DEPARTMENT OF AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCES

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

VARIATION OF FRUIT ANTHOCYANINS AND PHENOLIC ACIDS CONTENT IN ADVANCED STRAWBERRY BREEDING SELECTIONS.

TYPE OF DISSERTATION: EXPERIMENTAL RESEARCH

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DEDICATION

This study is wholeheartedly dedicated to my entire family who have been a source of inspiration and motivation.

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ABSTRACT

The modification of the lifestyle in modern society has underlined new crucial aspects from the nutritional point of view. In few decades there has been an increased interest in studies concerning all kinds of fruits. Particularly berry fruits, which are well studied as they contain the best dietary sources of bioactive compounds. Among berry fruits, strawberry received in the last years increasing attention, and a growing number of scientific evidence demonstrated how a short- or a long-term consumption of strawberries could be beneficial for the consumers. The nutritional properties of strawberry depend on the amount and the profile of the bioactive and antioxidant compounds it contains (e.g., polyphenols and vitamins). The recent aim of different breeding programs is to select new strawberry cultivars having fruit with high concentrations of antioxidant compounds. In this study, 12 commercial cultivars plus 42 advanced selections for 2018-2019, as well as 13 cultivars plus 86 selections for 2019-2020 seasons from the UPM-D3A breeding program have been deeply evaluated for their nutritional quality for 2019 and 2020 harvest seasons. The concentration of key bioactive compounds: anthocyanins and phenolic acids were measured through HPLC-UV. Generally, the average phenolic acids contents of both 2019 and 2020 genotypes were higher than the anthocyanin content. The average phenolic acids content for 2019 strawberry selections and cultivars, as well as 2020 selections and cultivars were: 102.91, 106.63, 65.19 and 57.94 mg/ 100g FW, respectively. The average anthocyanins content for 2019 strawberry selections and cultivars, as well as 2020 selections and cultivars were: 17.66, 16.77, 20.24 and 20.52 mg/ 100g FW, respectively. Amongst the best performing strawberry genotypes, 'Francesca' consistently proved to have superior quality in terms of phenolics acids content for 2019 and 2020 harvesting years. Overall, 'Monterey' proved to be the best performing genotype in terms of both anthocyanins and phenolic acids contents for 2019 and 2020 harvesting years. 'AN07,105,53', 'FVG', 'AN13,13,55', 'AN13,44,52' were the best selections. The results obtained demonstrate the high variability in anthocyanins and phenolic acids composition among cultivars and selections and the possibility to generate new cultivars producing fruit with high contents of polyphenols, stable at the different cultivation cycles, to be labelled with a compositional claim.

Keywords: strawberry, anthocyanin, phenolic acids, cultivar, selections, variation.

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CHAPTER 1: INTRODUCTION

1.1 Background

Strawberries (*Fragaria* × *ananassa*, Duch.) are the most cultivated and consumed berry fruits worldwide, with an annual fruit production surpassing 13.3 million MT cultivated on a surface area of 522527 ha (FAOSTAT, 2019). Several studies have shown that strawberry fruits have high *in vitro* antioxidant activity linked with the high polyphenol content, particularly anthocyanins and phenolic acids (Heinonen *et al.*, 1998; Wang and Jiao, 2000; Wang and Lin, 2000). Strawberry fruits are well known as highly rich source of phenolic compounds (anthocyanins, flavonoids and phenolic acids) and bioactive compounds (vitamin B9 and C) (Giampieri *et al.*, 2012; Ariza *et al.*, 2016). All these compounds have shown huge biological potential in humans: antioxidant capacity, antihypertensive, anti-inflammatory, and anti-proliferative abilities (Mazzoni *et al.*, 2016; Battino *et al.*, 2019; Cianciosi *et al.*, 2019; Prasain *et al.*, 2020; Zabaleta 2020; Muceniece *et al.*, 2019; Gasparrini *et al.*, 2017).

Strawberries are among the most important crops in Europe with strongly increasing production to meet consumer demand. The main antioxidant compounds of the strawberry contributing the high TAC of the fruit are polyphenols and vitamin C (Giampieri *et al.*, 2012). However, the composition of strawberry fruits varies, depending on the individual genotypes, environmental factors, cultivation techniques, maturity stage and pre- and post-harvest practices (Tulipani *et al.*, 2011). In the past years, fruits sensorial and nutritional quality have become the major breeding targets in the commercial strawberry (Mezzetti *et al.*, 2016; Ariza *et al.*, 2018). According to Capocasa *et al.* (2008), the success of a breeding program depends on the heritability and variability of interesting bioactive compounds from parents to progenies. 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.

In recent times, 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. Diamanti *et al.* (2012) revealed that the inclusion of wild species in breeding programs, having a genetic background capable of giving progeny increased phytochemicals is important. In this study, 14 commercial cultivars and 5 advanced selections from the UPM-D3A breeding program have been evaluated for their nutritional quality for 2019 and 2020 harvesting years. The concentration of the main bioactive compounds being anthocyanins and phenolic acids were measured using HPLC-UV.

CHAPTER 2: LITERATURE REVIEW

2.1 Origin, Taxonomy and Morphology of Strawberry

Strawberry is a wide crop spread overall the world. Strawberries (family: Rosaceae, genus: Fragaria, cultivated: *Fragaria* × *ananassa*, wild: *F. virginiana*) belong to berries that are popular due to their desirable sweet taste and attractive aroma, with red colour and smooth texture. What generally is regarded as strawberry fruit is actually a swollen part of the strawberry flower known as receptacle. The true strawberry fruits are the achenes which are fixed in the outer layer of the receptacle and generally denoted as seeds. The unique aromatic flavour and taste of strawberry is highly appreciated by many people, hence widely produced for fresh fruit consumption.

First traces of this berry genus were found in strawberry fossils from Neolithic period. However, the ancient documents that reported strawberry presence belong to Romans. This fruit was well known for its good flavour and perculiar aroma. At the end of sixteenth century, strawberry was not so popular in the agronomic scene; in Europe, just three species Fragaria spp were present: *F. vesca* L. (known as wood strawberry), *F. moschata* Duch and *F. viridis* Duch. The most common specie was *F. vesca*, collected from woods to gardens. At the end of 1600 the strawberry fruit started to be identified as iconic element. For instance, it was used on the top of the table as an ornament.

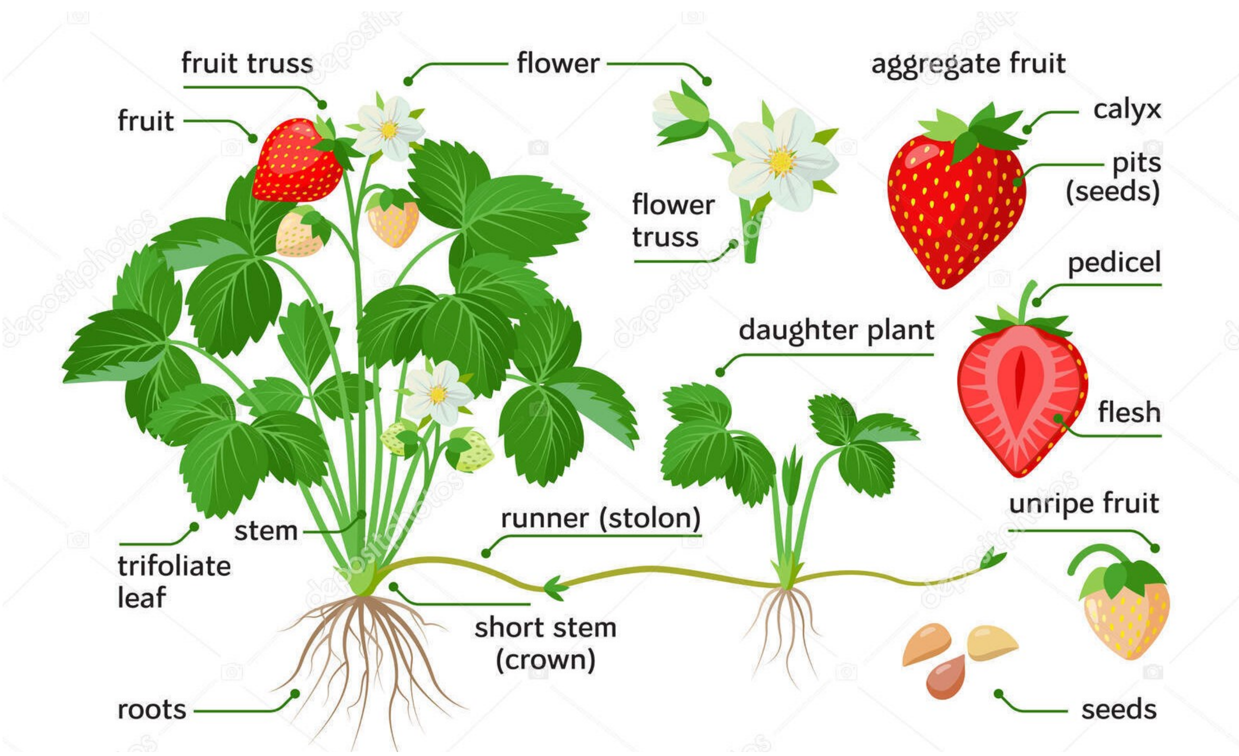

Figure 1: Strawberry plant parts

Strawberry is a perennial herbaceous plant which forms stolons; roots apparatus is assembled, composed of primary and secondary roots. The roots are generally shallow. The stem, also known as crown, is short (2 – 3 cm) and herbaceous during the early stages of the plant. However, it starts to lignify during the plant development.

The leaves are composed of three (3) minor leaves with barbed borders: they are placed in the same level and opened like a fan (palmed leaves). The flowers (Fig. 1) are white and they are collected in inflorescences that are generally set by primary axis, two secondary axes, four tertiary axes and eight quaternary axes. Axes length is influenced by heritage traces and formation period. The inflorescence with short primary axis shows more fruits and flowers, in the other hand the inflorescence with long primary axis is characterized by less flowers (Bucci *et al.*, 2010).

Strawberry fruit is considered as a false fruit, because it derives from the enlargement of the receptacle, while the real fruits are the green, yellow or red achenes placed on strawberry skin surface. The fruit shape changes based on the variety of fruit, it could be conic, long-conic, oblate, globose, globose-conic, necked, long-wedge and short wedge (Fig. 2).

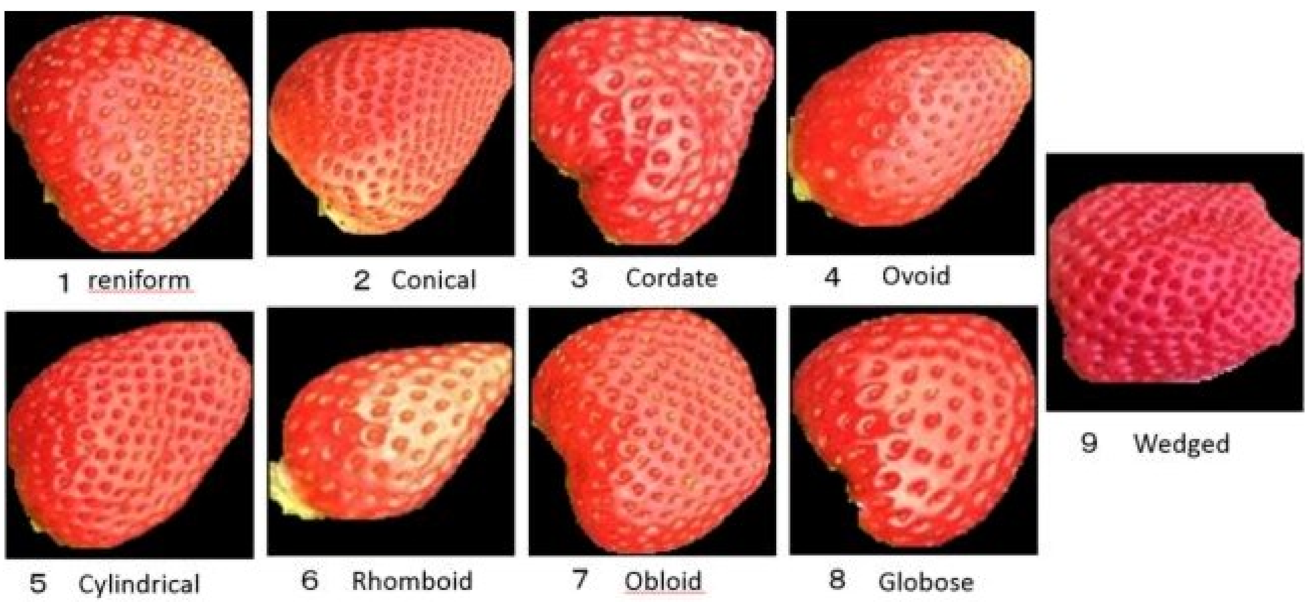

Figure 2: Strawberry fruit shapes

2.2 Genetic Diversity and Breeding for Improvement of Strawberry

Strawberries come in a wide assortment of commercially available cultivars (cultivated varieties). Differences between cultivars may include the date the fruit ripens, disease resistance, freezing quality, firmness, berry size, berry shape, and flavor. Many different cultivars have been developed over the years. This notwithstanding, almost all the strawberries are cultivars of *Fragaria* × *ananassa* (Strum *et al*., 2003). Increasing antioxidant levels in fruit through breeding is an important option to support higher antioxidant intake particularly when fruit consumption is low. Indeed, if nutritional components are also combined with a high standard of sensorial fruit quality, the perspective for consumer health can be further improved by encouraging more fruit consumption. Wild species are valued by strawberry breeders as sources of novel traits, especially for pest resistance and abiotic stress tolerance (Bosc, 2009). Furthermore, previous investigations have shown improvements in fruit nutritional quality in breeding material that originated from Fragaria virginiana ssp. glauca (FVG) inter-species crosses. Recently, commercial varieties of strawberries have also shown interesting variability in fruit nutritional quality (Masny and Żurawicz, 2010).

