeding program was successful to create new genotype with increased nutritional quality with near agronomic standards required by the market.

Table2. 2. Mean values of all parameters of 2 years for best performed bc4 selections and f×a.

Year	Genotypes	SS	TA	Production	Fruit firmness	Total anthocyanin content	Total phenolic compounds	Total antioxidant capacity	Vitamin C	Phenolic acids	Anthocyanins (HPLC)	Folate content
2020	AN16,37,53	10.10	10.05	501.07	378.75	408.24	4465.75	27.95	30.92	28.31	27.65	15.42
2021	AN16,37,53	10.40	12.30	219.00	593.13	126.87	1319.21	11.89	55.67	32.06	35.14	80.31
2020	AN16,37,60	8.90	12.17	921.60	390.21	242.46	2142.38	16.39	34.57	27.24	17.37	11.49
2021	AN16,37,60	10.20	11.30	503.00	561.67	172.08	1092.97	8.98	62.77	46.72	25.43	92.84
2020	AN17,12,51	8.23	14.55	713.44	406.46	314.68	2827.30	20.44	38.60	29.40	24.38	12.05
2021	AN17,12,51	9.00	15.60	422.00	393.75	392.02	2511.11	27.74	52.04	53.55	70.08	93.02
2020	AN17,12,52	10.10	14.97	486.14	504.79	264.13	3196.95	21.04	64.25	31.48	18.63	12.10
2021	AN17,12,52	10.40	14.80	452.00	688.54	204.01	2071.20	22.09	106.29	74.08	50.09	127.28
2020	AN17,12,55	8.00	14.83	383.28	322.92	382.03	3605.17	25.90	72.92	42.71	28.70	12.31
2021	AN17,12,55	10.10	15.40	284.00	393.13	232.74	2156.94	22.78	78.46	44.21	39.65	77.63
2020	f×a	8.78	10.74	695.31	450.27	286.03	1508.94	17.25	30.47	34.27	20.46	12.07
2021	f×a	8.49	11.16	911.00	508.08	379.58	1471.31	18.02	48.79	46.61	37.26	56.62

Chapter 3.

Resistance of different cultivars to *Botrytis cinerea* and its relation with fruit quality

Abstract

Strawberries are a delicate, high-value nutritional fruit with an extremely short shelf life and high susceptibility to tissue infection, mainly by Botrytis cinerea. Control of the disease requires an extensive amount of fungicide that is applied in varying complexes because the pathogen easily develops resistance against the active compounds. Planting of resistant cultivars seems to be a promising alternative for fruit growers, but there are currently no cultivars available combining resistance to B. cinerea with attractive horticultural traits. In this study four well-defined strawberry cultivars (Romina, Cristina, Silvia and Sibilla) were selected and tested under treatment with *Botrytis cinerea* to determine the susceptibility of each cultivar and its relation with fruit quality. The result shows that 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 and Romina cultivar resulting in the cultivar most susceptible to the treatment, with a McKinney Index equal to 100%. The results of the study also highlighted important qualitative changes in the four strawberry cultivars contaminated with *Botrytis cinerea*, by a decrease in the soluble solids content and increase of acids. Generally, in all cultivars phenolic acids and vitamin C decreased in both control and treated but there was strong decreased in treated fruit compare to control. Anthocyanin content increased in control fruits but strong decrease in treated. Strong positive correlation was obtained from nutritional quality with resistance to decay, as high amount of nutritional content more tolerance to botrytis cinerea.

3.1. Introduction

Strawberry is one of the most cultivated and widespread fruits in the world and represents an important 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.

Strawberries have been part of human diet for centuries and they are considered as a very rich dietary source of bioactive compounds (vitamin C and B9) and phenolic compounds (phenolic acids, and flavonoids and anthocyanins) (Oszmianski et al., 2009; Giampieri et al., 2012; Ariza et al., 2016; Mazzoni et al., 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 main strawberry pathogen is Botrytis cinerea, the causal agent of gray mold, followed by Rhizopus stolonifera, Mucor spp., Colletotrichum spp., and Penicillinum spp. (Feliziani and Romanazzi, 2016). Postharvest diseases caused by these pathogens are the result of latent infections that are initiated in the field during the growing season and infections from wounding during harvest and handling operations (Michailides et al., 2009). Early detection of fungal infected fruit is very important both for producers and consumers. Fungal contamination is especially dangerous in packing houses during storage, transport and marketing procedures because even a very small number of infected fruit can spread the infection to adjacent healthy strawberries. The presence of fungal diseases on fruit surfaces not only causes loss of quality, but also diminishes the safety of the final product. Some fungal genera and species can produce mycotoxins, which cause infections or allergies in susceptible individuals (Gallo et al., 2015).

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. 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.

For the present study, four well-defined strawberry cultivars (Romina, Cristina, Silvia and Sibilla) were selected 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,

anthocyanins, and phenolic acids; and to evaluate the response and susceptibility of each cultivar, in response to the disease caused by the fungal pathogen.

3.2. Material and Methods

3.2.1. Plant Materials

The production of strawberries took place at the "P. Rosati" Experimental Farm (43°31'N 13°36'E. 46 m altitude) Agugliano (Ancona, Italy), on non-fumigated soil, having the following main characteristics: pH 7.9, active calcium 9% and texture composed at 40% clay, 25% sand and 35% silt, following the procedure described in Capocasa et al. (2016). 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).

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. 108 fruits were sampled for each variety and the fruits are selected from various plots and collected during the third or fourth harvest.

Table3. 1. Strawberry genotypes analyzed

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

3.2.2. Inoculation and damage assessment from *Botrytis cinerea*

For evaluating the resistance of different varieties to Botrytis cinerea, we evaluated based on the protocol of (Siedliska et al. 2018). Before inoculation procedure Botrytis cinerea was grown on agar for two weeks at room temperature. The fungal conidia were scraped from the surface of the agar with a sterile glass rod in a small volume of sterile water and diluted to a concentration of about 10^6 spores mL^{-1}. During the inoculation, strawberry fruit of the different varieties were immersed in botrytis cinerea (10^6 spores mL^{-1}) or in sterile distilled water for the control, for 30s and dried in room temperature under air cabin. During the whole experiment (4 days), control and inoculated fruit samples were stored in dark chambers at 20 ± 1 °C and 90% relative humidity (RH), according to the procedure proposed by Wang et al. (2012) and Guidarelli et al. (2011). 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. The decay index were

calculated based on I%= $[\sum \frac{(d \times f)}{(N \times D)}] * 100$ (McKinney, 1923). After each day evaluated fruits were stored at -80˚C for other sensorial and nutritional parameters analysis.

3.2.3. Soluble solid content

Determined as described in chapter 2.2.3.

3.2.4. Titratable acidity

Determined as described in chapter 2.2.3.

3.2.5. Methanolic extraction

Fruit extracts were prepared as described in chapter 2.2.4.1.

3.2.6. Vitamin C extraction

Vitamin C of fruits was extracted as described in chapter 2.2.4.2.

3.2.7. HPLC determination of Phenolic acids

Phenolic acids were analysed as previously described in chapter 2.2.4.7.

3.2.8. HPLC determination of Anthocyanins

Anthocyanin content was analysed following the method as described in chapter2.2.4.7.

3.2.9. HPLC determination of Vitamin C contents

Vitamin C content was measured as described in chapter 2.2.4.7.

3.2.10. Data analyses

The results are presented as the values ± standard error

3.3. Results and Discussion

3.3.1. Inoculation and damage assessment from *Botrytis cinerea*

Figure 3. 1 and Figure 3. 2 show the susceptibility index of Romina, Cristina, Silvia and Sibilla cultivars against Botrytis *cinerea* in control and treated fruits, respectively. As result shows in Figure 3. 1, Cristina was the most sensitive against Botrytis *cinerea* with almost 70% of damage index and Romina was the most resistance in control trail (35%). However, the interesting result which was found in treated strawberries, shows that Romina is very sensitive (almost 100%) when treated it with Botrytis *cinerea* and Silvia was the most resistant against Botrytis cinerea in treated trail with sensitivity of

71.7% (Figure 3.2).

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 increases the McKinney's Index and the severity decay index.

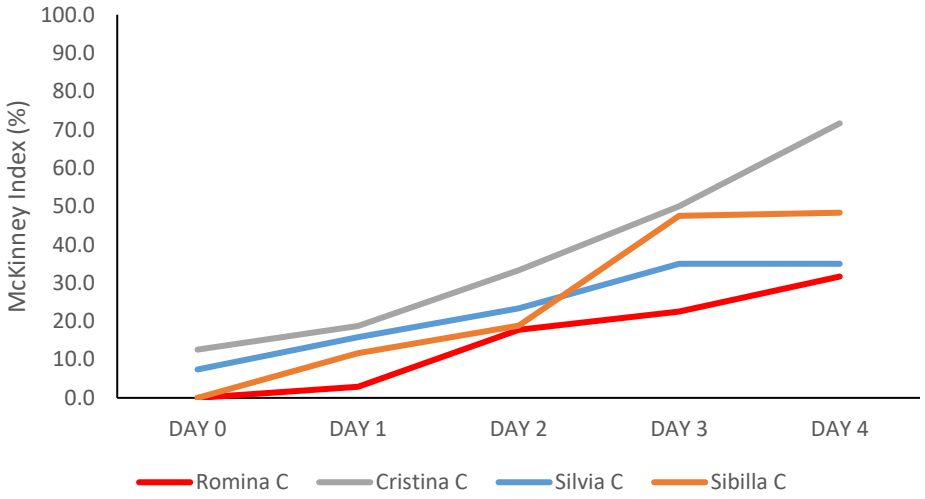

Figure 3. 1. Susceptibility of strawberry cultivars to Botrytis cinerea (control)

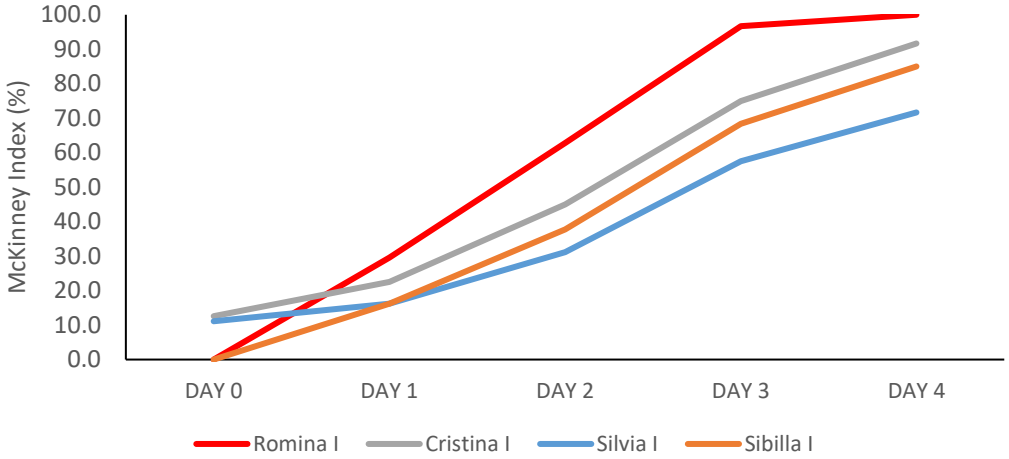

Figure 3. 2. Susceptibility of strawberry cultivars to Botrytis cinerea (treated trial).

