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Variation of Fruit Vitamin C and Vitamin B9 content in Advanced Strawberry Breeding Selections

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DEDICATION

This research is dedicated sincerely to my beloved parents Mr. Isaac Boateng and Mrs. Jackline Boateng and my dear two brothers Clinton Boateng and Clinsman Boateng

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I am much grateful that the good Lord has been so wonderful to me, guided me, given me strength, knowledge and insight throughout my research work. I would like to offer sincere gratitude and appreciation to my supervisors; Prof Bruno Mezzetti and Dr. Luca Mazzoni, Dr Francesca, Dr Silvia from Department of Agricultural, Food and Environmental Science and special friends; Francis Aheto and Boakye-Yiadom Kofi Armah and any other person who contributed to the successful completion of my project work through their enthusiasm, encouragement, lessons, inspiration, support and patience they shown towards me. All that I want from God to you all is his everlasting blessings, strength and renewal of energy you lost. Amen.

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 recent aim of strawberry breeding is to produce new cultivars combining high plant adaptability and yield with high sensorial, nutritional and antioxidant properties of the fruit. The main aim of this present study was to ascertain the variation of vitamin C and vitamin B9 content in well-defined strawberry cultivars and in advanced strawberry breeding selections. In this study, (12 commercial cultivars and 42 advanced selections) and (13 cultivars and 86 advanced selections) were from 2019 and 2020 strawberry samples for vitamin C study, whereas for folate study there were 12 cultivars and 42 selections in the 2019 strawberry samples. These genotypes studied from the UNIVPM-D3A have been deeply ascertained for their nutritional quality.

HPLC-UV-FD was used to assess the concentration of the vitamin B9 (folate) and vitamin C content. The results revealed high variability in vitamin C and vitamin B9 among cultivars and breeding materials. Generally, there were significant differences (P> 0.05) among the cultivars and the selections for both years and among different years. The cultivars namely "Cristina, Aurea, and Alba'' for the 2020 strawberry sample had high variability of vitamin C**.** Also, the best new selections for vitamin C from both years combined were "AN,13,20,52, "AN,17,12,55" and "AN,13,15,57". Also, the best selections for vitamin B9 for the 2019 strawberry sample were; "AN,12,29,54", "AN,13,16,56" and "AN,12,13,58". Based on the results, there is the possibility to generate new potential cultivars with high contents of folate and vitamin C on the commercial market.

Keywords: vitamin C, vitamin B9 (folate), cultivars, selections, strawberry, breeding, HPLC-UV-FD.

Contents

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

MPA- Meta-phosphoric acid

DTPA- Diethylenetriaminepentaacetic acid

HPLC- High Pressure Liquid Chromatography

KH_2PO- Potassium phosphate

KH2PO4- Potassium Phosphate monobasic

K2HPO4- Potassium phosphate dibasic.

BAC- Bioactive Compounds

RDA- Recommended Daily Allowance

LDL- Low Density Lipoprotein

HCA-Hydroxycinnamic acid

HBA- Hydroxybenzoic acid

TAC- Total Antioxidant Capacity

NQ- Nutritional Quality

PUFA- Polyunsaturated Fatty Acid

DHFR- Dihydrofolate reductase

THF- Tetrahydrofolate

CVD-Cardiovascular disease

UNIVPM- Università Politecnica delle Marche

RDI- Recommended Daily Intake

CHAPTER 1

1.0 INTRODUCTION AND AIM OF THE THESIS

1.1 Background of the study

Strawberries (*Fragaria* × *ananassa* Duch.) are the most popular, cultivated and consumed berry fruit worldwide among the small fruits, having an annual fruit production exceeding 8.3 million metric tons (MT), cultivated on a surface of 372,361 ha (Mazzoni *et al*., 2021). They are regarded as an important fruit and the most studied berry from genomic, agronomic and nutritional point of view, due to their economic and commercial impact (Šamec *et al*., 2016). Strawberries have been part of human diet for centuries and constitute one of the main and rich dietary sources of bioactive compounds (BAC) such as vitamin C and vitamin B9, phenolic compounds (flavonoids, anthocyanins and phenolic acid) (Mezzetti *et al*., 2021). Additionally, strawberries contain other vitamins which are also regarded as major nutritional relevance and they include vitamin E, A, K, niacin, thiamin and carotenoids etc. The amount of antioxidant compounds found in strawberries correlate with the total antioxidant capacity (TAC), which is responsible for measuring the radical scavenging activity, that indicates a good fruit quality parameter (Capocasa *et al*., 2016). Moreover, all these compounds have impact on the human health by preventing various disease such as diabetes, cardiovascular disease (CVD), inflammation, obesity, neurological disorders, metabolic syndrome (Giampieri *et al*., 2015). The importance of strawberry is not only to improve the health of consumers but also its production can provide employment opportunities for those in the rural areas, thus contributing to the rural economies (Mezzetti *et* al., 2016).