Breeding for improvement of strawberry cultivars is difficult. Many traits, like disease resistances, firmness and vulnerability of the fruit, productivity, nutritional quality and of course its taste, have to be considered in the selection of a successful strawberry cultivar. In addition, genetic variation in *F. x ananassa* has found to be very limited, while genetic variation is a prerequisite for progress in conventional breeding. Furthermore, breeding is hampered because strawberry is an octoploid, hybrid species, originating from a rather recent cross between two wild octoploid Fragaria species: *F. virginiana* and *F. chiloensis*. The complicated genetic constitution of the strawberry genome has kept most researchers from investing in the development of methods that can assist breeding of strawberry. Only recently, the first results towards the production of a genetic map for strawberry have been published (Haymes *et al*., 2000; Lerceteau-Kohler *et al*., 2003).

For several years, the objective of many breeding programs on strawberries all around the world was to improve the common marketed strawberry, also known as *Fragaria* × *ananassa*, in order to obtain greater, stronger and better plants. Those breeding program continues from two centuries ago until nowadays. Actually, breeding programs are giving every year new good results, because strawberry has a high ploidy value (eight-ploidy) and it can easily interact with the surrounding environment; this character allows a wide phenotypic variability (Mezzetti *et al*., 2010). *Fragaria* × *ananassa* comes from two eight-ploidy species hybridization (*F. chiloensis* and *F. virginiana*), but just few plants obtained from this hybridization gave 7 rises to the overall varieties currently available. Thanks to this crossbreed, strawberries are highly adaptable to the cultivation in many environments with different climates. Those breeding programs share many common objectives, like: perfect flower, high production level, good balance between production and resistance to biotic stress. However, they could also pursue more specific objectives, for instance the ripening period, the consistency, the size, the fruit quality and the diseases resistance.

The D3A Department of Università Politecnica delle Marche (UNIVPM) has been engaged in strawberry breeding programs for many years, with the initial objective of obtain new genotypes with high-production levels and high-quality fruits, and adaptability to resilient conditions. Also, the market, during the last years, has driven breeders to get higher quality fruits with typical flavours and good aromas. The correlation between these two parameters (high production and high quality) is extremely difficult to get, making breeding programs not properly efficient. The main problem about high production level is that not always can get sweeter fruits, contrariwise strawberries may have less sugar concentration.

In the last period, consumers' knowledge about beneficial properties of strawberry phytochemical compounds on human health and their ability to fight various diseases has boosted the breeding process toward the research and creation of new genotypes richer in bioactive compounds with antioxidant activity. Among fruits, in fact, strawberry is considered one of the best sources of these bioactive compounds. For this reason, the current D3A breeding program is focusing the attention, besides the plant production and fruit quality, also to the nutritional properties of new strawberry genotypes, through the improvement of the concentration of ascorbic acid (vitamin C), folates (vitamin B9), and antioxidants (polyphenols).

Finally, breeding program wants also to help growers to obtain new genotypes with high resistance to diseases. For instance, the abolition of methyl bromide fumigations 8 on field gave institutions the awareness to find genotypes with higher resistance to roots diseases. Concluding, the breeding programs aim to the improvement of many productive and qualitative aspects of strawberry. In the case of D3A, the implementation of the breeding program over years led to the registration of 4 new commercial varieties ("Adria", "Cristina", Romina" and "Sveva") and hundreds of new selections currently evaluated for their high commercial value.

2.3 Strawberry Cultivation Techniques

The strawberry plant is acclimatized to different environments and, therefore, could be cultivated worldwide, intensively in Europe and North America in open fields, whereas in China it is cultivated mainly in greenhouses (Wang *et al*., 2015). The production of strawberry relies on a limited number of genotypes, which are initially selected for adapted growth characteristics to different geographic and climatic conditions (Strik *et al*., 2007). For example, in North-West Europe for 60-95% of the strawberry production the cultivar Elsanta is used, while this cultivar is less suitable for commercial production in either Scandinavia or in the South of Europe.

Strawberry is a particular fruit species that can grow in different soils; it prefers generally clay soils with a good and proper drainage. It also prefers sub-acid or acid soils with a basic pH, between 5.5 and 7. The presence of pH higher than 7 can cause some disorders to the plant: for instance, the strawberry plant may hardly absorb iron, and other damage may be related to less marked yellowing of leaves, also known as chlorosis. The first step of strawberry cultivation is the preparation of soil: the species requires a careful preparation of the soil, in order to avoid stagnant water that can lead to roots diseases (Claire, 2018). It is necessary to level the soil that has never hosted strawberries, facilitating the outflow of excess water. Once the soil is levelled, it is possible to apply an organic fertilizer useful for the development of the roots and for the elimination of unwanted weeds.

The rotation is an important process to set up between different cultivation seasons. It is necessary to avoid stunted development problems, stress or collapse of the plant. The objective of rotation is to improve soil structure, maintaining chemical fertility and reducing the presence of pathogens. Other important aspect is related to the crop's rotation choices: for strawberry is recommended the rotation with pea and bean, that improve both structure and fertility of the soil. If the rotation is not possible, the sterilization of the soil is an important and widespread practice and represent an important aspect that should be taken in account before strawberry cultivation. The solarization is one of the most used sterilization methods. To obtain a proper solarization action it is necessary to cover the soil with green or transparent polyethylene films (PE), for 4-8 weeks. The proper solarization and the combination time/temperature should guarantee an optimal effectiveness against various telluric pathogens.

The strawberry plant is traditionally cultivated on baulate soil, with a height from 10 to 30 cm. This technique maintains a dried microclimate to the plant, avoiding water 6 stagnation and fungal infections (Muñoz, 2017). The baulation is realized with the aid of a special furrow opener, that at the same time places in the field the hose for the irrigation and the mulching film. The mulching film is a dark polyethylene plastic film with holes placed at a distance of 30-40 cm. The main advantage using this practice is to manage properly the development of unwanted weeds. The plantation period depends on the area and type of cultivation adopted. For instance, in mountain regions the planting begins in early June, in the northern plains environment the plantation begins in mid-June. In the southern regions the plantation operations begin in mid-August. The harvesting represents the last step of the entire cultivation. It is also the most delicate operation, and it requires special care. The fruits shall be harvested when they reached the red colour on the overall surface. The over-ripe fruits and/or those who have deformed shape should be sold separately. The detachment of the fruits shall be made manually for fruits destined to fresh consumption, while it may be done mechanically just for the products intended to industry (Feng, 2012).

2.4 Socio-Economic Importance of Strawberry Fruit

Strawberries are economically and commercially important and widely consumed fresh or in processed forms, such as jams, juices, and jellies. It is also utilized as flavours in sweets, pastry and dairy products, and as fragrance for perfumes. That is why they are among the most studied berries from the agronomic, genomic, and nutritional points of view. Strawberries are produced commercially in 76 countries. China is the largest producer and the top five producing nations also include USA, Mexico, Turkey and Spain. Production continues to increase, particularly in Asia, North and Central America, and North Africa with a matching increase in demand in many parts of the world.

The United States produces more than 3 billion pounds of strawberries each year, providing almost 20% of the world crop, and is a global leader in production per unit area. The farm gate economic value of strawberries is just shy of $3 billion per year (Hummer and Janick, 2009).

In 2018, the revenue of the strawberry market in the European Union amounted to $3.8B in 2018, rising by 1.9% against the previous year. This figure reflects the total revenues of producers and importers (Simpson, 2018). This excludes logistics costs, retail marketing costs, and retailers' margins, which will be included in the final consumer price. According to Global Trade (2020), in Carlifornia, USA, strawberry farming accounts for an estimated $108 million in annual tax revenue. This in turn supports local, state, and regional government services. Strawberry growing has a multiplier effect, creating jobs and generating revenue beyond the farm. For every farm dollar made, 97 cents is invested back into the community.

The countries with the highest volumes of strawberry consumption in 2018 were Germany (233K tonnes), Poland (203K tonnes) and the UK (183K tonnes), comprising 51% of total consumption. Italy, France, Spain, Belgium, Romania, Greece, Portugal, Austria and Sweden lagged somewhat behind, together comprising a further 39%. From 2007 to 2018, the most notable rate of growth in terms of strawberry consumption was attained by Greece, while strawberry consumption for the other leaders experienced more modest paces of growth (Global trade, 2020).

In value terms, Germany ($819M), the UK ($792M) and Italy ($360M) were the countries with the highest levels of market value in 2018, together comprising 52% of the total market. Poland, France, Spain, Romania, Belgium, Sweden, Austria, Portugal and Greece lagged somewhat behind, together comprising a further 36%. The countries with the highest levels of strawberry per capita consumption in 2018 were Poland (5,315 kg per 1000 persons), Belgium (2,900 kg per 1000 persons) and Germany (2,844 kg per 1000 persons).

Driven by increasing demand for strawberry in the European Union, the market is expected to continue an upward consumption trend over the next decade. Market performance is forecast to retain current trend, expanding with an expected CAGR of +1.1% for the period from 2018 to 2030, which is projected to bring the market volume to 1.4M tonnes by the end of 2030 (Global Trade, 2020).

2.5 Nutritional and Functional Composition of Strawberry

Strawberry is one of the most consumed fresh fruit all around the world with high nutritional value. During the past years, strawberries have been the object of several chemical analyses to appreciate the presence and quantity of nutritional and bioactive compounds (Giampieri *et al.*, 2012).

Table 1: Nutritional Composition of Fresh Strawberries (USDA, 2019).

Type	Nutrient	Per 100 g
Proximates	Water (g)	90.950
	Energy (kcal)	32.000
	Protein (g)	0.670
	Ash (g)	0.400
	Total lipid (g)	0.300
	Carbohydrate (g)	7.680
	Dietary Fiber (g)	2.000
	Sugars (g)	4.890
	Sucrose (g)	0.470
	Glucose (g)	1.990
	Fructose (g)	2.440
Minerals	Calcium (mg)	16.000
	Iron (mg)	0.410
	Magnesium (mg)	13.000
	Phosphorus (mg)	24.000
	Potassium (mg)	153.000
	Sodium (mg)	1.000
	Zinc (mg)	0.140
	Copper (mg)	0.048
	Manganese (mg)	0.386
	Selenium (µg)	0.400
Vitamins	Vitamin C (mg)	58.800
	Thiamin (mg)	0.024
	Riboflavin v	0.022
	Niacin (mg)	0.386
	Pantothenic acid (mg)	0.125
	Vitamin B6 (mg)	0.047
	Folate (µg)	24.000
	Choline (mg)	5.700
	Betaine (mg)	0.200
	Vitamin B12 (µg)	0.000
	Vitamin A, RAE (µg)	1.000
	Lutein + zeaxanthin v	26.000
	Vitamin E, α-tocopherol (mg)	0.290
	β-tocopherol (mg)	0.010
	γ-tocopherol (mg)	0.080
	δ-tocopherol (mg)	0.010
	Vitamin K, phylloquinone (µg)	2.200

According to Ganhão *et al.* (2019), strawberries contain nearly 91 % water, low in calories (32 kcal/100 g) and amongst the fruits, fresh strawberries are considered to have the highest content of vitamin C. Due to their dietary fibre (2.0 g/100 g) and fructose content (2.44 g/100 g), strawberries have a satiating effect and can help regulating blood sugar levels by slowing digestion. Additionally, strawberries are a good source of minerals. In particular manganese, potassium, magnesium, copper, iron and phosphorus can be found in ample quantities (Table 1).