3.3.2. Total Soluble solids content

The Figure 3. 3 represents the soluble solids content of control and Figure 3.4 represents the soluble solids content of treated strawberries of all cultivars (Cristina, Romina, Sibilla and Silvia) from day0 to day4. The highest sugar content in control was achieved by Sibilla cultivar with an average of 7.9 °Brix, and 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, and it shows a slightly decreasing trend variation by the increasing of shelf life in all cultivars. The least changes were in Silvia and the most decreasing trend was found in Romina.

In treated fruit, the highest sugar content was achieved by Sibilla cultivar with an average of 7.78 °Brix, and the lowest were 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. From the Figure 3.4, it is possible to deduce how the Cristina and Silvia cultivars show a significant decreasing trend variation till 4 days of shelf life, 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 (Figure3. 3).

Based on the result, 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. 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. Ripening increase with the increasing shelf life, and during ripening, the content of sugar in strawberries increases and therefore can serve as nutrients for B. cinerea. In unripe strawberries, the main sugars are glucose and fructose with low concentrations of sucrose. Sucrose levels increase rapidly during de-greening and red colouring (Jia et al., 2011). Soluble sugars are the major carbon source for the fungal growth and reproduction may promote the infection processes (Li et al., 2022).

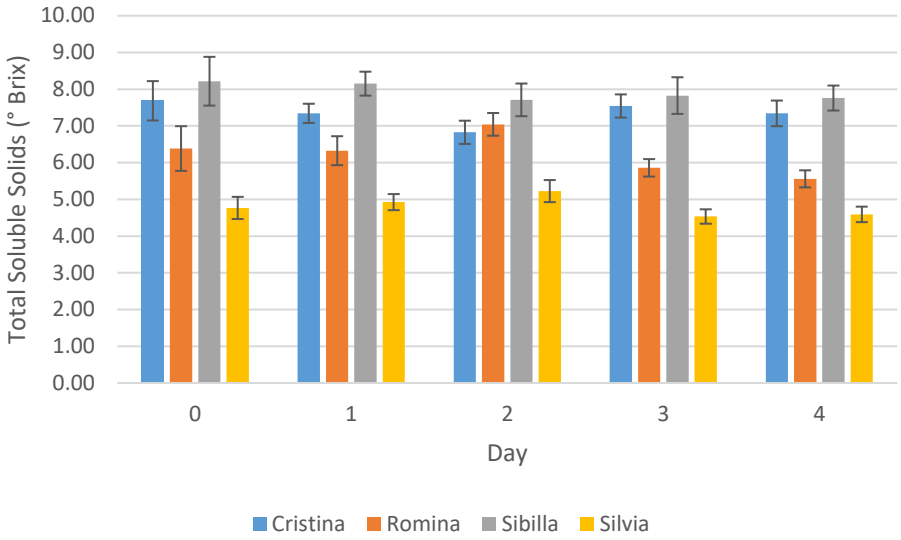

Figure 3. 3. Total Soluble solids content (° Brix) from day 0 to day 4 in control trials of Romina, Cristina, Sibilla and Silvia cultivars.

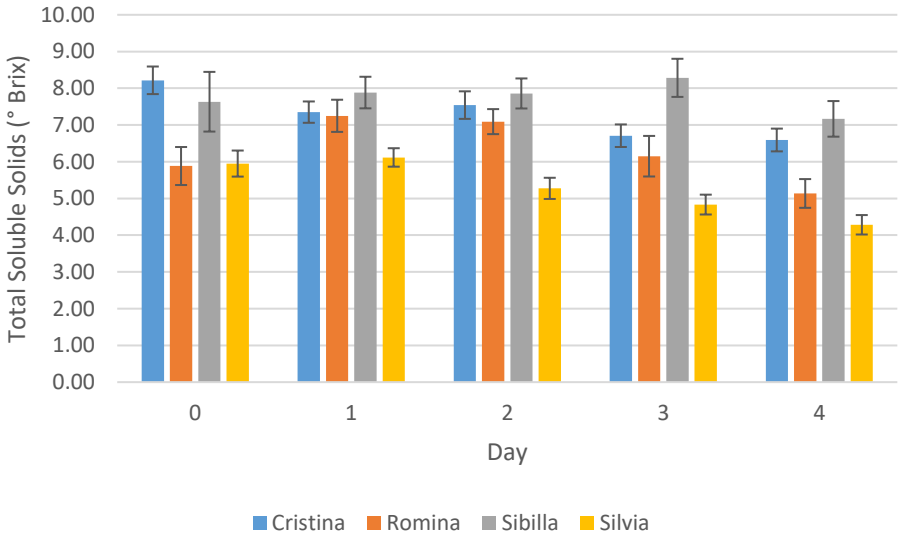

Figure 3. 4. Total Soluble solids content (° Brix) from day 0 to day 4 in treated trials of Romina, Cristina, Sibilla and Silvia cultivars.

3.3.4. Titratable acidity

Values of titratable acidity in control and treated strawberries of Romina, Cristina, Sibilla and Silvia cultivars from day 0 to day 4 are shown in Figure 3, 5 and Figure 3.6, respectively. In control trail, the fruits of the Sibilla cultivar had a higher citric acid content (0.77%), while the Romina fruits are less acid with the average values of 0.49%. The fruits of the Silvia and Cristina cultivars showed values of 0.66% and 0.72% respectively. The figure 3.5 shows how the evolution of the titratable acidity for the control cultivar Cristina had an increasing trend after 4 days of shelf life, while, Romina, Sibilla and Silvia control cultivars showed a significant decrease. The evolution of titratable acidity for the fruit infected with Botrytis cinerea, varied depending to the cultivar (Figure 3.6): 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, while the Silvia cultivar recorded decreased in values.

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. B. cinerea can alter neutral sugar and sugar acid levels in the infected host tissues, mainly by degradation and depolymerisation of cell walls (Zhang and van Kan, 2013)

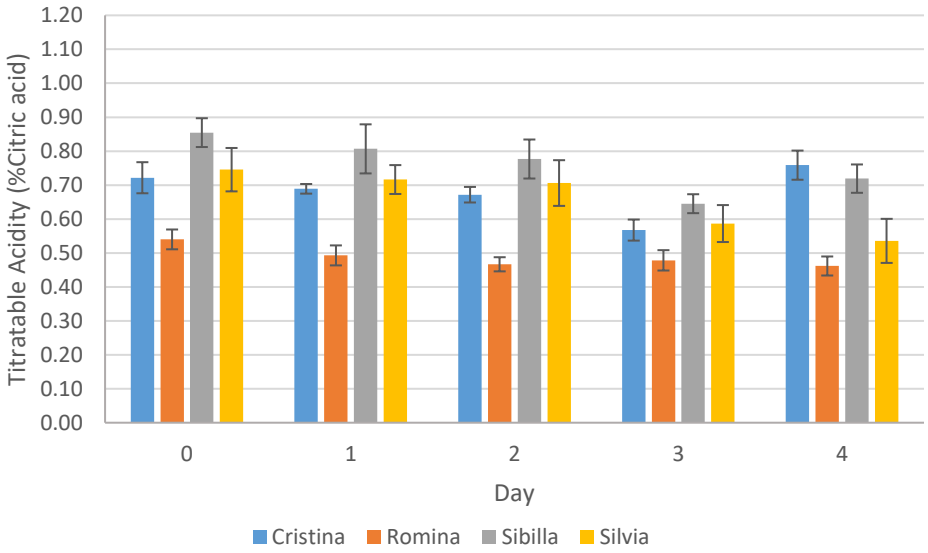

Figure 3. 5. Titratable acidity (% citric acid) of control fruits trail from day 0 to day 4 in Romina, Cristina, Sibilla and Silvia cultivars.

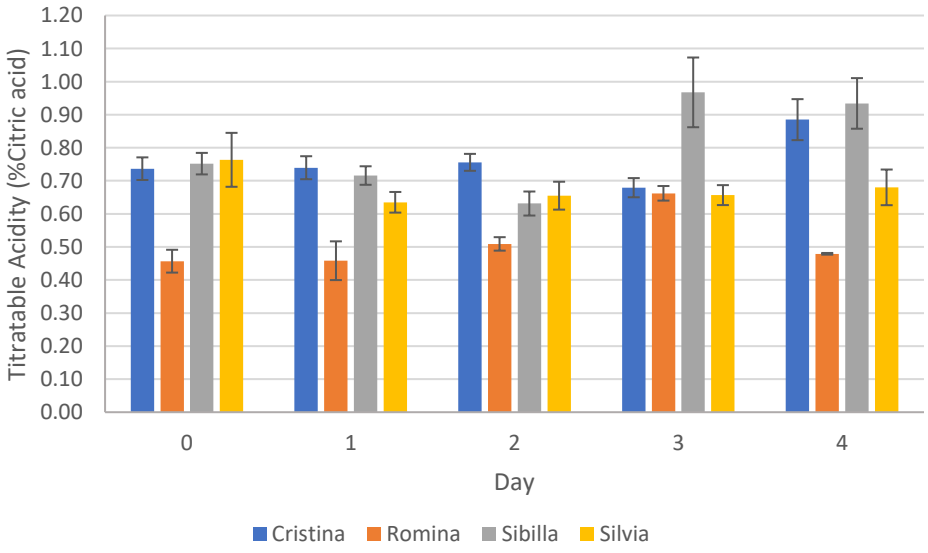

Figure 3. 6. Titratable acidity (% citric acid) of treated fruits trail from day 0 to day 4 in Romina, Cristina, Sibilla and Silvia cultivars.