Furthermore, the content of beneficial compounds in strawberry depends on several factors including the genotype, cultivation methods and techniques, environmental factors, pre- and post-harvest factors, maturity stage (Šamec *et al*., 2015). In fact, a cautious consumer decision to purchase and consume a particular fruit is based on the physical characteristics (color, shape, size and texture) and chemical composition (sugar contents, organic compounds, acidity, volatile compounds, macro and microelements, bioactive compounds). However, these characteristics could be compromised through degradation during prolonged storage. Thus, for these reasons, major breeding programs have been introduced in the last decade to solve the canker. More so, breeding programs for commercial strawberries are not only geared towards the release of new cultivars with increased resistance to pest and disease but also with improved nutritional and sensorial qualities (Mezzetti *et al*., 2018).

Breeding and biotechnological approaches are currently used to increase the content of specific bioactive components of plants. In fact, a successful breeding program approach is dependent on the heritability and variability of interesting bioactive compounds from parents to progenies, as cited in the study of Mazzoni et al. (2021). Moreover, according to the study of Capocasa et al. (2008), the availability of genetic diversity within compatible species of any given crop will enhance improvement. To extend this improvement, biotechnological approach is now an integrative option. However, in this approach, one must have knowledge in the molecular tools needed to modify specific biosynthetic pathways that will help promote and increase several metabolites (Penna, 2001). Notwithstanding, there are still limitation in the use of the biotechnological approach with regards to commercial exploitation of new products by public concern and biosafety rules. However, in order to succeed in using both approaches, the knowledge of the most useful wild and cultivated genetic diversity must be known. Recently, traditional breeding programs is the possible way to improve the nutritional and phytochemical composition of fruit, thus, is needful to accurately describe the genetic resources used in cross combination. In a breeding program, it is important to include wild species with a genetic background that is capable of producing progenies with elevated levels of phytochemicals (Diamanti *et al*., 2012).

1.2 AIM OF THE THESIS

In this study, there are 12 commercial cultivars, and 42 advanced selections also, 13 commercial cultivars and 86 advanced selections from the UNIVPM-D3A breeding program that have been evaluated for their nutritional quality for two consecutive years, from 2019 to 2020.
The main aim of this study was to ascertain the variation of vitamin C and vitamin B9 content in well-defined strawberry cultivars and in advanced strawberry breeding selections.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Overview of strawberry and health associated benefits

Strawberry (*Fragaria × ananassa Duch*.) is one of the valuable berries that plays essential role in human diet due to their BAC, nutritional properties, sensorial properties and chemical composition (Skrovankova *et al*., 2015). It belongs to the family *Rosaceae* and is of the most highly consumable berries. There have been a lot of studies on fruit berries and the reason is because they contain high bioactive compounds especially in the highly colored berries. Strawberry plant can adapt to different environmental condition and for that matter is widely cultivated worldwide. It is mostly cultivated intensively in open fields both in Europe and North America whereas in, China is cultivated in greenhouses (Wang *et al*., 2015).

Berries that contain the best dietary source of BAC are mostly consumed in fresh form when most of the BAC are in great amount and active, very delicious and have low energy content. They are also important dietary source of phytochemicals and micronutrients. Strawberries are among the richest natural source of vitamin C and folate (Tulipani *et al*., 2008). Among the berry species, the vitamin C content in strawberries is comparable to raspberries, higher than blackberries from the same *Rosaceae* family and four times higher than blueberries from *Ericaceae* family. Depending on the cultivar and other factors, the ascorbate content in strawberries differs and ranges from 5- 80 mg/100g fresh weight (Frankie *et al*., 2004). Consuming a handful of strawberry is adequate to cover the RDA of vitamin C. Furthermore, it was cited in the study of Tulipani and his authors (2008) that, consumption of 250-350 g of strawberries on the average can contribute to 60 -100 % of daily European folate intake recommendations thus, 200- 300 µg/day.

Strawberry is mainly rich in glucose and fructose and contains smaller amount of sucrose. Moreover, it contains acids of which citric acid is the dominant. The sugars and acids contribute to the taste and flavor of the strawberry fruit. Their sugar and acid content ranges from (4.66-8.4 3%) and (0.56-1.6 %) respectively. Furthermore, other sugars have also been identified as well namely, rutinose, arabinose and rhamnose. It is a rich source of omega-3 fatty acids, vitamin K, vitamin B6, B2 and B5. It is a good source of beta carotene and vitamin E. Also, it contains minerals such as calcium, magnesium and potassium (the most abundant mineral) (Đilas *et al*., 2011).

Figure 1: Strawberry fruit

Furthermore, in the pharmacopeia and folk medicine industry, strawberries are used as potential remedy due to their diuretic and astringent properties. Some of the health benefits include the use of the fruit paste to treat skin diseases and wounds. Also, the leaf extract possesses antioxidant, anti-diabetic and anti-inflammatory properties that helps to fight degenerative diseases. Furthermore, the juice of the fruit is known to treat lungs and nerves inflammation (Kunwar *et al*., 2010). Antioxidants found in strawberry helps fight against the damaging of cellular tissues, inhibits the oxidation of low LDL-cholesterol, reduce the risk of incidence of cardiovascular diseases, and improve the functioning of the vascular endothelial (Prasath and Subramanian, 2014).