Among the micronutrients, vitamin C and folate (compounds with similar chemical form as folic acid or vitamin B9) contents have to be emphasized, but in minor quantities also vitamin B1, B2, B3, B6, A, E and K were found in strawberries (Ganhão *et al.*, 2019). With an average of 24 µg folates per 100 g fresh weight (FW), strawberries are one of the fruits with the highest amount of this essential micronutrient for human health. Another good parameter that shall be taken in account is the total antioxidant capacity (TAC). This common parameter is used to determine the nutritional quality and it is related to the quantity of bioactive compounds with antioxidant characteristics in the fruits. So, in strawberry is possible to find vitamin C and phenolic compounds – above all anthocyanins – that are responsible for the scavenging of free radical molecules (Giampieri *et al.* 2013; Tulipani *et al.* 2008a).

Antioxidant compound have the ability to donate an electron to those highly reactive free radicals which thereby become neutral again, reducing the oxidative stress in cells and lowering the risk of disease (Tulipani *et al.*, 2009b). Strawberries are among the fruits with the highest TAC levels, exceeding those of raspberries, apples, peaches, pears, grapefruit and even oranges and kiwis. Only a few fruits such as blackcurrant and blueberry show a higher TAC (Halvorsen *et al.* 2006; Wang *et al.* 1996; Proteggente *et al.* 2009; Wang and Lewers, 2007; Wang and Lin 2000; Kalt *et al.*, 1999).

In vivo studies of Tulipani *et al.* (2011b; 2014) and Romandini *et al.* (2013) could demonstrate that strawberry consumption increases the antioxidant capacity in human plasma and the defence against oxidative stress. One of the main aspects that may influence phytochemicals concentration within fruits are the climatic conditions (Wang and Millner, 2009): among them we should remember the kind of soil where the plants are cultivated, UV-rays exposition, moisture and temperature conditions and cultivation techniques.

2.6 Polyphenols

The most represented category of the non-nutritional compounds in strawberries are called polyphenols. The term "non-nutritional" means that those compounds are not strictly necessary for the health of the humans, and their absence in the diet did not lead to any disease. However, their assumption with the diet is absolutely suggested, giving that they possess many interesting healthy properties and they can significantly help the human body in the prevention of many diseases (Balasundram *et al.*, 2006; Giampieri *et al.*, 2012; Tulipani *et al.*, 2009b; Alvarez-Suarez *et al.*, 2014; Giampieri *et al.*, 2013).

Polyphenolic compounds are the main responsible of the antioxidant properties of strawberries. In plants and vegetables, they constitute secondary metabolites and they are responsible for colouration, tastes and flavours, but the main characteristic is to act as fourteen (14) radical scavengers. Furthermore, they can also interact with proteins and protect the plant from an excessive UV radiation. Finally, polyphenols may help the host from other stress situations like pathogens attacks which is a leading role for chemical defence mechanism (Ozcan *et al.*, 2014; Dixon and Paiva, 1995; Bravo, 1998). Polyphenols are present in three different classes (Fig.3): flavonoids (e.g. anthocyanins), non-flavonoids (e.g. stilbens, lignans and tannins) and phenolic acids (Tappi, 2018).

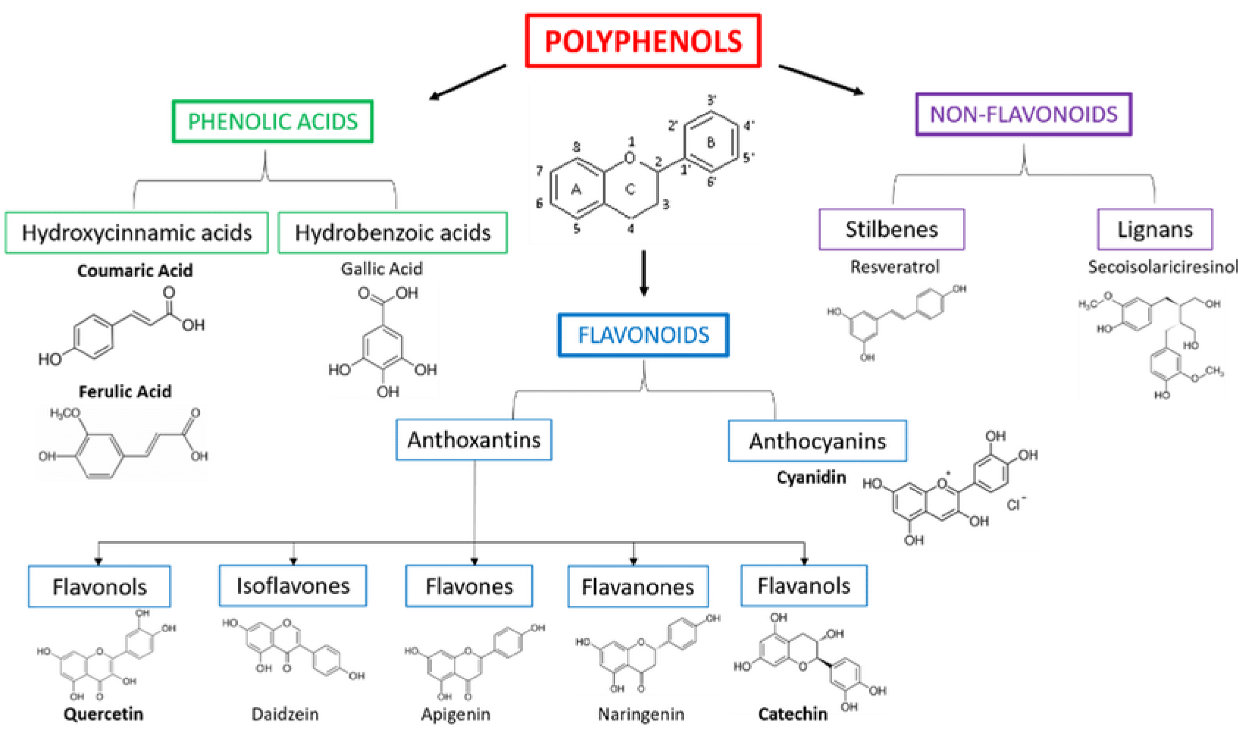

Figure 3: Classification of polyphenols (Basheer and Kerem, 2015).

2.7 Anthocyanins

Fruit anthocyanins have been a focus of several scientific researches in the past years (Aaby *et al*., 2012). Anthocyanins are well-known to possess high radical scavenging properties, prevent oxidative stress and thereby helping to maintain physiological functions (Du and Wang, 2008). More often, a key aspect of studies on fruit anthocyanins is related to strawberry (*Fragaria* × *ananassa* Duch.) fruits, since anthocyanins are also responsible the fruit's antioxidant activity; one of the highest among several fruits (Cordenunsi *et al*., 2005).

Strawberry fruits (*Fragaria* × *ananassa* Duch.) have been proven to have high *in vitro* antioxidant activity which has been positively linked to polyphenolic compounds content, particularly, anthocyanins. Quantitatively, anthocyanins are the type of polyphenolic compounds considered the most important in strawberry (Heinonen *et al*., 1998; Wang and Jiao, 2000; Wang and Lin, 2000). Strawberry fruit's anthocyanin content has been the object of several research studies. However, it is still not fully characterized regarding minor pigments. According to Mazza and Miniati (1993), strawberry anthocyanins collectively stem from pelargonidin (Pg) and cyanidin (Cy) aglycones (Fig. 4a). Previous investigations have revealed that the main anthocyanin compound in strawberry fruit is Pg-3-glucoside (Pg 3-gluc) which was initially identified by Robinson and Robinson (1931).

The presence of Cy 3-glucoside (Cy 3-gluc) also appears constant in all varieties in lesser quantities (Bridle and Garcia-Viguera, 1997; Hong and Wrolstad, 1990a; Lukton, *et al*., 1955). Also, Pg 3-rutinoside (Pg 3-rut) is usually present (Bakker *et al*., 1994; Co and Markakis, 1968; Hong and Wrolstad, 1990b). Moreover, Pg 3-arabinoside (Fiorini, 1995; Goiffon *et al*., 1999) and Cy 3-rutinoside (Bridle and Garcia-Viguera, 1997) have been quoted in some cultivars of strawberry, as well as various acylated anthocyanins. Specifically, Pg 3-(6-malonylglucoside) was clearly identified by Tamura *et al*. (1995) and shown as one of the major pigments in most Japanese cultivars, consisting 5–30% of the total anthocyanin content (Tamura *et al*., 1995; Yoshida *et al*., 2002). Other acylated anthocyanins also reported in strawberry are Pg 3-acetylglucoside (Hong and Wrolstad, 1990b) and Pg succinylglucoside (Bakker *et al*., 1994).

Small quantities of anthocyanin-related pigments have also been detected and identified in strawberries, including 5-carboxypyranopelargonidin 3-glucoside (Fig. 4b) (Andersen *et al*., 2004) and four (4) purple anthocyanin–flavanol complexes consisting of pelargonidin 3-glucoside C–C linked to (epi)catechin and (epi)afzelechin moieties (Fig. 4c) (Fossen *et al*., 2004). That was the initial proof of the occurrence in a natural plant source of this kind of condensed pigments, whose formation was related to reactions that take place during maturation and ageing of red wines (Jurd, 1969; Salas *et al*., 2004; Somers, 1971; Vivar-Quintana *et al*., 1999). González-Paramás *et al.* (2005) also provided more evidence about the presence of anthocyanin–flavanol condensed pigments in strawberry fruits and other plants.

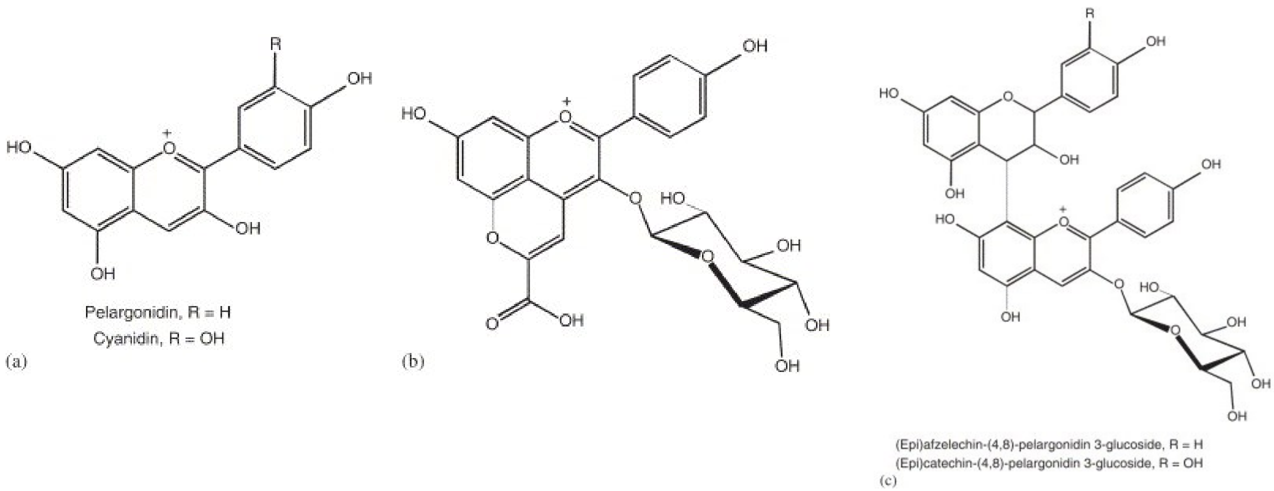

Figure 4: Structures of pigments found in strawberry. (a) Anthocyanin aglycones; (b) 5-carboxypyranopelargonidin 3-glucoside (Andersen et al., 2004); and (c) anthocyanin-flavanol condensed pigments (Fossen et al., 2004).

2.8 Phenolic Acids

Phenolic acids constitute another important group of beneficial compounds in strawberries belonging to phenolic compounds. As affirmed before, they can be divided in two subgroups. The first one is known as hydroxybenzoic acids: these acids content is generally very low in fruits and vegetables, with the exception of certain red fruits (strawberry), black radish and onion, which can have high concentration (several tens of milligrams per kilogram FW). Among these acids, the most common is gallic acid: for instance, tea is an important source of gallic acid, due to tea leaves that may contain up to 4.5 g/Kg FW. The second subgroup is recognized as hydroxycinnamic acids: these are more commonly present in many foods than hydroxybenzoic acids. They are: p-coumaric acid, caffeic acid, ferulic acid and sinapic acid. These acids are rarely found in the free form, except in processed food that has undergone freezing, sterilization or fermentation (Giampieri *et al*., 2012, 2013; Määttä-Riihinen *et al*., 2004; Mattila *et al*., 2006; Tappi, 2018).