3.3.5. Phenolic acids

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.

Figure 3.7 and Figure 3.8 represents the phenolic acids content in control and treated strawberries of Cristina, Romina, Sibilla and Silvia cultivars from day 0 to day 4, respectively. In control the highest phenolic acid content was detected in Silvia (25.67mg/100g FW), while the lowest amount was achieved in Cristina with 16.64mg/100g FW. Romina and Sibilla had 17.9 mg/100g FW and 22.75 mg/100g FW at day 0, respectively.

All cultivars in control fruits had increased phenolic acids from day 0 till day4, with the exception of Cristina in day 2 (6.55 mg/100g FW). In details, Cristina fruits had significantly increased phenolic acids from 16.64mg/100g FW to 27.03 mg/100g FW at day 4, with exception decrease at day 2 and in Romina, Sibilla and Silvia phenolic acids progressively increased from 17.9 mg/100g FW, 22.75 mg/100g FW, 25.67mg/100g FW at day 0 to 22.68 mg/100g FW, 34.42 mg/100g FW and 33.80 mg/100g FW at day 3, then decreases to 21.97 mg/100g FW, 29.19 mg/100g FW and 30.37 mg/100g FW, respectively.

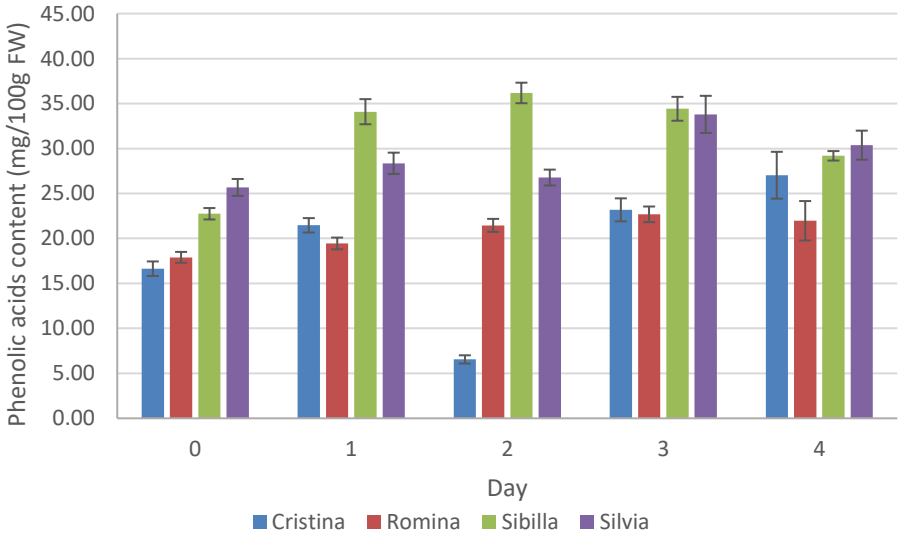

Figure 3. 7. Phenolic acids content of control fruits of Cristina, Romina, Sibilla and Silvia cultivars

Regards on treated fruits the highest phenolic acid content was detected in Silvia (29.99mg/100g FW) and the lowest were Romina (16.31mg/100g FW). and the amount of phenolic acids of Cristina and Sibilla were 18.25 mg/100g FW and 23.58 mg/100g FW, respectively at day 0.

Generally, in all cultivars there was an increased trend by increasing shelf life from day 0 to day 3 and at day 4 phenolic acids were decreased in all cultivars. In Cristina the phenolic acids increased from day 0 to day 4 with exception of day2 that observed lowest content during shelf life (9.91 mg/100g FW), similar to control trail. And in Romina, Sibilla and Silvia, phenolic acids increased from day 0 to day 2 and from day 2 to day 4 had decreased trend.

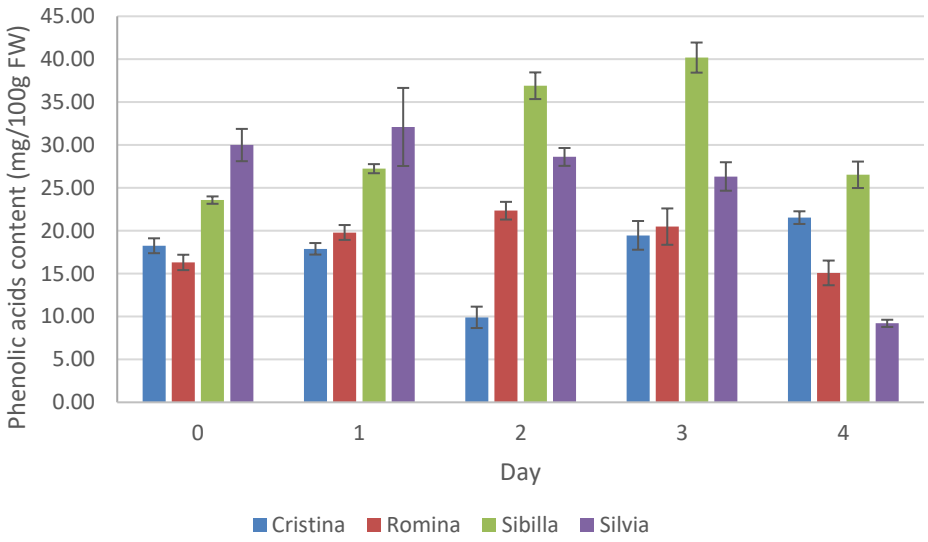

Figure 3. 8. Phenolic acids content of treated fruits of Cristina, Romina, Sibilla and Silvia cultivars

As a comparison, treated fruits had more phenolic acid content compare to control at the middle shelf life period but high phenolic acid content was achieved in control at the end of day4 compare to treated fruits.
Strawberry fruits are a good source of natural antioxidants whose extracts exhibit high enzymatic activity against free radicals and for oxygen detoxification (Wang et al., 2005). Numerous studies have reported that the antioxidant properties of constitutive and induced phenolic compounds provide good defence plant tissue against pathogen intrusions (Nicholson & Hammerschmidt, 1992; Prusky, 1996).

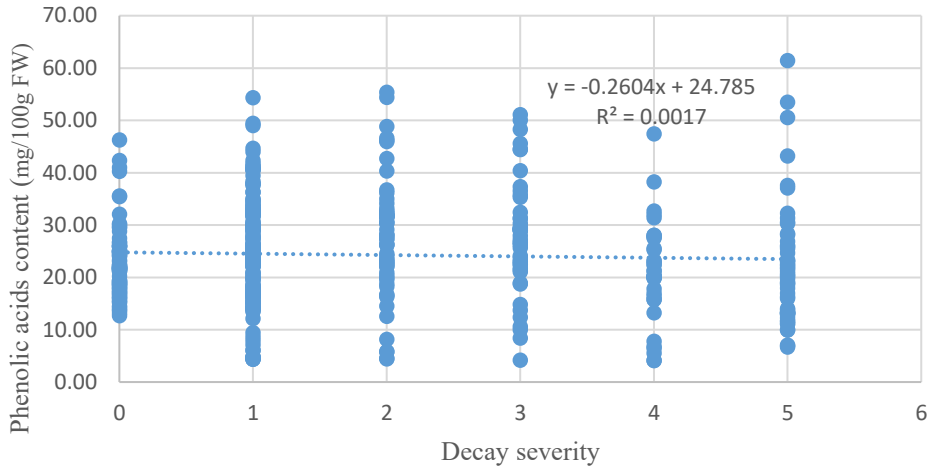

Figure 3. 9. Distribution between fruit phenolic acids content and Decay severity

In strawberry cultivars, the main phenolic components having antioxidant properties are: ellagic and gallic acids, which confers a better resistance to B. cinerea. Phenolic compounds reduce the susceptibility of the fruits to the *botrytis cinerea*. In a study by Hegyi Kalo et al., (2019) observed that the inhibition of *botrytis cinerea* growth was more profound on dark berries than white berries that contain lower concentration of phenolic compounds. In another study, Harshman et al., (2014) reported that total phenolics has the greatest positive correlation with resistance to decay, as phenolics increase, resistance to decay also increase.
In summary, the phenolic acids of strawberry may be due mainly to its genetic but also to their morphology and anatomy, which could also be in relation to its susceptibility to *Botrytis cinerea*. Further investigation is then required for a better understanding.

3.3.6. Anthocyanins

Figure 3.9 and Figure 3.10 represents the anthocyanin content in control and treated strawberries of Cristina, Romina, Sibilla and Silvia cultivars from day 0 to day 4, respectively.
In control fruits, Silvia had the highest anthocyanin content (45.73 mg/100g FW), while Cristina had the lowest amount of anthocyanin (27.56 mg/100g FW), and in Sibilla and Romina the amount of anthocyanin content was detected 34.45 mg/100g FW and 40.6 mg/100g FW, respectively at day 0. In Cristina, Sibilla and Silvia cultivars there was an increased trend from day 1 to day 3, and decreased in day 4, and Romina had a strong increased trend from day 1 to day4. Romina reached the highest amount of anthocyanin with 153.68 mg/100g FW at day4.