There are two forms of antioxidants: free and bound form. The free form are those compounds that do not bind to the cell wall of the strawberry, and they are free anthocyanins, hydroxycinnamic, flavonol glycoside, (+)-catechin. Also, there is the bound form namely the proanthocyanidins that functions by binding to polysaccharides and proteins and are present in strawberries as procyanidin and propelargonidin derivatives, (+)-catechin and (-)-epicatechin. Proanthocyanidin is used as indicator of gray mold resistance in strawberry and this indicator is useful to screen and properly select strawberry cultivars that are of high quality and improved shelf-life (Oszmiański and Wojdyło 2009).

There is little information with regards to the structural features of proanthocyanidins that affect the metabolism and bioavailability within the body. However, they exhibit more antioxidant potential that correspond to health protective actions than the monomeric phenolics. Moreover, several studies have shown that, the crude extract of the strawberries possesses the capability to modify the exposure of several genes that relates to the progress of oral cancer. Notwithstanding, the crude extract and pure compounds of anthocyanins exhibit anti-proliferative and could suppress the growth of colon and prostate cancer (Casto *et al*., 2013).

2.1.1 Brief statistics of strawberry production, exportation and importation

In summer, one of the most popularly consumed fruit in Europe are strawberries. Due to the greenhouse production and diverse varieties and several breeding programs integrated in the system of European countries, they are self-sufficient to produce, import and export to both developed and developing countries. Italy is one of the largest fruit and vegetable suppliers in Europe. **Figure 2** shows the recent statistics of strawberry production in 2020 in the major strawberry producing countries. The major producers in the descending order are, China, USA, Mexico, Turkey Spain, Egypt, Japan, South Korea, Rusia and Poland**.** The non-Eu countries, thus, China, USA and Mexico in that order are well noted for the top 3 strawberry producers in the world. However, for the European countries, it can be seen that, Turkey and Spain as the major producer of strawberry as compared to the others. Notwithstanding, only Egypt is the only African country that made it to the top 6 and this clearly shows that, in Africa, there are factors such as land, climatic conditions and other factors that are not so suitable for strawberry cultivation. In the North America, Asia and Europe there are two or more representative countries in the chart. To rate the continents in percentage-wise, it could be said that, the European countries are the most represented followed by Asia, North America and lastly Africa. This could be inferred that; the climatic and environmental conditions are favorable for the cultivation of strawberry in the top 3 producing continents.

Figure 3 shows gross production value of strawberry in Italy from 2002 to 2018 and from the data shown it could be seen the highest gross production value was in 2004 and the highest dip is in 2011. The crises faced by the country could be as a result of the 2011 major dip. However, it could be noted from the subsequent years that, things got to normal but still lesser than the value seen from 2002 - 2010.