Moreover, one of those acids (ellagic acid) is not so commonly find in foods. However, strawberries, and few other berry species, have a relevant quantity of this compound. Ellagic acid is a gallic acid dimer and is rarely present in its free form. More often, it occurs bound in ellagitannins, which belong to the phenolic group of hydrolysable tannins. Through acid hydrolysis, those complex compounds decay and ellagic acid is released (Koponen *et al.*, 2007; Tulipani *et al.*, 2008a; Nile and Park, 2014). The main characteristics of phenolic acids is their antioxidant and anti-inflammatory properties, but they can also exert other important activities, like anti-mutagenic, anti-carcinogenic and anti-allergy (Koponen *et al.*, 2007; Giampieri *et al.*, 2012, 2013).

The biosynthesis of phenolic acids is the same of anthocyanins, because natural phenols in plants are derived from shikimate pathways (Fig. 5). The polyphenolic substances derive from the biosynthetic pathway of shikimic acid and from the synthesis of the aromatic amino acids phenylalanine and tyrosine. Shikimic acid is the starting point for the synthesis of the chorismic acid, from which the aromatic amino acids derive (phenylalanine is the most important). From here, the phenylpropanoid pathway begins, through the production of cinnamic acid and para ceramic acid, precursor of all the following compounds. (Fig. 5).

Phenolic acids have great interest on plants life, especially gallic acid that contributes to plants defence mechanisms. It can act as feeding repellents to a great variety of animals. Phenolic acids have also other good defence and aid mechanism to help the plant in various stress conditions. Phenolic acids act specially against pathogen attacks, defending the plants from insects or pests; they can help the plant during and/or after wounding the edible parts of the plant. Here phenolic acids can interact lignin cells to re-build the leaves or other plant components. At least phenolic acids can interact on plants roots for the right and proper iron absorption (Tappi, 2018).

In human body, phenolic acids exert many other benefits that scientists and biologists have studied during last years. The top three phenolic acids that have been focused are: ellagic acid, chlorogenic acid and cinnamic acid. Main phenolic compounds produced by plants in many stressing conditions (Tappi, 2018). The ellagic acid has the capability to reduce the appearance of colon and oesophagus cancer disease, it inhibits the mutation of DNA and improves enzymes that linked cancerogenic compounds to excretion molecules. This acid is mainly present in strawberries and raspberries. Chlorogenic acid can regenerate vitamin E when it undergoes in oxidative form. Main sources of chlorogenic acid are: tomatoes, blueberries and grapes. Cinnamic acid is a powerful antibacterial, antifungal and pesticide compound. It is present mainly in cinnamon, where it confers the typical taste and flavour. Others phenolic acids present in fruit juices (such as blueberry and pomegranate) have the ability to reduce the adhesion capacity of some bacteria on teeth and on urinary tract. According to Baiano *et al.* (2010), phenolic acid may reduce the oxidation process of LDL cholesterol.

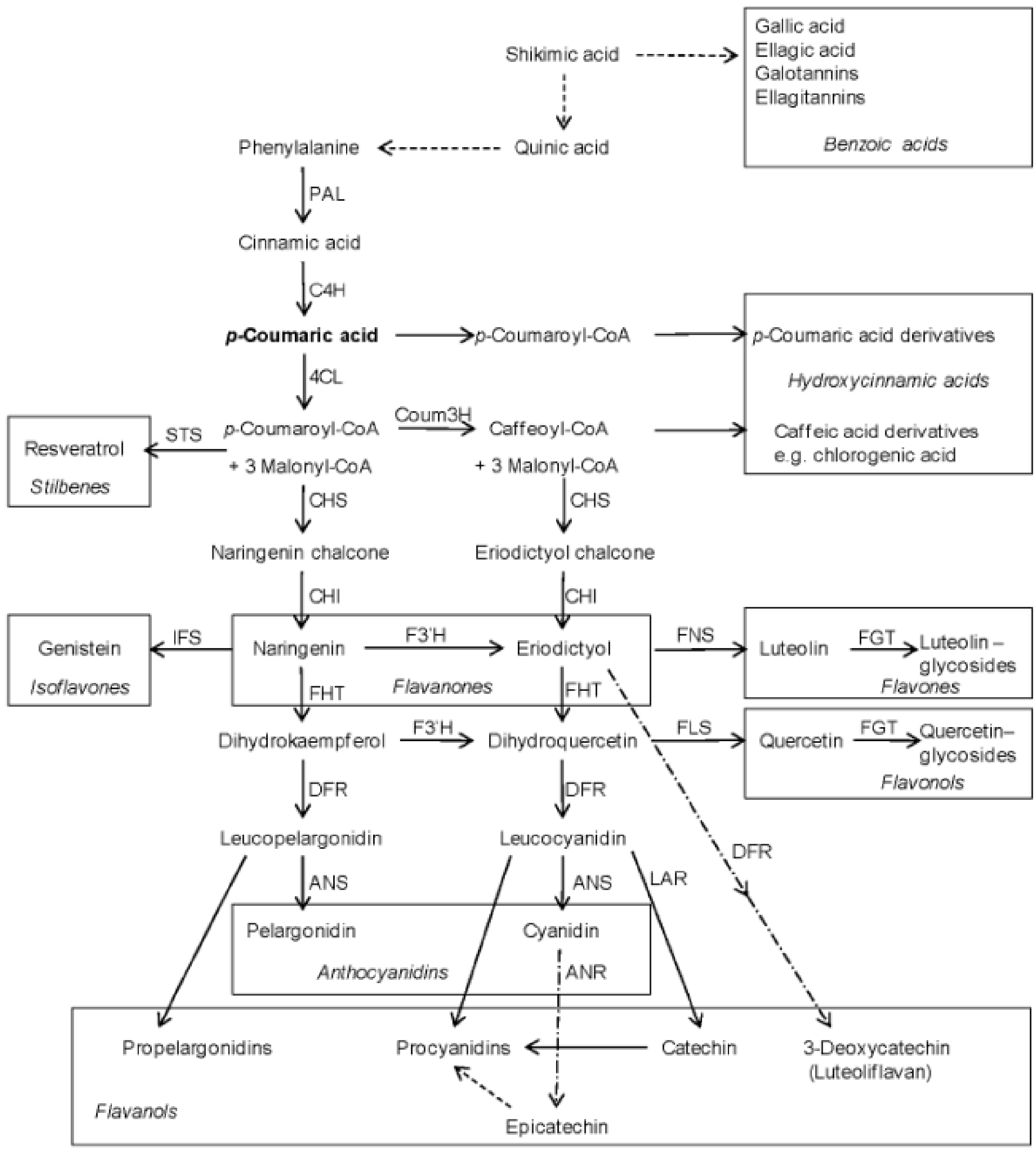

Figure 5: Biosynthetic pathways of the major phenolic compounds. Song et al. (2010).

2.9 Health Benefits of Strawberry Consumption

A considerable attention for health-promoting properties of fruits and vegetables arises because several studies could demonstrate a link between their consumption and a reduced risk of various chronic diseases (Diamanti *et al.* 2014; Tulipani *et al.* 2008a; Forbes-Hernandez *et al.* 2016). Biocative copmounds in strawberries also help to lessen the risk of cardiovascular incidents by inhibition of LDL-cholesterol oxidation or improved vascular endothelial function. This could reduce the risk of incidence of thrombosis (Basu *et al.*, 2010; Prasath *et al.*, 2014), It is known that some compounds present in strawberries, such as ellagic acid and quercetin (Edderkaoui *et al.*, 2013) have demonstrated anti-cancer activity in their purified forms or fractions, sometimes enriched with specific components. The preventative effect of berry fruits for human esophageal cancer is because of their potential to modify exposure of several genes relating to the progress of oral cancer (Chen *et al.*, 2012).

The protection from tumorigenesis upon pre-treatment with strawberry extracts was observed for breast cancer in mice too, but the mechanism by which it exerts the chemoprevention is still not clear. Protective effects of strawberry extracts on human dermal fibroblasts were also referred (Giampieri *et al.*, 2014, 2018) to (Wiseman *et al.*, 2002), strawberry (*Fragaria* × *ananassa*) is a relevant source of bioactive compounds because of its high levels of vitamin C, folate, and phenolic constituents. According to the available data (Tulipani *et al.*, 2008), the intake of dietary folate through strawberry consumption is interesting. For example, 250 g of strawberries (w60 mg of folate on average) can supply 30% of the daily European and U.S. folate recommended daily allowances. Moreover, the strawberry, although to a lesser extent, is a source of several other vitamins, such as thiamin, riboflavin, niacin, vitamin B6, vitamin K, vitamin A and vitamin E.

Low folate status has been associated with increased risk of cancers of the colon, cervix, lung, pancreas, prostate, mouth and pharynx, head and neck, stomach, and brain. According to Yang *et al.* (2012), there might be a potential benefit of stroke prevention with folic acid supplementation. Folic acid supplementation in patient with type 2 diabetes mellitus was seen to cause reduction in homocysteine levels and, therefore, contributed to better glycemic control (Title *et al.*, 2006). According to Imdad *et al.*, (2011), during the pre-conception period, risks of stillbirths secondary to neural tube defects (NTDs) were reduced with folic acid supplementation by approximately 41%.

CHAPTER 3: MATERIALS AND METHODS

3.1 Plant Material

The strawberry cultivars and new selections from the UPM-D3A breeding program, listed in Table 1, were planted in 2018 and 2019 in non-fumigated soil in "P. Rosati" experimental farm of Università Politecnica delle Marche, sited in Agugliano (Ancona, Italy), grown in open field conditions according to the plastic hill culture. Each cultivar and selection were planted in a single plot of six plants each, cultivated with the standard integrated pest management system, and harvested in May of the following year. Fruit samples were harvested at fully red stage, at the second, third and fourth seasonal pickings and stored at -20°C until analyses.

Table 2: List of the studied 2018-2019 strawberry genotypes

Selections		Cultivars
1. AN11,32,55	2. AN14,17,51	1. Romina
3. AN12,05,54	4. AN13,16,59	2. Sibilla
5. AN12,20,51	6. AN14,16,62	3. Janiss
7. AN12,23,58	8. AN13,13,62	4. Cristina
9. AN10,04,51	10. AN13,16,56	5. Laetitia
11. AN07,105,53	12. AN12,35,52	6. Francesca
13. AN12,23,66	14. AN14,20,51	7. Silvia
15. AN12,29,54	16. AN15,08,51	8. Lauretta
17. AN12,23,53	18. H106	9. Tea
19. AN12,24,52	20. H18	10. Monterey
21. AN12,13,58	22. H38	11. Galletta
23. AN12,20,53	24. AN14,27,62	12. Dina
25. AN06,164,52	26. AN14,21,62	
27. AN12,29,60	28. H41	
29. AN12,45,53	30. H81	
31. AN13,13,55	32. H33	
33. AN13,16,51	34. H71	
35. AN13,21,56	36. H107	
37. AN12,44,60	38. H2	
39. AN12,44,51	40. AN14,21,55	
41. AN14,12,58	42. H97	

Table 3: List of the studied 2019-2020 strawberry genotypes

Selections				Cultivars
1. AN00,239,55	2. AN12,51,56	3. AN15,08,51	4. AN17,07,52	1. Alba
5. AN06,164,52	6. AN13,13,55	7. AN15,09,57	8. AN17,07,53	2. Aurea
9. AN11,05,53	10. AN13,13,62	11. AN15,13,53	12. AN17,07,54	3. Cristina
13. AN11,05,58	14. AN13,15,57	15. AN15,19,55	16. AN17,07,55	4. Dina
17. AN12,05,54	18. AN13,16,57	19. AN16,04,52	20. AN17,12,51	5. Francesca
21. AN12,13,58	22. AN13,20,52	23. AN16,04,53	24. AN17,12,52	6. Janiss
25. AN12,20,51	26. AN13,20,58	27. AN16,22,52	28. AN17,12,53	7. Lauretta
29. AN12,20,53	30. AN13,44,52	31. AN16,22,53	32. AN17,12,54	8. Monterey
33. AN12,23,53	34. AN14,01,52	35. AN16,22,56	36. AN17,12,55	9. Romina
37. AN12,23,58	38. AN14,08,55	39. AN16,27,52	40. AN17,19,51	10. Scala
41. AN12,23,66	42. AN14,12,58	43. AN16,27,53	44. AN17,19,52	11. Sibilla
45. AN12,24,52	46. AN14,16,62	47. AN16,27,54	48. AN17,19,53	12. Silvia
49. AN12,29,54	50. AN14,20,51	51. AN16,32,51	52. AN17,19,54	13. Talia
53. AN12,29,60	54. AN14,21,55	55. AN16,32,53	56. AN17,19,55	
57. AN12,29,62	58. AN14,21,56	59. AN16,32,55	60. AN17,19,56	
61. AN12,44,51	62. AN14,21,62	63. AN16,37,51	64. AN17,19,57	
65. AN12,44,60	66. AN15,01,57	67. AN16,37,52	68. BL13,9,5 FB5	
69. AN12,45,53	70. AN15,01,60	71. AN16,37,53	72. FVG	
73. AN12,48,54	74. AN15,04,54	75. AN16,37,57	76. H71	
77. AN12,49,53	78. AN15,07,51	79. AN16,37,58	80. H97	
81. AN12,49,65	82. AN15,07,53	83. AN16,37,60		
84. AN12,50,52	85. AN15,07,57	86. AN17,07,51		

Fig. 4.0: Strawberry breeding fruit sample

3.2 Double Methanolic Extraction Method

Fruit phenolic fraction was extracted following the method of Diamanti *et al.* (2012). 10 g of frozen fruits were homogenized in 20 mL of methanol and shake for 30 min in the dark. The suspension was centrifuged at 4500 rpm for 10 min and the supernatant was collected, then the pellet of the fruit was extracted a second time by adding another 20 mL of methanol and repeating the procedure. The second supernatant was added to the first one and then stored at -20°C (Fig. 5).