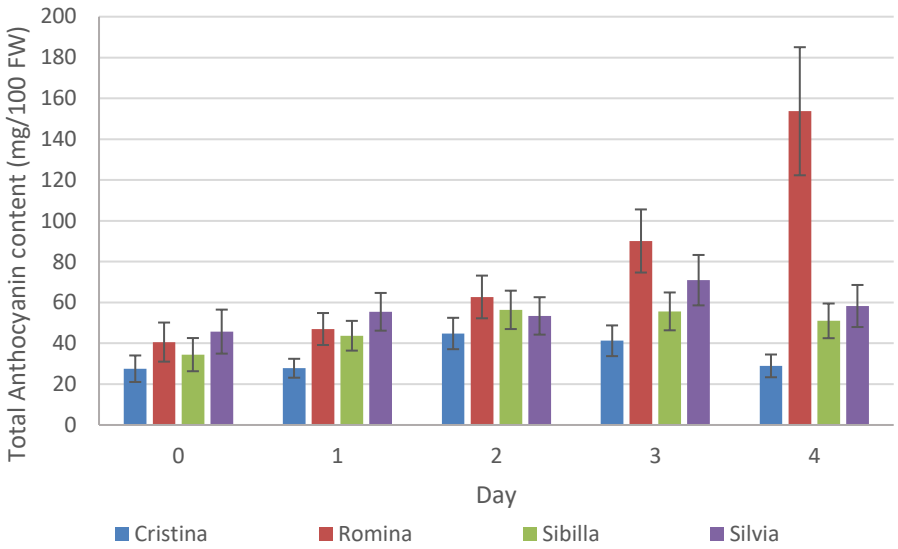

Figure 3. 10. Total anthocyanin content of control fruits trails of Cristina, Romina, Sibilla and Silvia cultivars

Regards on treated fruits, Silvia had the highest amount of anthocyanin content with 51.01 mg/100g FW and Cristina had 29.11 mg/100g FW at day 0 while in Romina and Sibilla observed 45.81 mg/100g FW and 49.17 mg/100g FW, respectively.
In treated fruits in all cultivars there is no clear anthocyanin content trend of increase or decrease, during the 4 days of shelf life. Each cultivar seems to respond differently. In details: Cristina had slightly increased from day 0 to day 2 and then decreased till day 4 with the amount of 17.37 mg/100g FW. Romina had a trend of strong increased at day 1 (113.64 mg/100g FW) to strong decreased to day4 (3.4 mg/100g FW). In Sibilla fruit, the anthocyanin content reaches a maximum peak on day 0 of 49.17 mg/100g FW, then from day1 till day 4 had decreased (11 mg/100g FW), and in Silvia fruit, anthocyanin content increased at day 1 (72.54 mg/100g FW) and decreased from day 2 till day4.
As a comparison to control trail, treated fruits in all cultivar had much less amount of anthocyanin content compare to control. In control fruits anthocyanin content was increased because of increasing the ripening while in treated fruit decreased due to enzyme activity. High amount of anthocyanin content reduce the susceptibility to botrytis due to antioxidant activity (Zhang et al., 2013). Our results are agreeing with this finding, as strawberry cultivar displaying lower spoilage rates had more anthocyanins. In addition, the distribution between anthocyanin and Botrytis shows that, there is positive correlation: as anthocyanins increase, resistance to decay also increases (Figure 12).

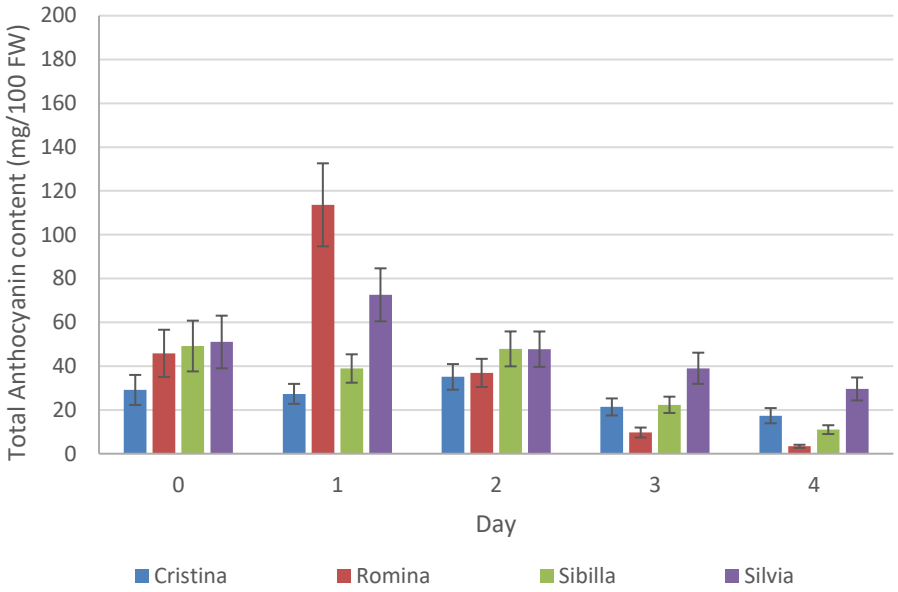

Figure 3. 11. Total anthocyanin content of treated fruits trails of Cristina, Romina, Sibilla and Silvia cultivars

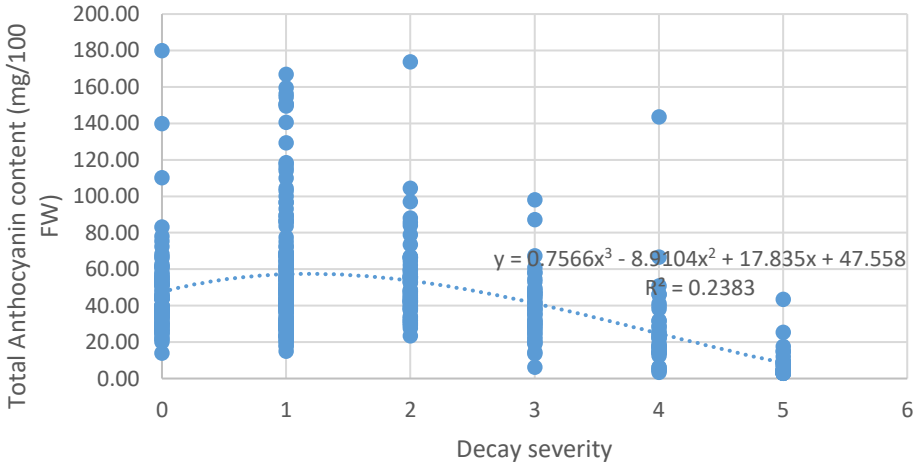

Figure 3. 12. Distribution between total anthocyanin content and Decay severity

3.3.7. Vitamin C contents

Figure 3.11 and Figure 3.12 shows the vitamin C content in control and treated strawberries of Cristina, Romina, Sibilla and Silvia cultivars from day 0 to day 4, respectively.

In control fruits, the highest content of vitamin C was detected in Sibilla (36.29 mg/100g FW) and the lowest amount was achieved in Cristina (13.41 mg/100g FW), and Romina and Silvia had 28.44 mg/100g FW and 30.53 mg/100g FW, at day 0, respectively.

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 FW 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 FW, 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 FW, then from day1 till day 4 had almost constant value.

In Silvia fruit, the vitamin C content shows an oscillating trend, consisting of decreases and increases that follow one another from day 0 to day 4, recording a maximum peak on day 0 of 30.53 mg/100g FW.

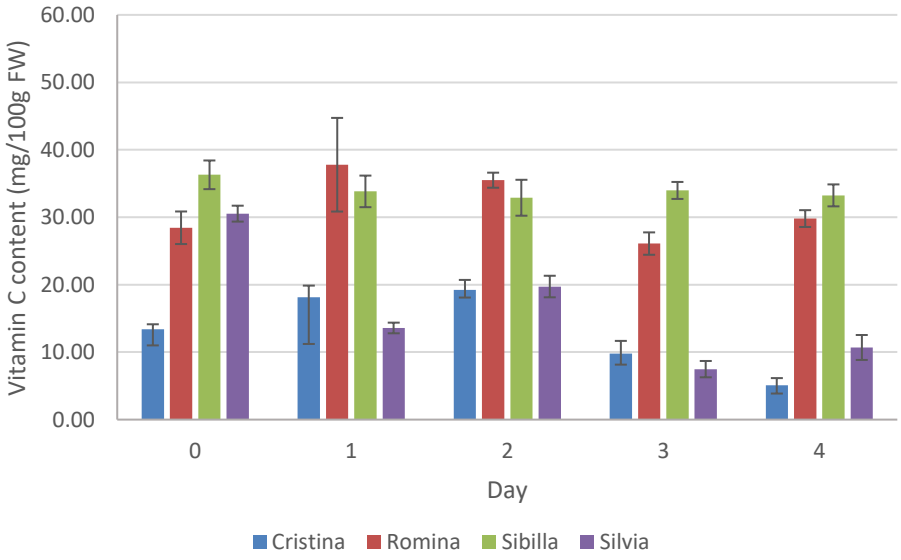

Figure 3. 13. Vitamin C content of control fruits trails of Cristina, Romina, Sibilla and Silvia cultivars.

In treated fruit, the highest vitamin C content was detected in Sibilla fruit, with an average of 36.2 mg/100g FW, the lowest in the fruits of the Silvia cultivar with an average of 12.18 mg/100g FW. Romina and Cristina reach an average of 27.7 and 17.57mg/100g FW, respectively at day 0. From the Figure 3.12, it is possible to deduce how the Cristina, Sibilla and Silvia cultivars show a marked decreasing trend after 4 days of shelf life, and Romina had increased at day 1, then progressively decreased till day 4. The treatment with *Botrytis cinerea* significantly affects the vitamin C content, that at the end of day 4 all cultivars had decreased almost all vitamin C content. As a comparison control fruits had much vitamin C content compare to treated fruits.