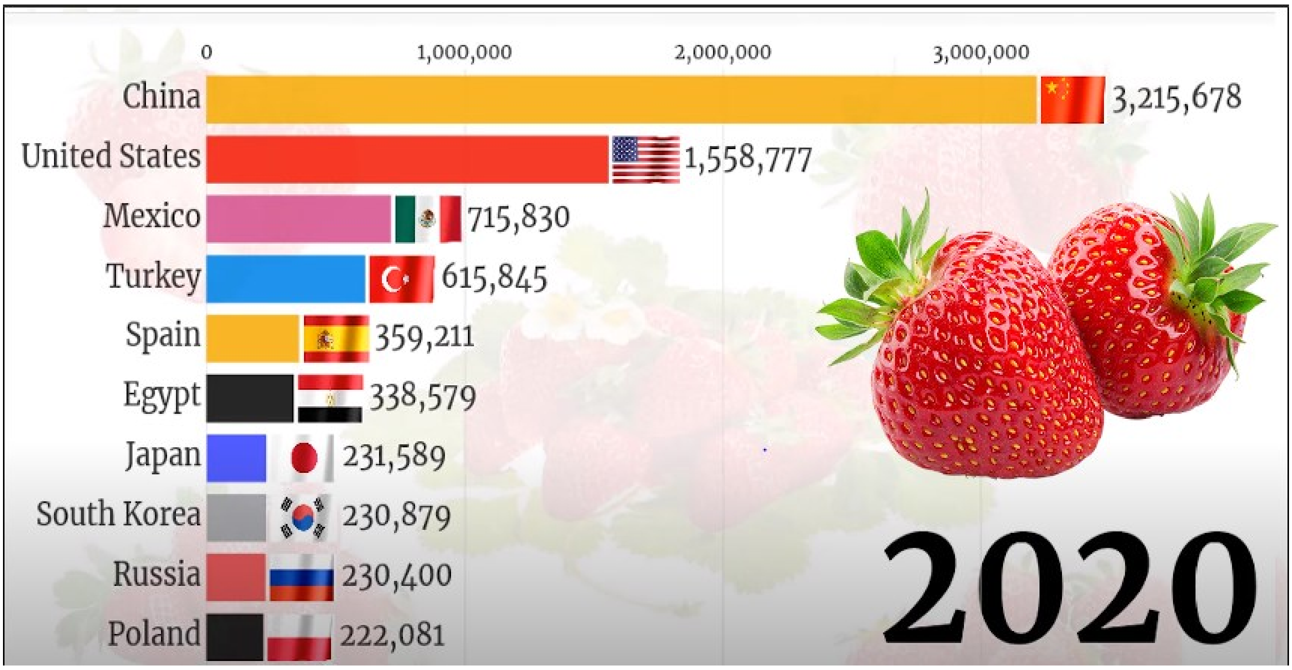


Figure 2: Strawberry production in the major strawberry producing countries

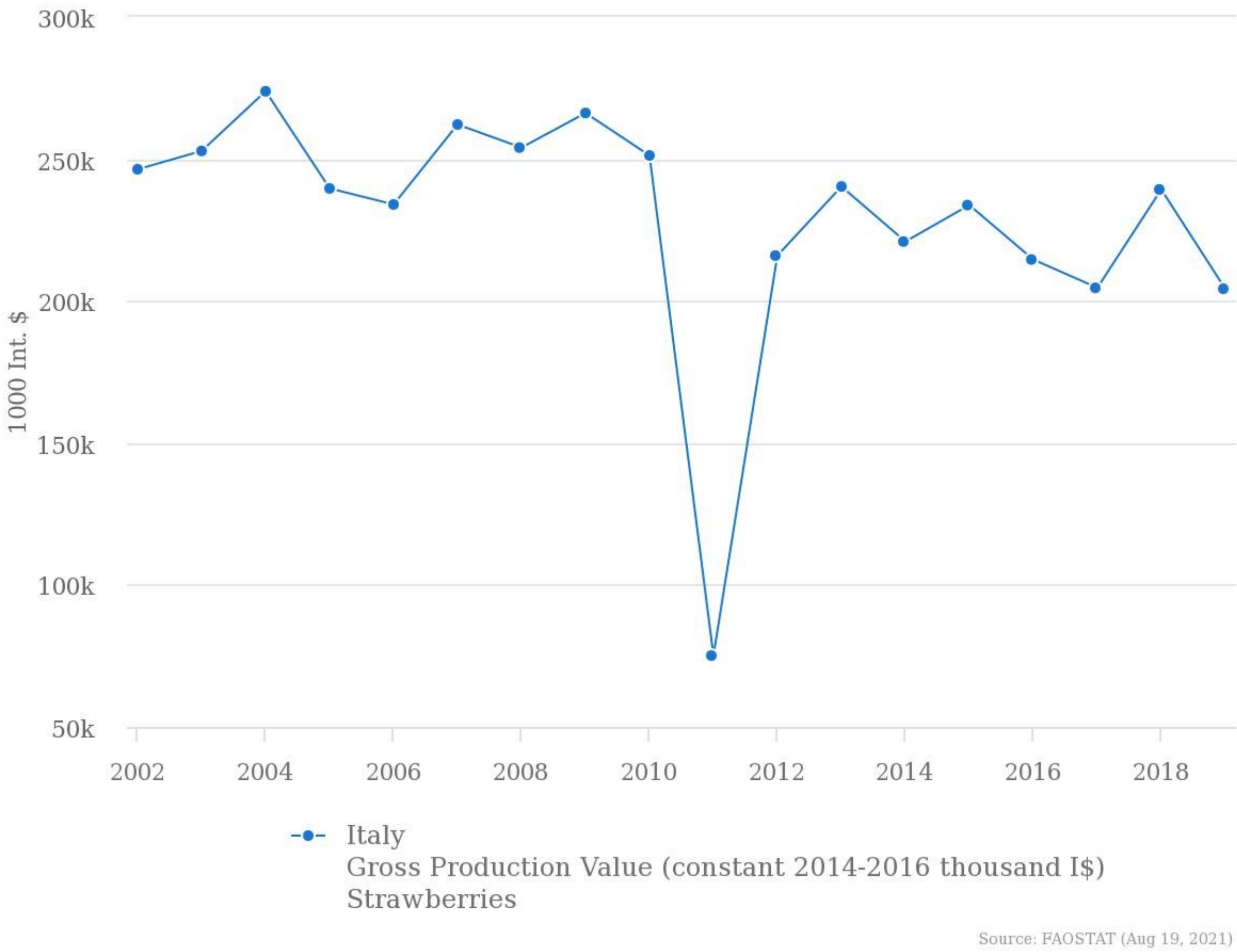


Figure 3: Gross production value of strawberries in Italy

Strawberry consumption in Europe is estimated around 1.2 million tonnes and countries including Italy, Germany and UK have the highest per capita with 3 kilos per year.

According to the report of CBI, 2019, as seen in the **figure 4** below, developing countries exported a lot of strawberries to Europe in the 2017 period, about almost 25,700 tonnes as compared to the non-EU countries. The quality of strawberry in Europe is high, the demand is high, and the market is large as well.