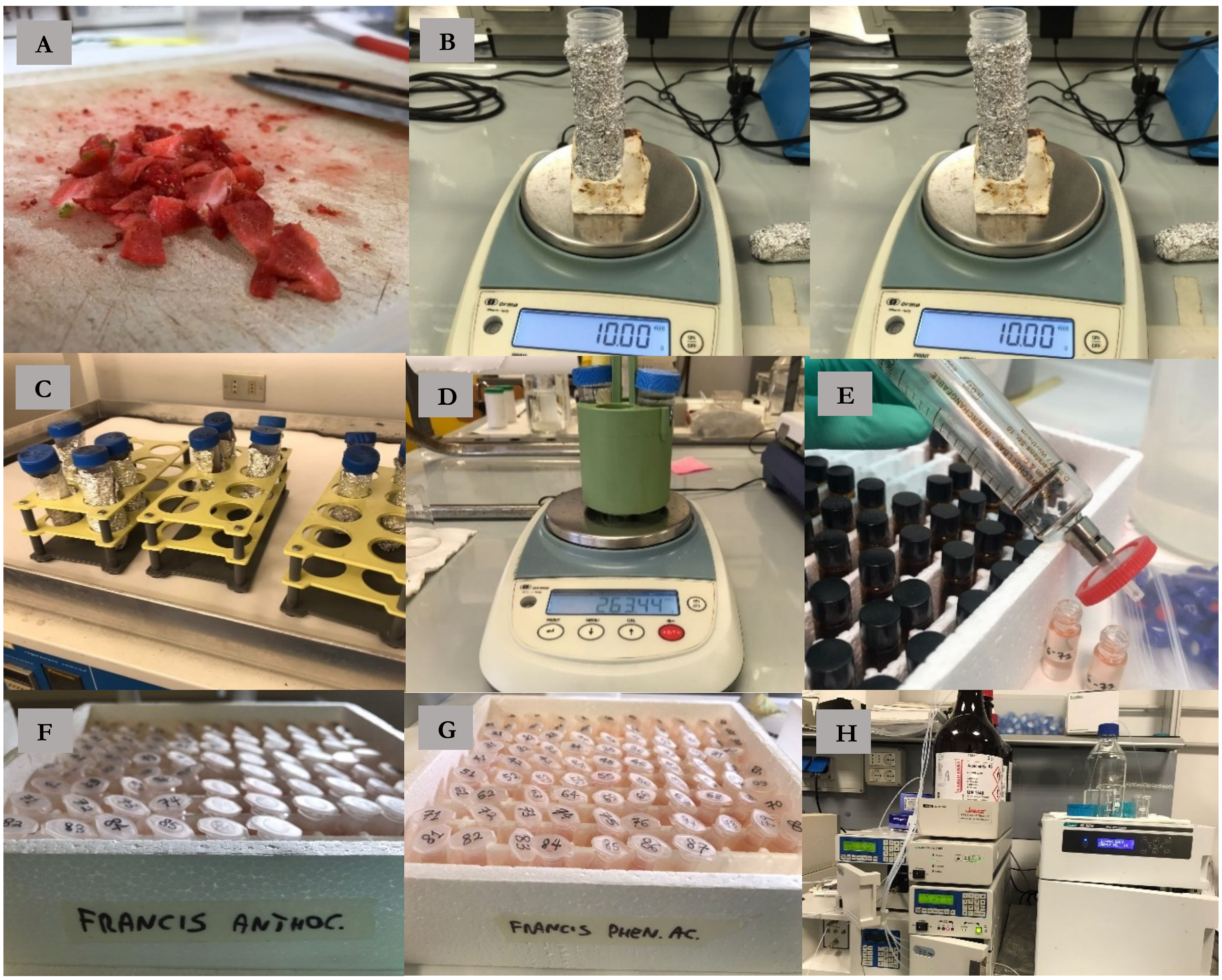

Fig. 5.0: A – sample cutting; B – sample weighing for anthocyanins and phenolic acid determination; C – sample agitation in warm room; D – sample centrifugation weight precheck; E – syringe filtration; F & G – filtered sample solutions for anthocyanins and phenolic acid determination, respectively; H – HPCL setup.

3.3 HPLC Determination of Anthocyanin Content

Anthocyanin content was analyzed following the method of Fredericks *et al.* (2013). The methanolic extract previously obtained was analyzed through HPLC-UV system consisting of a Jasco PU-2089 plus controller, and a Jasco UV-2070 plus ultraviolet (UV) detector (Jasco Easton, MD, USA). The compounds were separated on an Aqua Luna C18 (250×4.6 mm) reverse-phase column, with a particle size of 5 μm (Phenomenex, Lane Cove, NSW, Australia) protected by a Phenomenex 4.0×3.0 mm C18 ODS guard column. UV monitoring was performed at 520 nm. Anthocyanins were quantified using external standards of cyanidin-3-glucoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside, and were expressed as mg of total anthocyanins per 100 g of fresh weight (mg 100 g-1 FW).

3.4 HPLC Determination of Phenolic Acids Content

Phenolic acids were analyzed as previously described in Schieber *et al.* (2001) and Fredericks *et al.* (2013). The methanolic extract previously obtained was subjected to HPLC-UV analysis through an HPLC system (Jasco PU-2089 plus), and a Jasco UV-2070 plus ultraviolet (UV) detector (Jasco Easton, MD, USA). The UV detector was set at 320 nm, and the column used for the separation was an Aqua Luna C18 (250×4.6 mm) reverse-phase column, with a particle size of 5 μm (Phenomenex, Lane Cove, NSW, Australia), protected by a Phenomenex 4.0×3.0 mm C18 ODS guard column. The quantification of phenolic acid content was performed using external standard of chlorogenic acid, caffeic acid, and ellagic acid (EA). Values were expressed as mg of total phenolic acids per 100 g of fresh weight (mg 100 g-1 FW).

3.5 Statistical Analysis

Data from the above analyses were analyzed statistically using “Statistica 7” (Stasoft, Tibco Software, Palo Alto, California, USA). One-way analysis of variance (ANOVA) for mean comparison and intergenotype significant differences (calculated according to LSD test) was used. Differences at $p \leq 0.05$ were considered statistically significant.

CHAPTER 4: RESULTS AND DISCUSSION

From the results, it is possible to understand the variation of fruit anthocyanins and phenolic acids content of the strawberry cultivars and selections obtained from UPM-D3A breeding program. Recently, much attention is given to the antioxidant capacity of fruits as a qualitive parameter, closely related to phenolic compounds. Therefore, phenolic acids and anthocyanins play vital roles in controlling oxidative reactions in human body (Schijlen *et al*., 2006; Tulipani *et al*., 2008).

Phenolic Acids Content of 2019 Strawberry Fruit Selections

A significant number of the 2019 strawberry fruit selections had phenolic acids content above the average value of 102.91 mg/100g FW (Fig. 6). 'AN07,105,53' was the selection with the highest phenolic acids fruit content, followed by 'H106', 'AN13,16,56' and 'AN13,16,51'. Many studies have confirmed many antiproliferative, cardiovascular and neurologic benefits associated with phenolic acids, hence consuming strawberry fruits with high levels of this compound will potentially have a great impact on human health (Giampieri *et al*., 2012). 'AN14,17,51' had the least phenolic acids content, below 50.00 mg/ 100g FW. Ellagic acid was the predominant phenolic acid among all the selections, in sync with Jakobek *et al*. (2012) and Kähkönen *et al*. (2001). Statistically, there was significant variation in the phenolic acids content of the 2019 strawberry fruit selections.

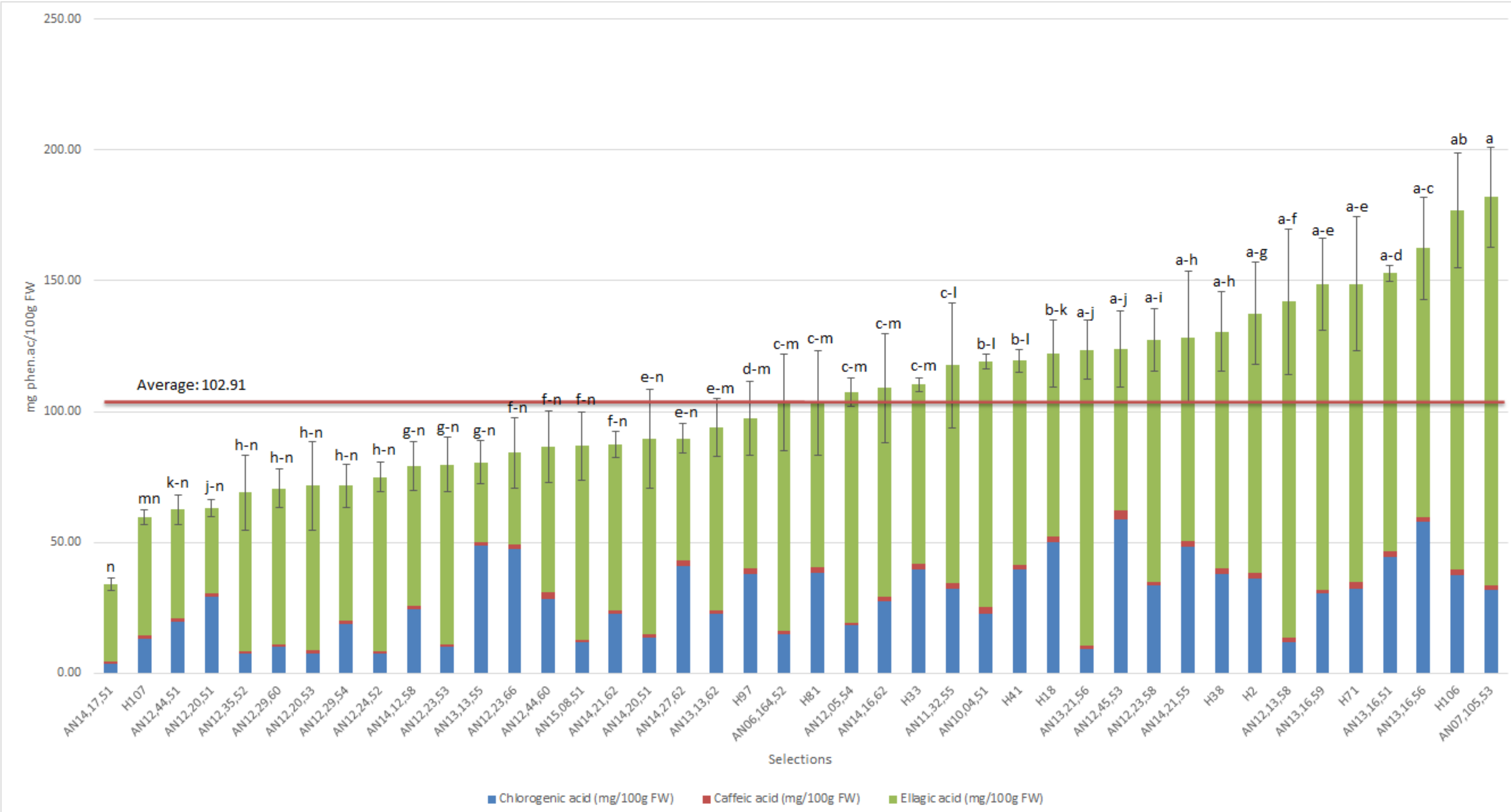

Figure 6: Phenolic acids content of 2019 strawberry fruit selections. Data are the means of triplicate values ± standard error. Different letters indicate statistical difference (LSD test, p≤0.05). FW = Fresh weight.