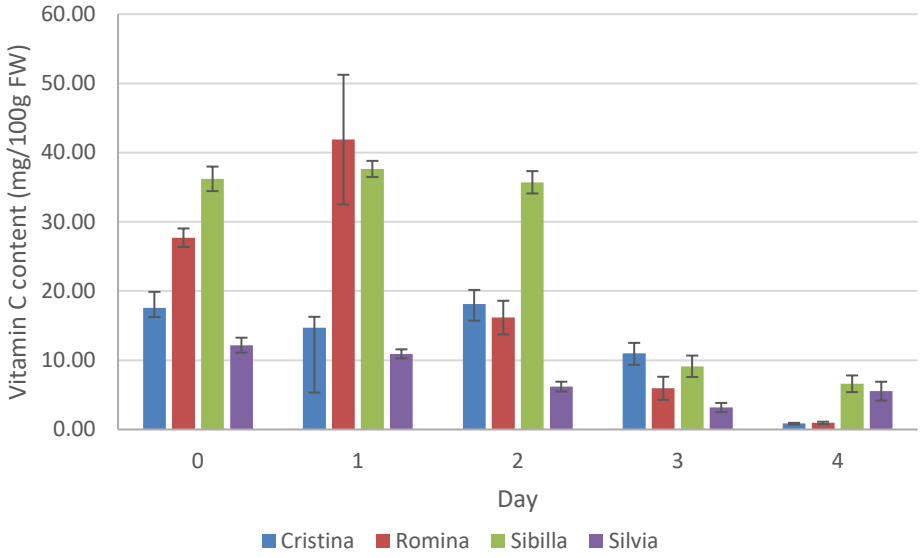

Figure 3. 14. Vitamin C content of treated fruits trails of Cristina, Romina, Sibilla and Silvia cultivars

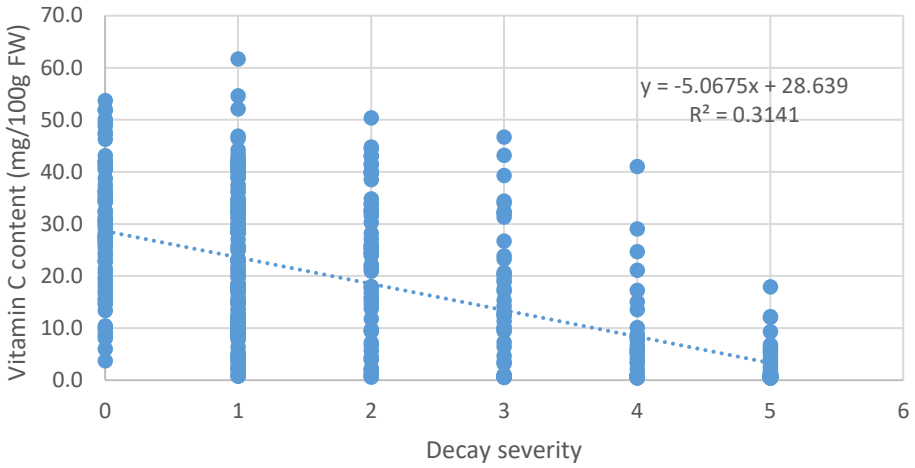

Figure 3. 15. Distribution between fruit Vitamin C content and Decay severity

Bui et al. (2019) observed an increased enzymatic activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX), accompanied by a decrease in the ascorbic acid level in fruit during the infection by B. cinerea. They proposed that the increase in SOD activity could be originated from fruit or *Botrytis cinerea* in response to the oxidative stress as SOD is important for detoxification of reactive oxygen species either fruit or fungus-produced (López-Cruz et al., 2017). Ascorbic acid is no longer recycled but consumed by APX in fruits with increasing severity of *Botrytis cinerea* infection, might be due to disruption in redox equilibrium.

3.4. Conclusions

For the present study, four well-defined strawberry cultivars (Romina, Cristina, Silvia and Sibilla) were tested under treatment with *Botrytis cinerea*, the fungus that causes gray mold; to evaluate the response and susceptibility of each cultivar, in response to the disease caused by the fungal pathogen and determine the nutritional and qualitative changes, evaluating the content of soluble solids, titratable acidity, vitamin C, phenolic acids and anthocyanin content to check the relation *Botrytis cinerea* with fruit quality. The analyses carried out 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 4 days of shelf life. The McKinney's index of strawberry fruits was 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 and

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 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.
- The contamination with *Botrytis cinerea* has been shown to be effective also in increasing the content of free acids of strawberry cultivars after 4 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.
- The results of the study also highlighted important nutritional changes in the four strawberry cultivars contaminated with Botrytis cinerea.
- Generally, in all cultivars phenolic acids and vitamin C decreased in both control and treated but there was strong decreased in treated fruit compare to control.
- Anthocyanin content increased in control fruits but strong decrease in treated.
- Strong positive correlation was obtained from nutritional quality with resistance to decay, as high amount of nutritional content more tolerance to *Botrytis cinerea*.

Chapter 4.

Stability of Strawberry Fruit (*Fragaria x ananassa Duch.)* Nutritional Quality at Different Storage Conditions

Abstract

Strawberry fruit is a very rich source of vitamins and phenolic compounds, which determine its nutritional properties. Strawberries are a highly perishable non-climacteric fruit, and their perishable nature can lead to physical and chemical damage during storage. Therefore, the large market of fresh fruit relies on the capacity of fast distribution and marketing under a continuous cold-storage chain. In this study, we applied different cold-storage temperatures (domestic −20 °C and industrial −80 °C) on different treatments (whole fruits and dried fruits) of three strawberry cultivars (Arianna, Francesca, and Silvia), for up to seven months, and evaluated the influence of different storage conditions and lengths on the stability of the fruits' nutritional compounds (vitamin C, phenolic acids, anthocyanins, and folate). The results show that the nutritional quality of the fruits was significantly affected by storage temperature (with −80 °C storage preserving more nutritional compounds), while storage time did not greatly affect the composition of the nutritional compounds in the whole or dried fruits. Oven drying the fruits dramatically affected their vitamin C content, almost completely degrading this compound (from 731.8 to 23.2 mg/kg at time 0 for fresh Arianna fruit, the cultivar with the highest amount). The amount of folate was increased during storage (from 126.17 at time 0 to 190.61 µg/kg at time 7 for fresh whole Arianna fruit). The interesting results obtained in this study are worth considering in future studies, to better plan fruit-storage conditions and time, for maintaining better fruit nutritional quality.

4.1. Introduction

Strawberries are the most cultivated berry fruit worldwide, with an annual production of 13.3 million tons, on a surface area of 522,527 ha (Fao, 2019). 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 (Giampieri et al., 2019; Gasparrini et al., 2021). Berries, including strawberries, have an important role among fruits because of their high phytochemical content (Giampieri et al., 2012; Sabbadini et al., 2021. 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 (Capocasa et., 2016).

Strawberries are a highly perishable non-climacteric fruit, which can lead to physical damage during storage and transportation. Therefore, the large market of fresh fruit relies on the capacity of fast distribution and marketing under a continuous cold-storage chain. Fruit overproduction, especially during off-season periods, is addressed mostly toward the processing industry to produce bakery products such as jam, jellies, juice, puree, flavor additives, etc. (Giampieri et al., 2012; Bhat and stamminger, 2015), with a consequence being a price reduction for the product. Strawberries' post-harvest decay can be due to physical, physiological, or pathological factors that may happen in the pre-harvest period (and then take place during storage) or directly in the post-harvest period, and their shelf life is diminished by firmness loss, fruit desiccation, and the growth of spoilage microorganisms (Bhat and Stamminger, 2015).

Freezing is a simple but common and effective method for long-term preservation and storage of fruits and vegetables, while maintaining many of their fresh-like qualities (Prochaska et al., 2000; Kamiloglu, 2019). In addition, freezing is less destructive than other available preserving methods (Chaves and Zaritzky, 2018). Given the nutritional value of fruits and vegetables, it is essential that bioactive compounds and nutrients are considered healthy when frozen and stored. These methods support the food supply chain and producer income when fresh food is not available. Long-term storage is becoming a more common in-home consumer behaviour, and labs freeze food prior to analysis, both for convenience. Analytical labs and home kitchens typically have −20 °C freezers. Although −80 °C can be used in a laboratory, this equipment is expensive and has high operating costs, mainly due to energy consumption (Dias et al., 2014). The freezing rate is the most important factor in the freezing process to prevent fruit-tissue damage, loss of bioactive compounds, and drip loss in thawing. Faster freezing results in small ice crystals and better frozen-fruit quality (Alexandre et al., 2013). The effect of freezing fruits on microbial and enzymatic activity can influence the chemical composition, but storage temperature and storage period could also have an effect on the chemical composition of the strawberry (Sahari et al., 2004).

Dehydration is another method for long-term preserving and increases the shelf life of delicate fruits. Oven drying fruits has been the most common preserving method used for many years (Erbersdobler, 1986), and, in the past few decades, considerable efforts have been made to understand the chemical and biochemical changes that occur during dehydration and to develop methods for preventing undesirable quality losses. Drying reduces water activity, and avoids microbial growth and deteriorative chemical reactions. The effects of heat on microorganisms and on the activity of enzymes are also important when drying fruits (Rahman, 2007).

In this study, we evaluate the effect of fruit treatment on whole fruits and dried fruits (WF and DF) at different storage times (from 0 to 7 months) and temperatures (−20 and −80 °C) on the nutritional quality (vitamin C, anthocyanins, phenolic acids, and folate) of three strawberry cultivars.

4.2. Materials and Methods

4.2.1. Plant Material

Three commercial cultivars (Arianna, Francesca, and Silvia) were planted in July 2020 in non-fumigated soil in "P. Rosati" experimental farm of Università Politecnica delle Marche, sited in Agugliano (Ancona, Italy), with the following main characteristics: pH 7.9, active calcium 9%, and texture composed of 40% clay, 25% sand, and 35% silt, following the procedure described in Mezzetti et al. (2021). Plants were grown in open field conditions according to the plastic hill culture production system. Fruit samples were harvested at fully red stage, at the second, third, and fourth main seasonal pickings, and immediately treated according to the experimental storage design. The environmental data of the picking season (April–June 2021) were registered and are reported in Table 4.1.

Table4. 1. Monthly rainfall (sum) and average daily maximum, average, and minimum temperatures registered in April, May, and June 2021

Month	Rainfall (mm)	Maximum Temperature (°C)	Average Temperature (°C)	Minimum Temperature (°C)
April	33.0	17.9	12.2	7.1
May	23.8	24.1	18.2	12.7
June	7.8	30.8	25.0	19.1

4.2. 2. Experimental Storage Design

For all cultivars analysed, two fruit treatments were taken into consideration: whole fruits (WF) and oven-dried fruits (DF). Regarding the fruit treatments, for the WF, we collected fresh ripe fruit and directly put them into a normal −20 °C domestic refrigerator (Zoppas, Vittorio Veneto, Italy) and a laboratory −80 °C refrigerator (HFU B series, Thermo Fisher Scientific, Milan, Italy). For the DF, after the collection of fruit from the field, we put them in a laboratory oven (Pbi international, Milan, Italy) at 65 °C for 1 week. The loss of water and the complete dryness of fruits was monitored by checking the fruit weight at intervals of 24 h, until no further weight reductions were detected. Then, dried fruits were placed at −20 °C and −80 °C, as indicated before for WF.