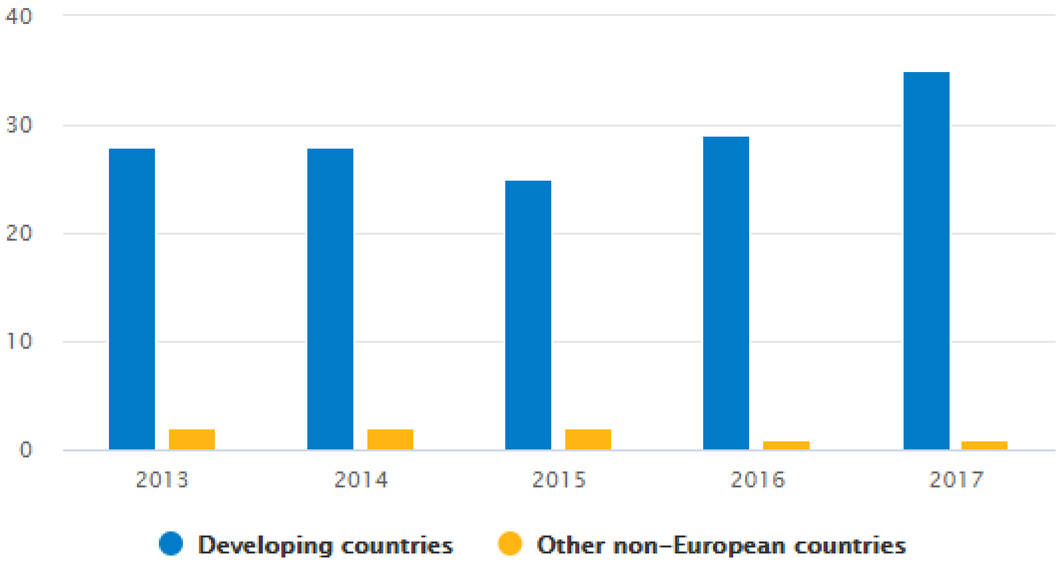


Figure 4: European Import of Fresh strawberries in 1000 ton **(CBI, 2019)**

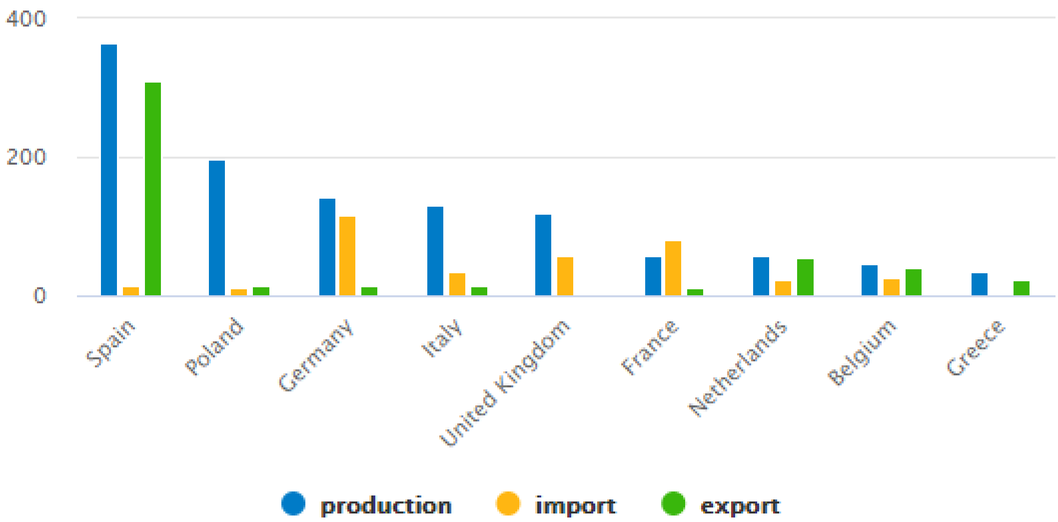


Figure 5: Main European producers, importers and exporters of strawberry in 2016 in 1000 tonnes **(CBI, 2019)**

From the **figure 5**, it could be seen that Spain is the leading exporter and producer of strawberries than any other nation, mostly to Europe and import least, and the revenue generated from this export is about €400 million per year (Goni, 2011). Countries including Poland, Germany and Italy are the second, third, fourth producers respectively. More so, aside Spain, Belgium and Netherlands do more export as compared to the other nation. Germany, France, UK and Italy import strawberries from other countries more than they export respectively in that order.

2.2 Nutritional content of strawberry

Consumers interest nowadays are consume food products that are higher in nutrients in order to improve their health thus, strawberries represent a healthy food choice. Firstly, the nutrient composition of fresh strawberry is presented in **figure 6** below. It could be seen that strawberries are rich source in fructose dietary fiber which aid to regulate blood sugar levels by slowing down digestion. Moreover, their fiber content has a satiating effect that aids to control intake of calorie. Furthermore, strawberry seeds are a rich source of PUFA which is essential to the human health (Giampieri *et al*., 2012). More so, they are rich in carotenoids, tocotrienols, vitamin C, minerals, tocopherols and other fat-soluble vitamins (Table 1). They are also important source of vitamin C (which is extremely high) and folate for human nutrition (Scalzo *et al*., 2005). The report of Tulipani et al. (2008) highlighted that, serving 250 g of strawberry is equivalent to 60 µg of folate and this can provide 30 % of daily European and USA folate RDA. This fruit is a good source of manganese, for instance if 8 (eight) medium strawberries are served, that correspond to 144 g, may provide more than 20 % of daily intake for manganese. Together with all the nutritive compounds discussed, strawberries contain phytochemicals. These are the non-essential and non-nutritive components that provide health benefit to consumers and include polyphenols above all (in particular flavonoids and phenolic acids).