Phenolic Acids Content of 2019 Strawberry Fruit Cultivars

All the 2019 strawberry cultivars had phenolic acids content exceeding 50.00 mg/100g FW, with an average of 106.63 mg/100g FW (Fig. 7). Thus, compared to the 2019 strawberry selections, the 2019 strawberry cultivars had higher average phenolics acids fruit content. Among the 2019 strawberry cultivars, 'Monterey' had the highest phenolic acids content (146.55 mg/100g FW), outstandingly higher than the average value and similar to the results obtained by Dunja *et al.* (2016). With such high phenolic acid content, 'Monterey' cultivar would be vital to human diet with several health benefits to consumers besides antioxidant activity. According to Tsao and Deng (2004), phenolic acids possess much higher *in vitro* antioxidant activity than well-known antioxidant vitamins.

Next to 'Monterey', the 2019 strawberry cultivars with interesting phenolics acid content were 'Tea', 'Francesca', 'Lauretta' (both from the UNIVPM breeding program), 'Sibilla', 'Laetitia', 'Galletta' and 'Janiss'. These cultivars also had similar phenolic acids contents. Unfortunately, 'Romina', 'Dina', 'Cristina' and 'Silvia', the other four cultivars deriving from the UNIVPM breeding program, were the cultivars with the least phenolic acids content, also having little variation in values. Just like the 2019 strawberry selections, ellagic acid was the predominant phenolic acid among all the selections, followed by chlorogenic acid. Notably, the contents of caffeic acid throughout the strawberry selections and cultivars were the least.

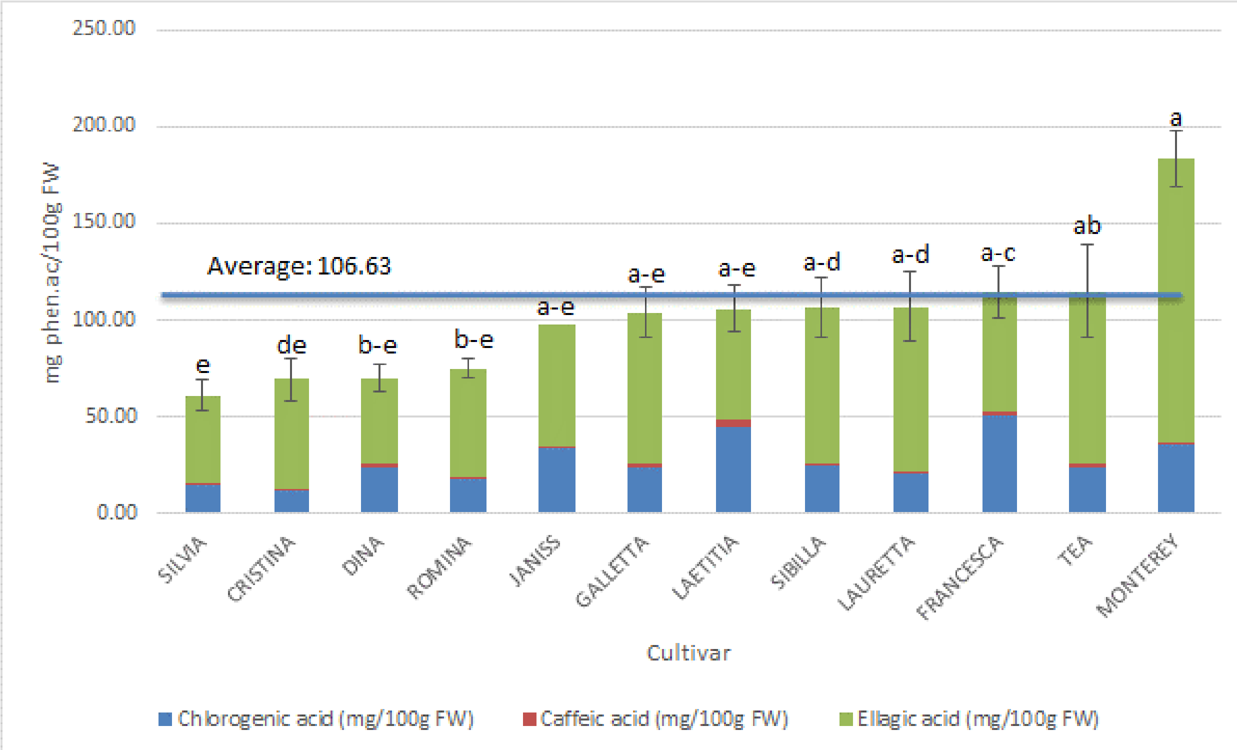

Figure 7: Phenolic acids content of 2019 strawberry fruit cultivars. Data are the means of triplicate values ± standard error. Different letters indicate statistical difference (LSD test, $p \leq 0.05$). FW = Fresh weight.

Anthocyanins Content of 2019 Strawberry Fruit Selections

A wide range of results was obtained for the anthocyanin content of the 2019 strawberry selections (Fig. 8). Far exceeding the average value of 17.66 mg/ 100g FW, 'AN13,13,55' 'AN12,23,58' and 'AN13,16,59' were the strawberry selections with the highest anthocyanins fruit content (29.34, 28.44 and 26.00 mg/ 100g FW, respectively). It is well established that anthocyanins mediate various biological effects; they possess antioxidant, anti-inflammatory and DNA protective properties associated with health benefits (Williamson, 2017). Over the years, strawberries have been bred for higher anthocyanin content which is a major contributor to the characteristic colour and nutritional value of the ripe fruit (Fredericks, 2013). Thus, the selections with high amounts of anthocyanins would be considered superior in nutritional quality with great health benefits upon consumption. With an anthocyanin content of 8.37, 10.04 and 10.46 mg/ 100g FW, 'AN12,35,52', 'AN14,17,51' and 'AN11,32,55' were the selections with the least amounts. Pelargonidin-3-glucoside was the predominant anthocyanin compound among all the selections. Generally, just like the phenolic acids contents, there were significant variations in the anthocyanins contents of the 2019 strawberry fruit selections. Albeit, the average phenolic acids content of the 2019 strawberry fruit selections (102.91 mg/ 100g FW) was far higher than that of the average anthocyanin content (17.66 mg/ 100g).

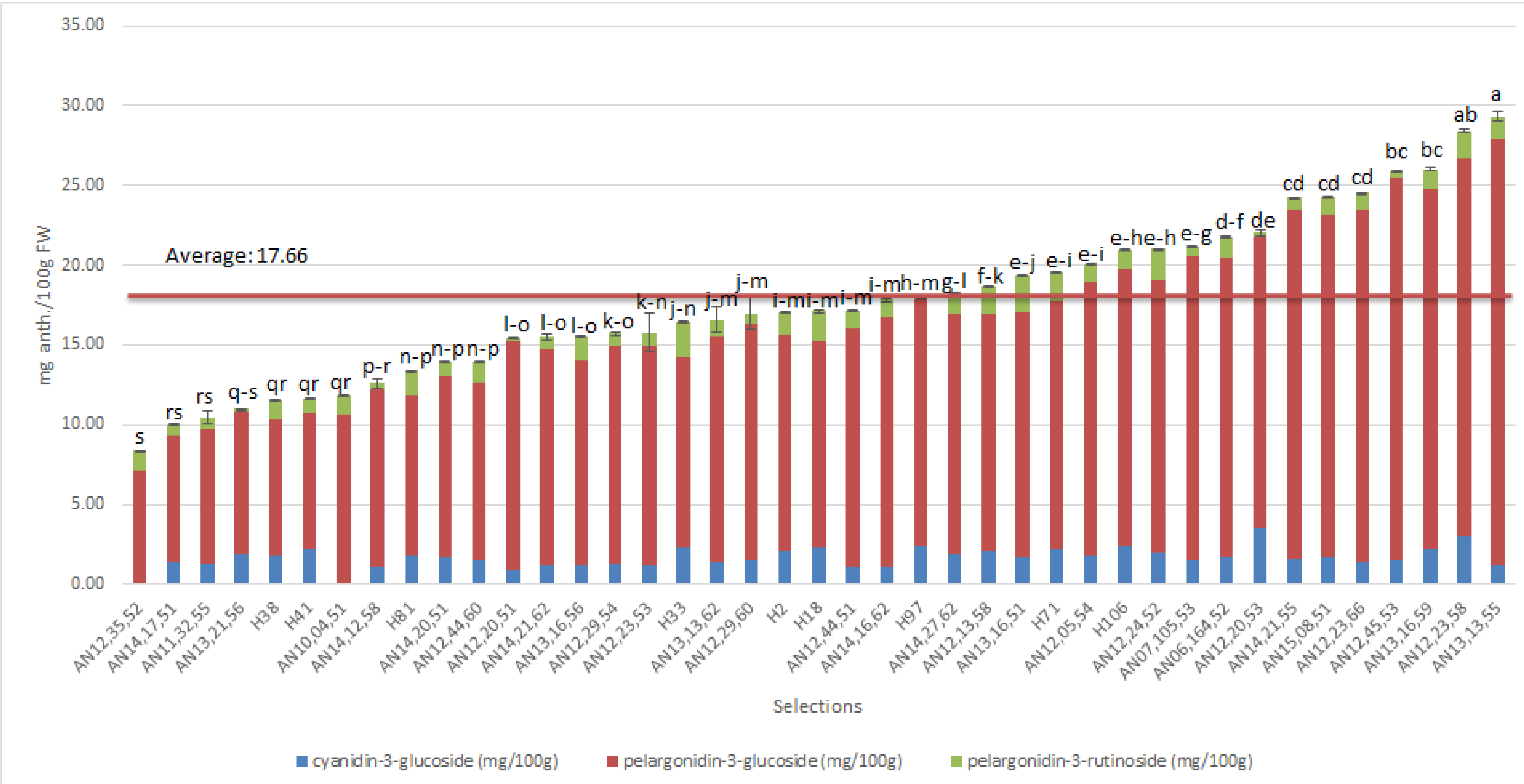

Figure 8: Anthocyanins content of 2019 strawberry fruit selections. Data are the means of triplicate values ± standard error. Different letters indicate statistical difference (LSD test, p≤0.05). FW = Fresh weight.

Anthocyanins Content of 2019 Strawberry Fruit Cultivars

As shown in Fig. 9, all the 2019 strawberry cultivars had anthocyanins content above 10.00 mg/100g FW, with an average of 16.77 mg/100g FW. Thus, compared to the 2019 strawberry selections, the 2019 strawberry cultivars had relatively lower average anthocyanins content. To explain this observation, selections have some wild germplasm which usually transfer vital traits such as high anthocyanins composition with an associated dark red colour (Longhi *et al.*, 2014). Among the 2019 strawberry fruit cultivars, 'Galletta', 'Monterey' and 'Romina' (this one from UNIVPM breeding program) had the highest anthocyanins content (20.42, 20.15 and 19.78 mg/100g FW, respectively). With such high anthocyanin content, 'Galletta', 'Monterey' and 'Romina' cultivars would have a deeper characteristic colour and associated health benefits to consumers due to the potentially higher antioxidant activity.

'Francesca', 'Laetitia', 'Cristina' and 'Janiss' were the cultivars with the least anthocyanins fruit content. Just like the 2019 strawberry selections, pelargonidin-3-glucoside was the predominant anthocyanin compound among all the 2019 strawberry cultivars. Notably, the contents of pelargonidin-3-rutinoside in all 2019 strawberry selections and cultivars were the least. Compared to the 2019 strawberry selections, the cultivars had less variations in the anthocyanins contents.