Fruits were stored for five different storage times: 0, 1, 3, 5, and 7 months after the harvest day. During each storage time (except at 0, when the fruits were immediately

analyzed), fruits of three cultivars were stored at −20 °C and −80 °C temperature conditions. For each combination of cultivar/storage time/treatment/storage temperature, three repetitions of 5 fruits were made. A summary of the experimental storage design is described in Table 2. For the analyses of fruit nutritional quality, the WF and DF were extracted and analyzed after 1, 3, 5, and 7 months of refrigerated storage; for the "time 0", WF were immediately extracted and analyzed after collection, while DF were immediately extracted and analyzed after oven-drying. The analyzed nutritional parameters were anthocyanins, phenolic acids, vitamin C, and folate. All the parameters were analyzed by HPLC-UV-FD.

Table4. 2. Storage experimental design applied to each of the three studied cultivars. WF: whole fruit; DF: dried fruit.

Storage Time	Fruit Treatment	Storage Temperature	Number of Fruits
0 M	WF	-	5
	DF		5
1 M	WF	−20	5
		−80	5
	DF	−20	5
		−80	5
3 M	WF	−20	5
		−80	5
	DF	−20	5
		−80	5
5 M	WF	−20	5
		−80	5
	DF	−20	5
		−80	5
7 M	WF	−20	5
		−80	5
	DF	−20	5
		−80	5

4.2.3. Methanolic Extraction

Fruit extracts were prepared as described in chapter 2.2.4.1.

4.2.4. Vitamin C Extraction

Vitamin C of fruits was extracted as described in chapter 2.2.4.2.

4.2.5. Extraction of Vitamin B9 (Folate)

Following the method described in chapter 2.2.4.3

4.2.6. HPLC Determination of Vitamin C Content

Vitamin C content was measured as described in chapter 2.2.4.7, and the results were expressed as mg vit-C per 1 kg fresh weight of strawberries (mg/kg FW).

4.2.7. HPLC Determination of Phenolic Acid Content

Phenolic acids were analyzed as previously described in chapter 2.2.4.7. Values were expressed as mg corresponding to phenolic acid per kilogram of fresh weight of strawberries (mg/kg FW).

4.2.8. HPLC Determination of Anthocyanin Content

Anthocyanin content was analyzed following the method as described in chapter2.2.4.7, and were calculated as mg per 1 kg of fresh weight of strawberries (mg/kg FW).

4.2.9. Quantification of Folate Content

Folate was quantified using the HPLC as described in chapter 2.2.4.8. Results are expressed as µg 5-CH3-H4 folate per 1 kg of fresh weight of strawberries (µg 5-CH3-H4folate/kg FW).

4.2.10. Data Analyses

The results are presented as the values ± standard error and were subjected to one-way analysis of variance (ANOVA), at a confidence level of 95%. Significant differences were calculated according to Tukey's tests, and differences at $p < 0.05$ were considered to be significant. A correlation matrix has also been developed among the nutritional parameters to check their inter-relationship, with $p < 0.05$. Statistical analyses were performed by using Statistica 7 software (StatSoft, TIBCO Software, Palo Alto, CA, USA).

4.3. Results and Discussion

4.3.1. Vitamin C

The vitamin C amounts of the Arianna, Francesca, and Silvia fruits at different storage times with different treatments are shown in Table 4.3. The highest vitamin C amount was detected in Arianna WF (731.8 mg/1 kg FW) at time 0, and the oven-drying process caused a high loss of total vitamin C (almost 97% at time 0 for DF). The vitamin C amount in the Arianna and Silvia WF during storage at −20 °C decreased from 1 M until the end of storage, with the lowest amount detected at 5 M (436.1 mg/kg FW and 388.1 mg/kg FW, respectively). However, in Francesca WF, vitamin C slightly increased after 1 M (599.8 mg/kg FW) and was maintained until the end of storage. Regarding the DF in −20 °C storage, there was a slight increasing trend in the vitamin C amount for all

cultivars until the end of storage, but the values remained dramatically lower than those of the WF.

At −80 °C, similar to −20 °C, the Arianna and Silvia WF had a decreasing trend for vitamin C during storage, but presented a higher value than at −20 °C; the Francesca WF showed an increasing trend for vitamin C until the end of storage, except at 5 M. The DF also presented higher vitamin C values (with some exceptions) at −80 °C until the end of storage for all cultivars compared to −20 °C, but with dramatically lower values than the WF. There was a significant difference in fruit vitamin C content between fruits stored at −20 °C and −80 °C during storage and between treatments.

Ancos et al., (2000), reported that vitamin C was highly preserved after freezing fruits, but the degradation increased during storage, confirming some of our results. The chemical changes that may occur after the freezing of berries, as a result of oxidation and enzymatic activity, might influence the degradation and loss of vitamin C content (Zhao, 2007). The concentration of reagents in the non-frozen phase of frozen fruits and crystallization can favor chemical and enzymatic oxidation reactions (Stevanovic et al., 2021). Regarding the drying process, vitamin C is highly sensitive to and unstable in heat, so it was degraded during the sample preparation and oven-drying process. Vitamin C is also hydrosoluble and, as 90% of strawberries are water, the loss of vitamin C occurs during the drying process (Phan et al., 2021).

Table4. 3. Average of vitamin C content (mg/kg FW) in fruits of Arianna, Francesca, and Silvia cultivars. The data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each cultivar during storage. Different uppercase letters indicate significant differences for $p \leq 0.05$ for all cultivars for a specific storage time. WF: whole fruit, DF: dried fruit. 0 M: 0 months of storage; 1 M: 1 month of storage; 3 M: 3 months of storage; 5 M: 5 months of storage; 7 M: 7 months of storage.

Storage Time	Storage Temperature	Treatment	Arianna	Francesca	Silvia
0 M		WF	731.8 ± 6.5$_{a}$A	580.4 ± 3.6$_{f}$B	519.3 ± 0.3$_{c}$C
		DF	23.2 ± 0.3$_{j}$D	18.8 ± 0.3$_{m}$D	16.5 ± 0.3$_{l}$D
1 M	−20	WF	650.6 ± 3.9$_{b}$B	599.8 ± 2.8$_{d}$C	402.2 ± 1.8$_{g}$E
	−80	WF	737.4 ± 4aA	736 ± 2$_{a}$A	592.1 ± 0.9$_{a}$D
	−20	DF	26.8 ± 0.7$_{j}$F	22.8 ± 0.4l$_{m}$F	16.4 ± 0.3$_{l}$G
	−80	DF	27.9 ± 0.1$_{j}$F	25.1 ± 0.3$_{ijkl}$F	14.6 ± 0.1$_{l}$G
3 M	−20	WF	554.4 ± 0.1$_{d}$D	594.3 ± 3.9$_{e}$B	423.4 ± 0.2$_{f}$F
	−80	WF	571.7 ± 13.4$_{c}$C	609.5 ± 1.6$_{c}$A	523.7 ± 2.3$_{c}$E
	−20	DF	24.2 ± 0.3$_{j}$HI	28 ± 0.1$_{ijk}$H	19.4 ± 0.2$_{l}$HI
	−80	DF	93.5 ± 5.3$_{h}$G	24.3 ± 0.3j$_{kl}$HI	14.1 ± 0.1$_{l}$I
5 M	−20	WF	436.1 ± 0.8$_{g}$D	517.2 ± 0.2$_{g}$A	388.1 ± 0.3$_{h}$F
	−80	WF	507.1 ± 0.5$_{e}$B	500.3 ± 0$_{h}$C	430.1 ± 0.1$_{e}$E
	−20	DF	25.4 ± 0.2$_{j}$I	23.1 ± 0.2$_{klm}$I	39.3 ± 0.8$_{j}$G

	−80	DF	29.2 ± 0.7$_{j}$H	30 ± 0.7$_{i}$H	31.3 ± 2.3$_{k}$H
7 M	−20	WF	544.6 ± 1.2$_{d}$C	576.8 ± 1$_{f}$B	509.4 ± 0.3$_{d}$D
	−80	WF	473 ± 0.5$_{f}$E	664.1 ± 1.5$_{b}$A	537.5 ± 8.2$_{b}$C
	−20	DF	46.3 ± 7.6$_{i}$F	29.3 ± 0.5$_{ij}$G	26.7 ± 0.1$_{k}$G
	−80	DF	27.3 ± 1$_{j}$G	26.1 ± 2i$_{jkl}$G	49.6 ± 0.7$_{i}$F

4.3.2. Phenolic Acids

Phenolic acids are a part of the large group of phenolic compounds, widely distributed in strawberry fruits. They are considered important ingredient of strawberries, contributing to taste, color, and nutritional properties (Tomás-Barberán and Espín, 2001).

The phenolic acids' content in the WF of all three cultivars during storage is shown in Table 4.4. The Arianna, Francesca, and Silvia fruits had a content of 396.4 mg/kg FW, 569 mg/kg FW, and 170 mg/kg FW phenolic acids at time 0, respectively. The oven-drying process reduced the amounts of phenolic acids in the fruits at time 0 to 192.6 mg/kg FW, 288.1 mg/kg FW, and 138.2 mg/kg FW, respectively. The phenolic acids' content in Francesca and Silvia WF increased at 1M during storage at −20 °C (643 mg/kg FW and 206.9 mg/kg FW, respectively) and had a decreasing trend until the end of storage. Differently, in Arianna, the highest amount was detected after 3 M of storage at −20 °C, with the value of 427.2 mg/kg FW. Regarding the DF, there was a decreasing trend until the end of storage, with a peak of phenolic acid content in fruits after 3 M of storage in all the cultivars.