Type	Nutrient	Per 100 g
Proximates	Water (g)	90.95
	Energy (kcal)	32
	Protein (g)	0.67
	Ash (g)	0.40
	Total lipid (g)	0.30
	Carbohydrate (g)	7.68
	Dietary fiber (g)	2.0
	Sugars (g)	4.89
	Sucrose (g)	0.47
	Glucose (g)	1.99
	Fructose (g)	2.44
Minerals	Calcium (mg)	16
	Iron (mg)	0.41
	Magnesium (mg)	13
	Phosphorus (mg)	24
	Potassium (mg)	153
	Sodium (mg)	1
	Zinc (mg)	0.14
	Copper (mg)	0.048
	Manganese (mg)	0.386
	Selenium (μg)	0.4
Vitamins	Vitamin C (mg)	58.8
	Thiamin (mg)	0.024
	Riboflavin (mg)	0.022
	Niacin (mg)	0.386
	Pantothenic acid (mg)	0.125
	Vitamin B6 (mg)	0.047
	Folate (μg)	24
	Choline (mg)	5.7
	Betaine (mg)	0.2
	Vitamin B12 (μg)	0
	Vitamin A, RAE (μg)	1
	Lutein + zeaxanthin (μg)	26
	Vitamin E, α-tocopherol (mg)	0.29
	β-tocopherol (mg)	0.01
	γ-tocopherol (mg)	0.08
	δ-tocopherol (mg)	0.01
	Vitamin K, phylloquinone (μg)	2.2

Figure 6: Nutritional composition of strawberry (Giampieri et al., 2012)

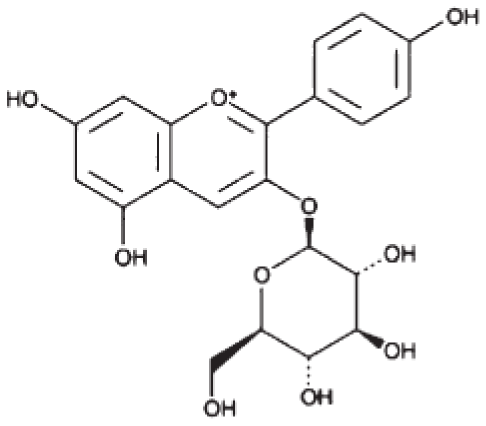
2.3 Phytochemical composition of strawberry

Phytochemicals are secondary metabolites that are produced by plants for their growth, development and for their defense. Some of the roles are to protect the cells from damage, helps the plant to resist from plant viral, bacteria and fungi infections. Also, other biological function include they provide pigmentation, they protect the plant from UV radiation, they act as reducing agents that quench free radicals that are generated during photosynthesis (Alvarez-Suarez, *et al*., 2014).

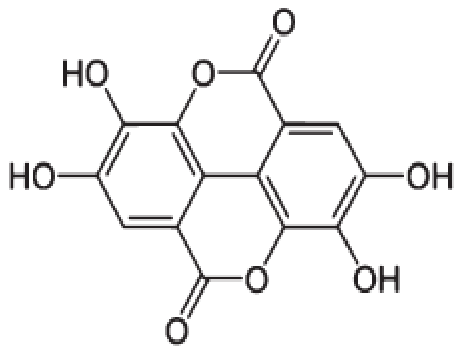
Strawberry is a rich source of BAC and among these compounds, phenolic is the main group of phytochemicals that contributes to the organoleptic, sensorial characteristics and nutritional value of the fruit (Kelebek and Selli, 2011). There have been several reported literatures on the phenolic profile of strawberry from diverse origins. Ellagic acid **(fig 7)** is one of the most abundant polyphenolic compounds in strawberry and this compound largely differ among cultivars of strawberries (Aaby and Wrolstad, 2005).

The ellagic acid and ellagic acid derivatives are known to play important role as antimicrobial activity against human pathogens as well as exhibit chemo preventive properties in the strawberry fruit (Aaby *et al*., 2007).

Furthermore, strawberry is a rich source of anthocyanins, and these compounds are the most important group of water-soluble pigment in plants. Anthocyanins in plant tissues produce pigments in blue, black, red, hues, purple in some cultivars of plants. The presence of co-pigment and the dependent of pH affect their hue and the structure (Musilová *et al*., 2013). The major anthocyanins in strawberry that are detected in almost all the varieties are pelargonidin-3- glucoside and rutinoside, cyanidin-3 glucoside. The pelargonidin-3- glucoside **(fig 7)** occurs in large proportion whereas the cyanidin-3-glucoside occurs in small proportion and these compounds are responsible for the bright color of the strawberry (Tulipani *et al*., 2008).