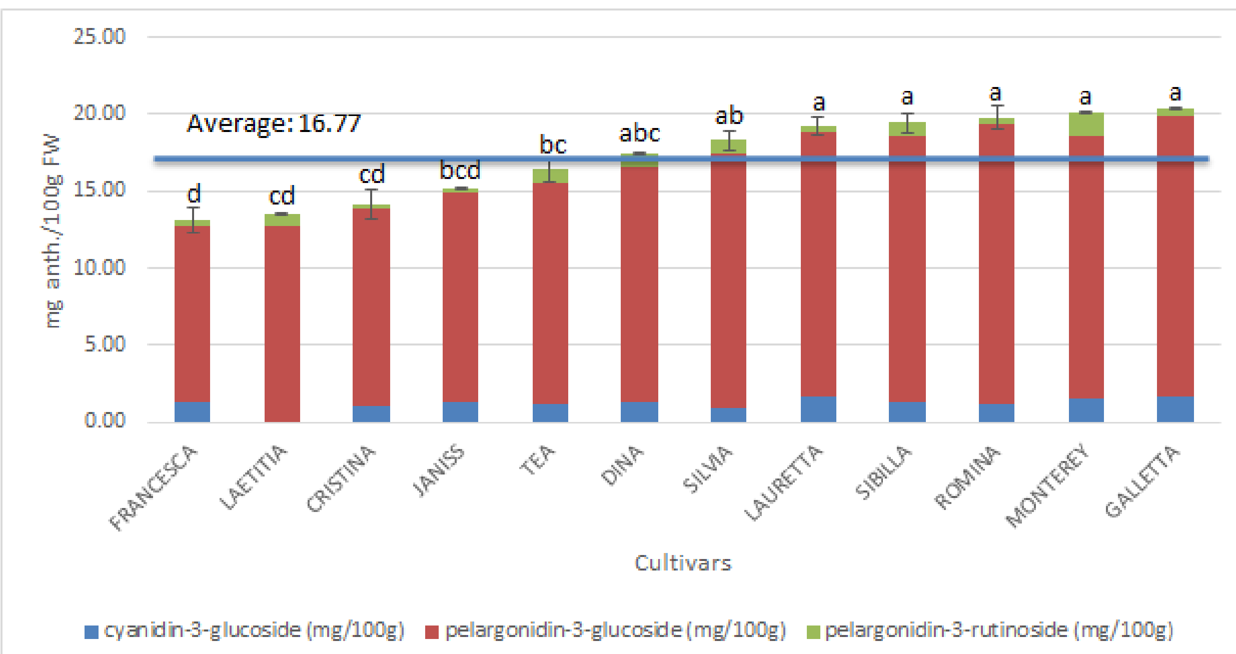

Figure 9: Anthocyanins content of 2019 strawberry fruit cultivars. Data are the means of triplicate values ± standard error. Different letters indicate statistical difference (LSD test, $p \leq 0.05$). FW = Fresh weight.

Phenolic Acids Content of 2020 Strawberry Fruit Selections

The phenolic acids content of the 2020 strawberry fruit selections had a significant variation of values, ranging from as low as 22.60 to as high as 243.33 mg/ 100g FW (Fig. 10). Quite a number of the 2020 strawberry fruit selections had phenolic acids content above the average value of 65.19 mg/100g FW. Far exceeding the average value, 'FVG' was the selection with the highest phenolic acids content (243.33 mg/100g FW). This was followed by 'AN13,20,53', 'AN13,20,58', 'AN16,32,55' and 'AN12,29,62' with phenolic acids contents of 168.37, 129.73, 110.03 and 108.21 mg/100g FW, respectively. 'BL13,9,5 FB5' (Bianca), 'AN16,37,52' and 'AN16,37,51' had the least phenolic acids contents, far below 50.00 mg/ 100g FW. Just like the 2019 strawberry selections and cultivars, ellagic acid was the predominant phenolic acid among all the 2020 selections. Similarly, caffeic acid was the phenolic acids with the least content in the 2020 strawberry selections. Despite the average phenolic acids content of the 2020 strawberry selections being lower than that of the 2019 strawberry selections (102.91 mg/100g FW), the selection with highest content for 2019 had relatively lower value compared to that of the highest 2020 strawberry selection. This result could be linked to the fact that the genotype with the highest phenolic acid fruit content in 2020 was the wild germplasm FVG, and it is known that wild germplasm is a very rich source of phenolic antioxidant compounds (Diamanti *et al*., 2012). Almost all the strawberry selections harvested in both 2019 and 2020 seasons had relatively higher phenolic acids contents in the 2019 season compared to the 2020 season ('AN06,164,52', 'AN12,05,54', 'AN12,13,58', 'AN12,20,53', 'AN12,23,53', 'AN12,23,58', 'AN12,23,66', 'AN12,24,52', 'AN12,29,54', 'AN12,29,60', 'AN12,44,60', 'AN12,45,53', 'AN13,13,55', 'AN13,13,62', 'AN14,12,58', 'AN14,16,62', 'AN14,20,51', 'AN14,21,55', 'AN14,21,62', 'AN15,08,51' and 'H97'). However, 'AN12,20,51' and 'AN12,44,51' were the only selections which had relatively lower phenolic acids contents in the 2019 season compared to the 2020 season.

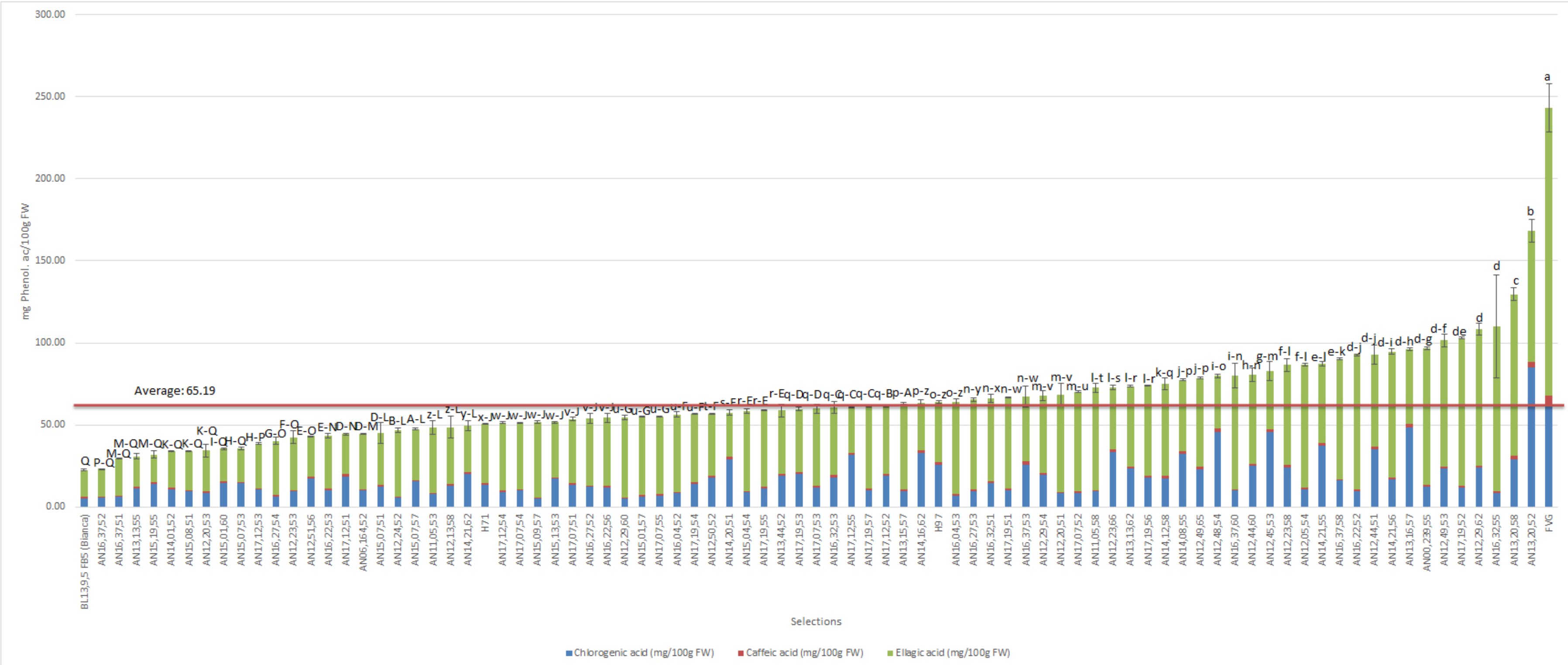

Figure 10: Phenolic acids content of 2020 strawberry fruit selections. Data are the means of triplicate values ± standard error. Different letters indicate statistical difference (LSD test, p≤0.05). FW = Fresh weight.

Phenolic Acids Content of 2020 Strawberry Fruit Cultivars

Evident in Fig. 11, all the 2020 strawberry cultivars had phenolic acids content exceeding 30.00 mg/100g FW. The average phenolic acids content of the 2020 strawberry cultivar was 57.94 mg/100g FW, lower than that of the 2019 strawberry selections being 106.63 mg/100g FW. Besides 'FVG', 'AN13,20,52' and 'AN13,20,58' were the selections which had phenolic acids contents double the value of that of the cultivars. Also, compared to the 2020 strawberry selections, the 2020 strawberry cultivars had lower average phenolics acids content. Among the 2020 strawberry cultivars, 'Alba' had the highest phenolic acids content (75.61 mg/100g FW). Next to 'Alba', the 2020 strawberry cultivars with interesting phenolics acid content were 'Francesca' (from UNIVPM breeding program), 'Aurea' and 'Janiss'. Unfortunately, 'Silvia', 'Romina', and 'Cristina' (all from UNIVPM breeding program) were the cultivars with the least phenolic acids fruit content. These cultivars were also least performing in phenolic acids content for the 2019 strawberry cultivars. Compared to the 2019 strawberry cultivars, the 2020 strawberry cultivars had higher variation of phenolic acids contents. Just like the 2019 strawberry genotypes and 2020 selections, ellagic acid was the predominant phenolic acid among all the 2020 cultivars, followed by chlorogenic acid. Similarly, the contents of caffeic acid in the 2020 strawberry cultivars were the least.

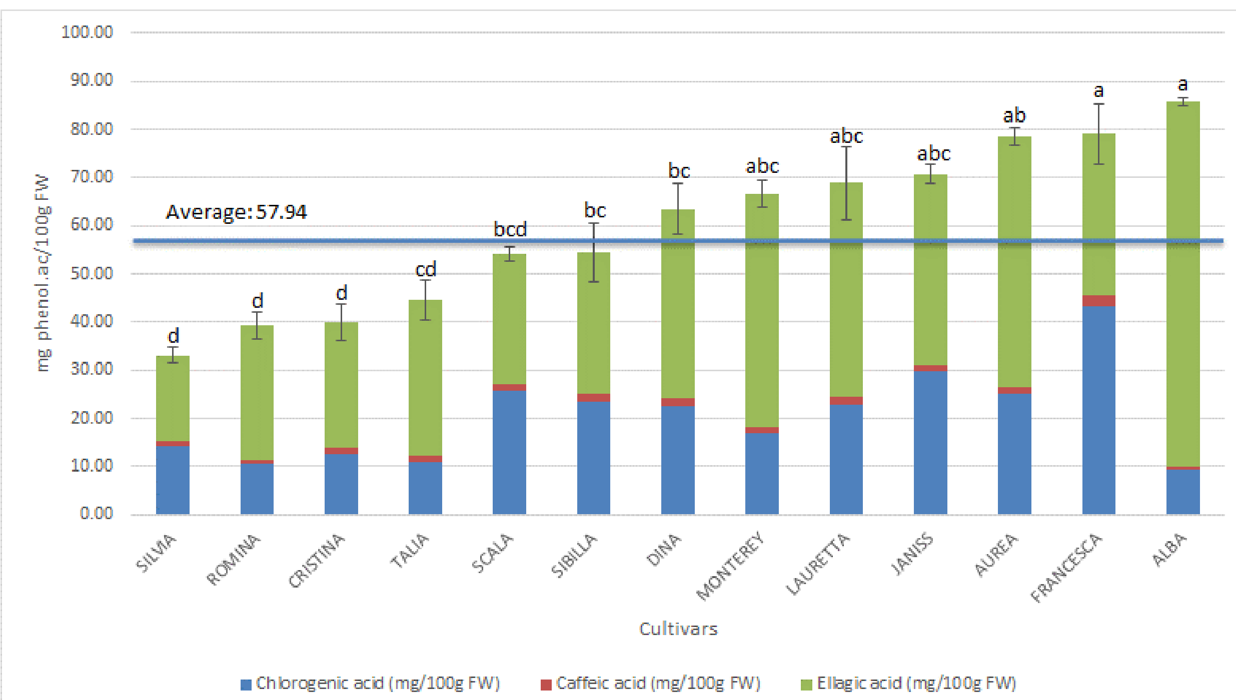

Figure 11: Phenolic acids content of 2020 strawberry fruit cultivars. Data are the means of triplicate values ± standard error. Different letters indicate statistical difference (LSD test, p≤0.05). FW = Fresh weight.

Anthocyanins Content of 2020 Strawberry Fruit Selections

A significant variation of results was obtained for the anthocyanin content of the 2020 strawberry selections (Fig. 12). Far exceeding the average value of 20.24 mg/ 100g FW, 'AN13,44,52' was the strawberry selections with the highest anthocyanins content (63.48 mg/ 100g FW). This was followed by 'AN12,49,65', 'AN17,07,55' and 'AN12,49,53'. With an anthocyanin content below 10.00 mg/ 100g 'AN16,22,52', 'AN16,22,53', 'BL13,9,5 FB5' (Bianca), 'AN16,22,56' and 'AN16,04,52' were the selections with the least amounts. The average anthocyanin content of the 2020 strawberry fruit selections (20.24 mg/ 100g FW) was higher than that of the average anthocyanin content of the 2019 selections (17.66 mg/ 100g). Similar to the 2019 strawberry selections, pelargonidin-3-glucoside was the predominant anthocyanin compound among all the 2020 selections. As observed with the 2019 strawberry selections and cultivars, the phenolics acids content of the 2020 selections was higher than the anthocyanin content of the 2020 selections.