At −80 °C storage temperature, the WF showed a similar reaction to −20 °C, with increased phenolic acids' content at the early stage of storage, then a decreasing trend until the end of storage. The highest amounts of phenolic acids were detected in Francesca fruit at 3M of storage, with the value of 646 mg/kg FW. Regarding the DF, the phenolic content of Francesca and Silvia fruits increased after 1M of storage, decreasing until the end of storage; Arianna showed a slightly different behavior, with increased phenolic acids' content from 1 M to 3 M, then decreasing until the end of storage. There was not a significant difference between the different temperatures during storage, but there was a significant difference between the treatments in all the cultivars, with the WF phenolic acids' content always being higher than those of the DF. The same effect was observed in a storage study of frozen strawberries by Oszmainski et al., (2009) They reported that the degradation of phenolic acids may be due to enzymatic oxidation. The degradation of phenolic acids during the oven-drying process were also confirmed in studies by Coklar et al., (2018), and Semenov et al., (2015). The oxidative and thermal degradation of phenolic compounds due to the increasing heat treatment led to decreases in the phenolic compounds in oven-dried fruits (Wojdylo et al., 2009).

Table4. 4. Average of phenolic acids' content (mg/kg FW) in cultivars of Arianna, Francesca, and Silvia fruits. The data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each cultivar during storage. Different uppercase letters indicate significant differences for $p \leq 0.05$ for all cultivars for a specific storage time. WF: whole fruit, DF: dried fruit. 0 M: 0

months of storage; 1 M: 1 month of storage; 3 M: 3 months of storage; 5 M: 5 months of storage; 7 M: 7 months of storage.

Storage Time	Storage Temperature	Treatment	Arianna	Francesca	Silvia
0 M		WF	396.4 ± 15.3$_{ab}$B	569 ± 1.9$_{c}$A	170.7 ± 9.7$_{d}$E
		DF	192.6 ± 1$_{ef}$D	288.1 ± 4.3$_{g}$C	138.2 ± 1.3$_{fgh}$F
1 M	−20	WF	305.2 ± 7.5$_{c}$B	643.2 ± 14.6$_{a}$A	206.9 ± 3.3$_{b}$EF
	−80	WF	273.4 ± 5.4cC	631.7 ± 11.5$_{a}$A	222.5 ± 1.9$_{a}$DE
	−20	DF	188.2 ± 2.2$_{ef}$F	313.2 ± 9.5$_{efg}$B	129.2 ± 0.1$_{ghijk}$G
	−80	DF	201.2 ± 2.3$_{de}$EF	237 ± 9.5$_{h}$D	131.2 ± 6.3$_{ghij}$G
3 M	−20	WF	427.2 ± 6.6$_{a}$C	601.2 ± 25.6bB	191.5 ± 2.5$_{c}$EF
	−80	WF	400.2 ± 6.6$_{ab}$C	646.8 ± 14$_{a}$A	191.6 ± 5.5$_{c}$EF
	−20	DF	230.6 ± 23.7$_{d}$E	386.2 ± 5.3$_{d}$C	164 ± 12.8d$_{e}$FG
	−80	DF	387 ± 11.7$_{b}$C	292.2 ± 8.9$_{fg}$D	140.9 ± 2.2$_{fg}$G
5 M	−20	WF	162 ± 3f$_{g}$E	321.4 ± 4.8$_{ef}$A	149.8 ± 3.3$_{ef}$E
	−80	WF	191.5 ± 4.4$_{ef}$C	325.5 ± 2.8$_{e}$A	129.7 ± 2.8$_{ghijk}$F
	−20	DF	91.4 ± 0$_{h}$G	177.1 ± 2.9$_{j}$D	123.1 ± 4.7$_{hijk}$F
	−80	DF	151.8 ± 10.4$_{g}$E	226.5 ± 2$_{hi}$B	117.5 ± 2.3$_{jk}$F
7 M	−20	WF	190.9 ± 6.1$_{ef}$B	294.3 ± 5$_{fg}$A	134.5 ± 2.4$_{ghi}$CDE
	−80	WF	186.8 ± 5.7$_{ef}$B	291.6 ± 7$_{g}$A	126.1 ± 1.6$_{ghijk}$DEF
	−20	DF	138.9 ± 4$_{g}$CD	198.7 ± 2.4$_{ij}$B	122.3 ± 4.9$_{ijk}$EF
	−80	DF	144 ± 3.3$_{g}$C	191.6 ± 5.2$_{j}$B	114.6 ± 2.3$_{k}$F

4.3.3. Anthocyanins

Anthocyanins are the most important phenolic compounds of the strawberry (Lopes-da- Silvia et al., 2002), and their concentration increases during the ripening progress (Tosun et al., 2008). The Arianna, Francesca, and Silvia WF had a content of 202.2 mg/kg FW, 108.8 mg/kg FW, and 300.7 mg/kg FW of total anthocyanins at time 0, respectively Table 4.5. The oven-drying process greatly reduced these amounts to 98 mg/kg FW, 77.4 mg/kg FW, and 108.6 mg/kg FW, respectively. Storage duration and temperature do not significantly affect the anthocyanins amount in the WF. There was a slight increase in the amounts of the anthocyanins in the Arianna and Francesca WF from 1 month to 7 months, while in Silvia fruit the anthocyanins amount was maintained during storage. Regarding the oven-dried fruits, the anthocyanin content of Arianna and Silvia fruits dramatically decreased after 1 month, and the values remained very low until 7 months of storage; however, in Silvia fruit, the anthocyanin content increased after the first month of storage, and, by increasing storage time, the anthocyanin content decreased in parallel. The lowest amount of anthocyanin was found in the Arianna DF after 5 months of storage at −20 °C. There was also no

significant effect of storage temperature on the anthocyanins' content of the dried fruit of all cultivars.

Freezing is commonly regarded as a technique that has a less deleterious effect on the anthocyanins of strawberries for long-term storage (Celli et al., 2015). Kamiloglu, (2019). studied the effect of different freezing methods on the bioaccessibility of strawberry polyphenols and concluded that freezing retains the bioactive compounds of strawberries and might enhance the total amounts of bioaccessible anthocyanins. Freezing rate and storage time are the most important parameters in the losses of anthocyanin in strawberries during the freezing process. It has been determined that a higher freezing rate is essential for the better preservation of the bioactive compounds in strawberries. Freezing should be done at an appropriate freezing rate to preserve the cell structure and the nutritional content of strawberries (Yanat and Baysal, 2018). The effects of heat on microorganisms and the activity of enzymes are important when drying fruits (Rahman, 2007). Some important properties of fruits change during dehydration such as texture, chemical changes affecting flavor and nutrients, and color, as the evolution of the color is strongly correlated with the anthocyanin concentration across various fruits and vegetables (Goncalves et al., 2007; Borochov-Neori et al., 2011; Shete et al., 2018. A high temperature during the drying process is an important factor for the loss of quality. Lowering the process temperature has great potential to improve the quality of dried products (Kumar and Sagar, 2009).

Table4. 5. Average of total anthocyanins' content (mg/kg FW) in cultivars of Arianna, Francesca, and Silvia fruits. The data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each cultivar during storage. Different uppercase letters indicate significant differences for $p \leq 0.05$ for all cultivars for a specific storage time. WF: whole fruits, DF: dried fruits. 0 M: 0 months of storage; 1 M: 1 month of storage; 3 M: 3 months of storage; 5 M: 5 months of storage; 7 M: 7 months of storage.

Storage Time	Storage Temperature	Treatment	Arianna	Francesca	Silvia
0 M		WF	$202.4 \pm 2.7_{b}E$	$108.8 \pm 5.5_{c}C$	$300.7 \pm 3.3_{a}B$
		DF	$98 \pm 1.8_{d}F$	$77.4 \pm 1.2_{e}E$	$108.6 \pm 0.5_{c}H$
1 M	−20	WF	$236.9 \pm 1.1ab_{c}C$	$127.3 \pm 1.8_{ab}D$	$266.6 \pm 9.1_{b}C$
	−80	WF	$224.7 \pm 14.4_{c}D$	$123.3 \pm 1.1_{de}B$	$300.7 \pm 7.5_{a}B$
	−20	DF	$61.1 \pm 0.8_{fg}G$	$52.9 \pm 1.5_{g}J$	$122.1 \pm 0d_{e}G$
	−80	DF	$50.8 \pm 0.7_{gh}G$	$74.9 \pm 1.5_{ef}F$	$109.4 \pm 3.8_{e}H$
3 M	−20	WF	$249 \pm 0.1_{a}A$	$101.4 \pm 0.6_{f}C$	$187.6 \pm 0.4_{d}F$
	−80	WF	$244 \pm 1.2_{ab}B$	$89.4 \pm 2.6_{g}D$	$207.4 \pm 1_{c}E$
	−20	DF	$45.8 \pm 0.8_{j}H$	$61.2 \pm 0.6_{hi}H$	$111.9 \pm 1.1_{gh}E$
	−80	DF	$56.4 \pm 1.5_{gh}I$	$64.8 \pm 0.4_{gh}H$	$92.1 \pm 1.9_{g}I$
5 M	−20	WF	$203.6 \pm 2.9_{c}E$	$132.7 \pm 0.3_{d}A$	$259.4 \pm 4.8_{b}C$
	−80	WF	$200.1 \pm 5.7_{c}E$	$108.9 \pm 6.3_{e}C$	$335 \pm 2.6_{a}A$

	−20	DF	$19.5 \pm 0_{g}I$	$69.2 \pm 1.4_{fg}I$	$72.1 \pm 0.8_{kl}G$
	−80	DF	$56.1 \pm 1.2_{gh}H$	$57.1 \pm 0.5_{ij}H$	$86.6 \pm 1_{ij}F$
7 M	−20	WF	$232.1 \pm 2b_{cd}B$	$124 \pm 1.5_{d}B$	$298.7 \pm 2.6_{c}B$
	−80	WF	$224.9 \pm 3_{cd}C$	$126.3 \pm 4.1_{ab}D$	$220.2 \pm 1.4_{c}D$
	−20	DF	$48.1 \pm 1.1_{gh}H$	$49.6 \pm 0.2_{gh}J$	$77.8 \pm 2.8_{jk}E$
	−80	DF	$44.7 \pm 0.8_{j}H$	$51.1 \pm 0.6_{g}J$	$62 \pm 1_{f}L$

4.3.4. Folate

The folate content of all three cultivars during storage is shown in Table 4.6. The folate content of the Arianna, Francesca, and Silvia WF at time 0 was 126 µg/kg FW, 171.55 µg/kg FW, and 113.72 µg/kg FW, respectively. The oven-drying process slightly decreased the folate content of the fruits of all cultivars, with the folate content being 120.96 µg/kg FW, 115.19 µg/kg FW, and 103.09 µg/kg FW in the DF at time 0, respectively. The folate content of the WF during storage at −20 °C had an increasing trend from 1M until the end of storage for all cultivars, and, at the end of storage, the folate contents of the Arianna, Francesca, and Silvia WF were detected to be 190.61 µg/kg FW, 208.4 µg/kg FW, and 132.06 µg/kg FW, respectively. In the DF, similarly to the WF, the folate content increased with the extension of storage for all cultivars.