Pelargonidin-3-glucoside

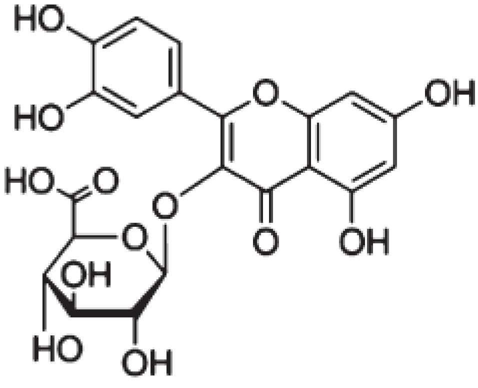


Ellagic acid

Figure 7: Chemical structures of pelargonidin-3-glucoside and Ellagic acid

Anthocyanidins are the aglycone or deglycosylated form of anthocyanins. The total anthocyanin content in strawberry fruits ranges from 150-650 mg/kg fresh weight. A lot of attention has been drawn to anthocyanins in strawberry due to their essential role in the antioxidant capacity of the strawberry fruits.

Moreover, flavonols are also present naturally in strawberries in a glycosylated form whereby the sugar moiety is often glucose or rhamnose. Other sugar moiety as substituents are arabinose, galactose, xylose and glucuronic acid. The derivatives of flavonols found in strawberry fruit are kaempferol, quercetin, and myricetin derivatives and the most abundant is the quercetin-3-glucuronide **(fig 8).**



Quercetin-3-glucoronide

Figure 8: Flavonols derivative; quercertin-3-glucoronide

There has been increasing research on flavonols composition in strawberry because studies show that these molecules are found in the urine and plasma at elevated concentrations as compared to the most abundant BAC as anthocyanins (Giampieri *et al*., 2012).

Flavonols play important role in the inhibition of the physiological enzymatic activities and receptor properties. Also, they exhibit direct and indirect antioxidant, anti-hypertensive, antimicrobial, anti-allergy properties (Santos-Buelga and 2000). In addition, other equally important phenolic acid found in strawberry is the derivatives of HCA and HBA mainly in the form of caffeic acid and gallic acid respectively. The principal difference between HCA and HBA is that, for HBA derivatives are found naturally in glucoside form unlike HCA. Research studies have shown that, derivatives of HCA in its soluble form are more common than the HBA ones. The soluble HCA comprised of *p*-coumaric, caffeic and ferulic acids and generally, they are found as glycoside and as esters with sugars or quinic acids (such as *p*-coumaroylhexose, *p*-coumaroylhexose-4-O-hexoside, or ferulic acid hexose derivatives) (Mattila and Kumpulainen, 2002).

2.4 Factors that affects the composition of strawberry

There are pre- and post-harvest factors that affect the composition of strawberry fruit such as cultivar, maturity degree, edaphic climatic conditions, growing conditions, genetic background, storage time and processing conditions (Skrovankova *et al*., 2015). Below briefly elucidates each of the factors that have been stated.

2.4.1 Maturity Degree

Generally, during the growth and ripening stage of strawberries, their polyphenol composition changes. The polyphenolic composition of unripe fruit (when they are green) is higher in terms of total phenolic content and flavonoids as compared to ripe fruit. Tulipani et al. (2011) reported the phytochemical composition of 4 (four) cultivars undergoing different ripening stages (full size green fruits, pink fruits and ripe red fruits). It was noted that, unlike the pink and red strawberry ripening stage, the full-size green strawberries of all the genotypes had higher content of phenolic compounds. In addition, other research study reported by Giné Bordonaba et al. (2010) shows that, different varieties of green strawberry fruits had higher phenolic content and flavonoids as compared to the pink and the red strawberry fruits and the composition declined during maturity stage. In contrast, the anthocyanin content of red strawberry fruit in all cultivars is higher than the pink and even absent in green strawberry fruits. Likewise, the vitamin C content of strawberry fruit can increase in the dark red stage but not always since it depends on other factors such as the storage and processing conditions, environmental factors and the genotype. The variation of the TAC of strawberry in all cultivars decreases during maturity and ripening stages and is highly linked to the decrease in tannins. However, vitamin C, which is polar non-phenolic antioxidants, in contrast increases during ripening stage but does not have significant impact on the decreasing trend (Olsson *et al*., 2004).

2.4.2 Storage time and processing conditions

During postharvest, one of the most important factors that affect the stability of micronutrients, vitamins as well as phenolic antioxidants in strawberry fruit is storage temperature. Short term storage has strong influence on the phytochemical profile, the nutritional quality and the micronutrients of strawberries. There have been several reports that highlight the changes in TAC, phenolic composition as well as anthocyanins of berries (Piljac-Žegarac, J. and Šamec, 2011, Gil *et al*., 2006). In the study of Tulipani et al. (2008), strawberry cultivars were stored for two days at 4 °C and one day at room temperature, in the dark, and folate content was assessed. It was found that, the level of folate content in all genotypes studied increased. Their study contrasts with many current available findings on the effect of storage temperature on folate content in strawberry and for that matter, it needs further evidence to support the results.