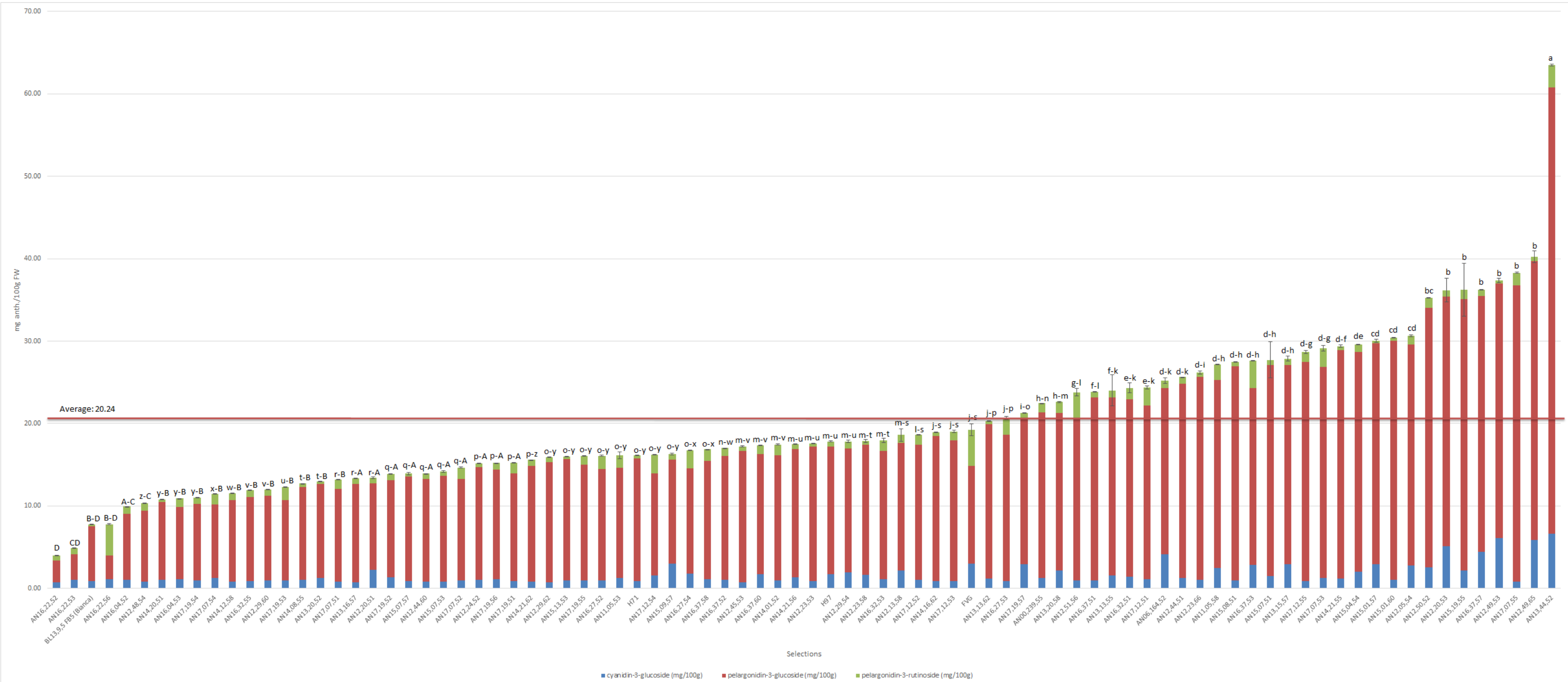

Figure 12: Anthocyanins content of 2020 strawberry fruit selections. Data are the means of triplicate values ± standard error. Different letters indicate statistical difference (LSD test, p≤0.05). FW = Fresh weight.

Anthocyanins Content of 2020 Strawberry Fruit Cultivars

Majority of the 2020 strawberry cultivars had anthocyanins content above 15.00 mg/100g FW, with an average of 20.52 mg/100g FW (Fig. 13). Thus, compared to the 2020 strawberry selections, the 2020 strawberry cultivars had slightly lower average anthocyanins content. The exact trend was observed between the 2019 strawberry selections and cultivars. Among the 2020 strawberry cultivars, 'Monterey' had the highest anthocyanins content (37.05 mg/100g FW). The other 2020 strawberry cultivars with relatively higher anthocyanins contents were 'Scala', 'Lauretta' and 'Dina' (both from UNIVPM breeding program). However, the best 2020 strawberry selections (AN13,44,52, 'AN12,49,65', 'AN17,07,55' and 'AN12,49,53') had double amount of anthocyanins compared to the best 2020 strawberry cultivars. 'Talia', 'Janiss', 'Francesca' and 'Alba' were the cultivars with the least anthocyanins content. As observed with the 2019 strawberry selections and cultivars, the phenolics acids content of the 2020 cultivars was higher than the anthocyanin content of the 2020 cultivar. Similarly, pelargonidin-3-glucoside was the predominant anthocyanin compound among all the 2020 cultivars. However, unlike the 2019 strawberry cultivars which showed less variation in results, the anthocyanin content of the 2020 cultivars had more variation. This indicates the wide diversity of genotypes under study.

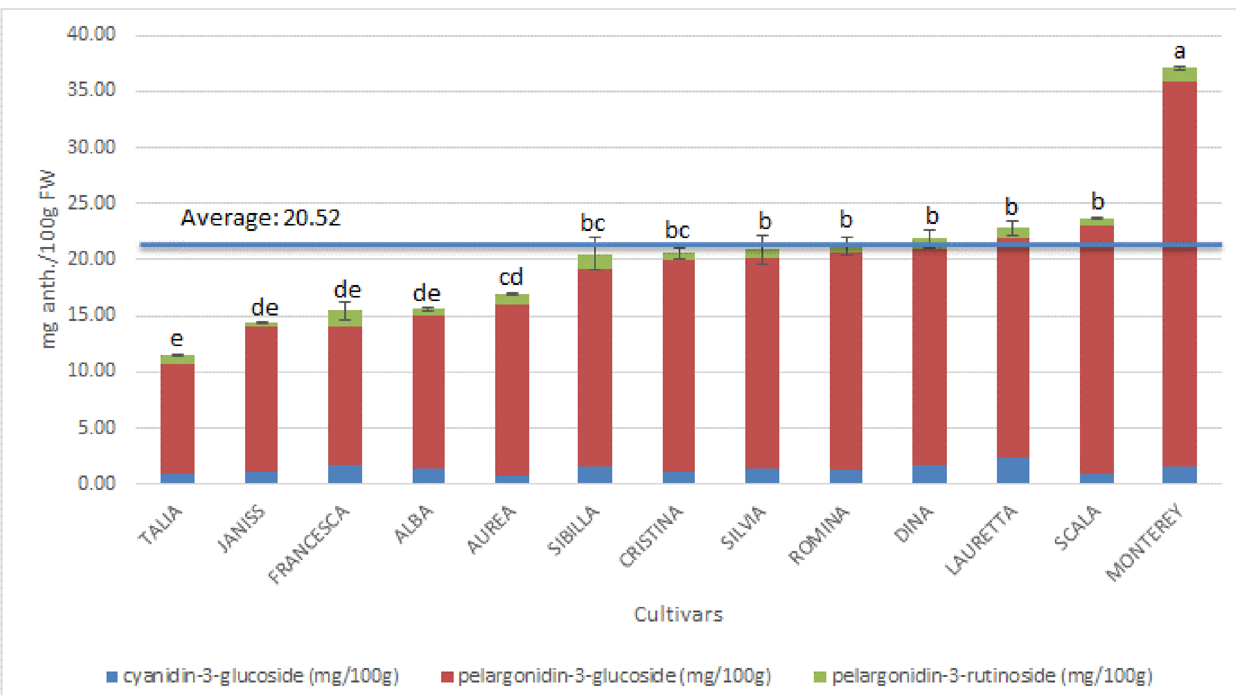

Figure 13: Anthocyanins content of 2020 strawberry fruit cultivars. Data are the means of triplicate values ± standard error. Different letters indicate statistical difference (LSD test, p≤0.05). FW = Fresh weight.

Table 4: Strawberry Genotypes with the Highest Phenolic Acids Content

2019		2020	
Best Selections (mg/100g FW)	**Best Cultivars (mg/100g FW)**	**Best Selections (mg/100g FW)**	**Best Cultivars (mg/100g FW)**
AN07,105,53 (181.94)	Monterey (183.57)	FVG (243.33)	Alba (85.75)
H106 (176.86)	Tea (114.88)	AN13,20,52 (168.37)	Francesca (79.06)
AN13,16,56 (162.45)	Francesca (114.52)	AN13,20,58 (129.73)	Aurea (78.57)

Table 5: Strawberry Genotypes with the Highest Anthocyanins Content

2019		2020	
Best Selections (mg/100g FW)	**Best Cultivars (mg/100g FW)**	**Best Selections (mg/100g FW)**	**Best Cultivars (mg/100g FW)**
AN13,13,55 (29.34)	Galletta (20.42)	AN13,44,52 (63.48)	Monterey (37.05)
AN12,23,58 (28.44)	Monterey (20.15)	AN12,49,65 (40.22)	Scala (23.65)
AN13,16,59 (26.00)	Romina (19.78)	AN17,07,55 (38.30)	Lauretta (22.81)

Amongst the best performing strawberry genotypes, 'Francesca' consistently proved to have the utmost quality in terms of phenolics acids content for 2019 and 2020 harvesting years (Table 4). Except for 'AN07,105,53', all the best strawberry selections for both 2019 and 2020 seasons had higher phenolic acids contents than the best cultivars. In terms of anthocyanins content, 'Monterey' was the genotype consistently showing interesting results for 2019 and 2020 harvesting years (Table 5). However, all the best strawberry selections for both 2019 and 2020 seasons had higher anthocyanins contents than the best strawberry cultivars. Thus, as a general trend, the best selections proved to be better than the best cultivars. Overall, 'Monterey' proved to be the best performing genotype in terms of both anthocyanins and phenolic acids contents for 2019 and 2020 harvesting years.

CHAPTER 5: CONCLUSION

Generally, the average phenolic acids contents of both 2019 and 2020 genotypes were higher than the anthocyanin content. Furthermore, the 2019 strawberry genotypes proved to have higher phenolic acids and anthocyanins content than the 2020 strawberry genotypes. The average phenolic acids content for 2019 strawberry selections and cultivars, as well as 2020 selections and cultivars were: 102.91, 106.63, 65.19 and 57.94 mg/ 100g FW, respectively. The average anthocyanins content for 2019 strawberry selections and cultivars, as well as 2020 selections and cultivars were: 17.66, 16.77, 20.24 and 20.52 mg/ 100g FW, respectively. The predominant phenolic acid and anthocyanin for the cultivars and selections of both harvesting years were pelargonidin-3-glucoside and ellagic acid, respectively. As expected, the variations between the phenolics acids and anthocyanins contents of the selections were generally higher than the cultivars, which tend to conform to market standards and consumer preference.

Amongst the best performing strawberry genotypes, 'Francesca' consistently proved to have superior quality in terms of phenolics acids content for 2019 and 2020 harvesting years. 'AN07,105,53', 'FVG', 'AN13,13,55', 'AN13,44,52' were the best selections, better than the best-performing cultivars. Overall, 'Monterey' proved to be the best performing genotype in terms of both anthocyanins and phenolic acids contents for 2019 and 2020 harvesting years. Therefore, 'Monterey' and 'Francesca' could be used as parents for further breeding programs to increase the nutritional values. Also, FVG, the wild germplasm which resulted high in phenolic acid fruit content, is confirmed as an important genetic source for increasing fruit pa content (Diamante *et al.*, 2012) This selection could be of interest as parent for the improvement of the nutritional quality. It is noted that the breeding program was successful to create new genotype with increased phenolic acids and anthocyanin contents: 'AN07,105,53', 'AN13,20,52', 'AN13,16,56', 'AN13,20,58', 'AN13,13,55', 'AN12,23,58', 'AN13,16,59', 'AN13,44,52', 'AN12,49,65' and 'AN17,07,55'. If these selections confirm their values also from productive and sensorial parameters, they could be good candidates as new cultivars. The release of a new cultivar is not only an ending point but also a starting point for further breeding programs for increasing the nutritional quality. Their potential can be better evaluated by analyzing also their data related to yield and fruit sensorial parameters.

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