At −80 °C storage, the folate content of the WF for all cultivars increased until the end of storage, as registered for the −20 °C storage. The Arianna and Francesca fruits had a peak of folate content after 3M of storage, with a content of 229.67 µg/kg FW and 223.59 µg/kg FW respectively, while the Silvia fruit had a peak after 5M of storage, with a content of 177.65 µg/kg FW. In the DF, there was also an increasing trend for folate content until the end of storage, with the highest content detected at the end of storage in the Arianna and Francesca fruits (188.04 µg/kg FW and 166.46 µg/kg FW, respectively); in the Silvia fruit, the highest folate content was detected after 5M of storage, with a value of 138.88 µg/kg FW. There was not a significant difference in folate content between temperature and treatments, but the WF presented more folate content than the DF during storage.

The strawberry is one of the most important sources of antioxidants and is considered a functional fruit due to the presence of a diverse range of bioactive components and high levels of vitamin C, vitamin E, folate, phenolic compounds, and fiber (Giampieri et al., 2015). In this respect, variations of the amount of vitamin C in strawberries can affect the folate content, as a higher vitamin C content can lead to increased stability of folate (Wilson and Horne, 1983). The retention of folate and ascorbic acid was affected by the same factors, and a high content of ascorbic acid could provide possible protection against folate degradation (Strålsjö et al., 2003). This assumption was also confirmed by Ringling and Rychlik (2017), who performed in vivo studies to simulate food folate digestion and found out that ascorbic acid stabilizes folate, particularly 5-CH3-H4 folate during digestion. The addition of ascorbic acid in physiological amounts improved the stability of some types of folate, depending on the food matrix (Ringling and Rychlik, 2017).

Table4. 6. Average of folate content (μg/kg FW) in cultivars of Arianna, Francesca, and Silvia fruits. The data are expressed as mean ± standard error. Different lowercase letters indicate significant differences for $p \leq 0.05$ in each cultivar during storage. Different uppercase letters indicate significant differences for $p \leq 0.05$ for all cultivars for a specific storage time. WF: whole fruits, DF: dried fruits. 0 M: 0 months of storage; 1 M: 1 month of storage; 3 M: 3 months of storage; 5 M: 5 months of storage; 7 M: 7 months of storage.

Storage Time	Storage Temperature	Treatment	Arianna	Francesca	Silvia
0 M		WF	126.17 ± 4.8$_{cd}$B	171.55 ± 1.3$_{abcd}$A	113.72 ± 0.79$_{cd}$D
		DF	120.96 ± 2.7$_{d}$C	115.19 ± 1.2$_{d}$D	103.09 ± 1.78$_{d}$E
1 M	−20	WF	133.58 ± 1.6$_{cd}$B	156.39 ± 1.8bcdA	114.88 ± 1.4cdCD
	−80	WF	132.27 ± 2.9$_{cd}$B	116.97 ± 0.5dCD	117.92 ± 1.4cdCD
	−20	DF	112.37 ± 0.8$_{d}$DE	131.47 ± 3.7dB	107.35 ± 0.9cdEF
	−80	DF	119.8 ± 3.1$_{d}$C	134.83 ± 0.6dB	104.04 ± 0.3dF
3 M	−20	WF	155.39 ± 2.8$_{bcd}$C	141.68 ± 3.5$_{cd}$D	126.53 ± 1.7$_{bcd}$EF
	−80	WF	229.67 ± 0.9$_{a}$A	223.59 ± 5.4$_{a}$A	123.39 ± 1.1$_{cd}$EF
	−20	DF	127.6 ± 2.9$_{cd}$E	164.8 ± 2.9$_{abcd}$B	106.5 ± 0.1$_{cd}$G
	−80	DF	139.74 ± 2.9$_{bcd}$D	119.27 ± 1.5$_{d}$F	110.13 ± 0.4$_{cd}$G
5 M	−20	WF	147.71 ± 7.2$_{bcd}$CDE	220.68 ± 3.3$_{a}$A	121.9 ± 24.3$_{cd}$EF
	−80	WF	131.23 ± 2.1$_{cd}$DEF	132.08 ± 2.1$_{d}$DEF	177.65 ± 5.6$_{a}$BC
	−20	DF	157.83 ± 12.8$_{bcd}$CD	150.22 ± 2.4$_{bcd}$CDE	121.43 ± 15.6$_{cd}$EF
	−80	DF	188.49 ± 7.1$_{ab}$B	109.92 ± 5.9$_{d}$F	138.88 ± 8.7$_{bc}$DEF
7 M	−20	WF	190.61 ± 3.6$_{ab}$AB	208.4 ± 7.2abA	132.06 ± 1.2bcdAB
	−80	WF	175.58 ± 7.3$_{bc}$AB	199.44 ± 51.7abcAB	157.55 ± 1.7abAB
	−20	DF	174.64 ± 40.4bcAB	156.41 ± 54.4bcdAB	124.8 ± 32.4cdAB
	−80	DF	188.04 ± 58.9abAB	166.46 ± 38.5abcdAB	102.66 ± 1.6dB

4.3.5. Correlation Matrix

The correlation matrix among the analyzed nutritional parameters indicated that there was a good positive correlation between anthocyanins and vitamin C content (0.70, $p < 0.05$). This means that these two classes of compounds presented a similar behavior in the tested conditions, decreasing dramatically with the oven-drying treatment, while they did not change greatly regarding storage temperature or length. Vitamin C also

presented a medium correlation with phenolic acids (0.52) and a lower but still significant correlation with folate content (0.27) (Table 4.7).

Table4. 7. Correlation matrix among the nutritional parameters analyzed. * indicates a significant correlation for $p \leq 0.05$.

	Anthocyanins	**Vitamin C**	**Folate**	**Phenolic Acids**
Anthocyanins	1.00	0.70 *	0.04	−0.03
Vitamin C	0.70*	1.00	0.31 *	0.52 *
Folate	0.04	0.31 *	1.00	0.27 *
Phenolic acids	−0.03	0.52 *	0.27 *	1.00

4.4. Conclusions

In this study, some interesting results were obtained, describing how the nutritional compounds of strawberries react during storage at different temperatures with different storage times and treatments.

- Higher amounts of nutritional compounds were detected in the WF compared to the DF.
- For preserving nutritional quality, the WF treatment was optimum, and it showed good results in −80 °C storage compared to −20 °C. This is an important indication for application in the laboratory analysis and processing industry.
- The anthocyanins' content in the WF seemed to not decrease during 7 months of −80 °C storage, with some exceptions.
- Oven drying was not an ideal treatment for preserving vitamin C, almost completely degrading this compound in strawberry fruits.
- In the WT and DF, the amount of folate increased during storage. More folate was detected in the WF.
- The different strawberry cultivars presented different amounts of nutritional compounds: The Arianna and Francesca fruits had more vitamin C, phenolic acids, and folates, but the Silvia fruit had more anthocyanins.
- At the end of storage (7M), there was more loss of vitamin C in the Arianna WF compared to the other two cultivars' WF.
- There was a slight increase in the amount of anthocyanin in the Arianna and Francesca WF after 7M of storage, but in the Silvia WF the amount of anthocyanin was retained.

Generally, it can be concluded that oven drying is not a recommended technique to treat fruits for preserving nutritional quality during storage. Storage time did not greatly affect the nutritional quality in whole fruits, though they presented a higher amount when stored at −80 °C.

Note: Qaderi, R.; Mezzetti, B.; Capocasa, F.; Mazzoni, L. **Stability of Strawberry Fruit (Fragaria x ananassa Duch.) Nutritional Quality at Different Storage Conditions.** Appl. Sci. 2023, 13, 313. https://doi.org/10.3390/app13010313.

CHAPTER 5.

GENERAL CONCLUSIONS OF THE STUDY

In this study, some interesting results were obtained and could be concluded as follow:

- The concept of fruit quality is complex and difficult to define, as it is influenced by different factors, disparate measurement indices and varies according to the opinion of the designated individual.
- the main descriptors of the concept of Strawberry fruit quality is mainly based on the Strawberry Plant Yield Efficiency, Organoleptic Quality and Nutritional quality
- The possible way to improve the fruit content of such phytochemicals is traditional breeding program and for this purpose, it is important to accurately describe the genetic resources used in cross combination.
- Wild germplasm is an interesting source of genes for improving fruit nutritional quality of new cultivars, but long-term backcross programs are needed to produce new pre breeding materials that can facilitate the release of new cultivars with fruits of superior quality.
- The release of new cultivar is not only an ending point, also it is a starting point for further breeding programs for increasing the nutritional quality.
- *Botrytis cinerea* is one of the main strawberry pathogen, increasing the postharvest decay.
- Botrytis cinerea significantly decrease the sugar content and nutritional compounds of the strawberry.
- For preserving nutritional quality, the WF treatment was optimum, compare to DF and it showed good results in −80 °C storage.

This study could be considered important for progress of breeding program, aiming improving continuously of strawberry new genotype including wild genotype germplasm has genetic diversity. The maintaining of quality be assure providing genotypes that tolerant to *botrytis*, and for this reason the cultivation of new cultivars and genotypes tolerance to *botrytis* must continue. Finally, the evaluation of nutritional quality for long term storage is an important starting to determine the best condition for maintaining quality in postharvest, also in this case the evaluation should continue to taking account other storage treatment.

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