Flavonoid concentration was higher in fruits after short term storage, while on the contrary, other nutritional compounds such as vitamin C, total phenolics, and anthocyanins were not affected by the storage according to the report of Alvarez-Suarez et al. (2014). The reason for these results could be attributed to the fact that there was early eluting polar phenolic antioxidant that occurred during storage. More so, the study cited in report of Alvarez-Suarez et al (2014) projected that there may be increase in total phenolic compounds and anthocyanin of strawberry fruits when the storage time and temperature is extended. Kal and his coauthors (1999) assessed the phytochemical composition in strawberry stored for 8 days at three different conditions (0, 10, 20, and 30 °C). Their findings showed an increase of anthocyanins of about more than 4-fold after 8 days storage and temperature accounts for the magnitude of the increase. Moreover, other authors study also showed that berries that were stored at the temperature of 10 °C for 7 days better retained higher level of anthocyanin and total phenolic content as compared to those stored at 0, 20, and 30 °C (Jin *et al.*, 2011). Their findings may be attributed to the postharvest phenolic metabolism of fruits and thus there could be a formation anthocyanin from a pool of precursors. In addition, short term storage has positive influence on the antioxidant capacity of strawberry fruit, and this is because, during postharvest period, there is ongoing complex reactions that take place and hence facilitate the formation of compounds with enhanced antioxidant capacity (Piljac-Žegarac *et al*., 2011). TAC of fruits during storage remains stable and even increase when the fruit is exposed to higher storage time and temperature (Ayala-Zavala *et al*., 2004).

Strawberry fruits are generally consumed when fresh; however, there are several transformed strawberries on the market such as jam, jellies, juice, nectar, puree etc. Some of the principal processing conditions that take place in strawberry derived products include cutting, blending, heating under vacuum, bottling, closing under vacuum etc. The processing steps such as heat treatment and production time have negative influence on the nutritional quality of strawberry. Comparing the nutritional quality (total phenolic content, anthocyanin, vitamin C and TAC) of fresh strawberry to strawberry derived products, several studies showed decrease of nutritional quality of the derived products (Klopotek *et al*., 2005). According to the study of Hartman et al. (2008), different processing steps showed negative effect on the quality parameters such as vitamin C, total phenolic content, and anthocyanins; however, TAC was affected slightly. The processing methods used were thawing, mashing and pasteurization. The authors attributed the reason of these results to the formation of new antioxidant compounds such as products of Maillard reaction which resulted during heating. The authors also reported that short enzymatic treatment is favorable to obtain maximum yield of TAC, total phenolics and anthocyanins. Processing of fruits into other products is an excellent way to enhancing diversity and ensure sustainability (by minimizing waste) if the processing condition and the holding time are efficiently executed, in order to better retain high amount of the nutritional quality of the fruit.

2.4.3 Genetic factors

Generally, environmental, technological and genetic factors cause variation in the phenolic composition, the total antioxidant and the nutritional quality of the fruits. For instance, genetic background plays an important role in the chemical and nutritional composition of berry fruits because the micronutrients and the phytochemical composition of strawberry fruit differ from cultivar to cultivar. For this matter, agronomist and researchers have characterized some genotypes by improving their nutritional quality through breeding programs in order to obtain the best and desirable fruits for human consumption. Notwithstanding, growing conditions such as exposure to sunlight, level of humidity, type of soil also affect the phytochemical composition and the micronutrients thereby affecting the NQ (Alvarez-Suarez *et al*., 2014). Tulipani et al. (2008) reported the total phenolic content for 4 (four) different genotypes of strawberry, and the results highlighted the variations of phenolic contents among each cultivar. In summary, Sveva cultivar had the highest of (2.83 mg GAE/g FW) while the Adria cultivar had the lowest value of (2.11 mg GAE/g FW). More so, the report by Capocasa et al (2008) on the combining quality and antioxidant attributes in the strawberry showed that different concentration of total phenolics among 20 strawberry genotypes were highlighted in the range of 1.80 to 3.20 mg GAE/g (FW). Furthermore, Scalzo et al (2005) analyzed 6 cultivated varieties and one (1) wild strawberry and observed important differences of their phenolic concentration among cultivars, in a range between 1093 mg GAE/L (cv Idea) and 2128 mg GAE/L (cv Patty).

Furthermore, the level of anthocyanin profile also varies among strawberry cultivars of the same or different species. Report by da Silva et al. (2007) showed that different varieties of strawberry showed different variability among several samples. More so, the individual concentration of Cyanidin 3-glucoside, Pelagonidin 3-glucoside, Pelagonidin 3-rutinoside in extract of five strawberry varieties ranged between 200 to 600 mg/kg (FW). In addition, research study reported by Tulipani et al. (2008) highlighted nine Italian strawberry cultivars and their anthocyanins concentration ranging between 99–296 mg/kg (FW). As evidently highlighted, the anthocyanins profile and the phenolic compounds may strongly vary even within the same cultivar thus depending on characteristics such as the post-harvest, ripening degree, climatic factors. When assessing these parameters as possible indicators of NQ of strawberry fruit, the stated characteristics are necessary. It could be seen that the authors reported different concentration of anthocyanins and phenolic profile found for the same cultivar could be attributed to different extraction methods used.

Moreover, there is a remarkable difference in TAC values among strawberry cultivars and the presence of vitamin C and phenolic compounds are closely linked to TAC. According to the results highlighted by Scalzo et al (2005), higher TAC value of about 2.5-fold were detected for the wild strawberry species *F. vesca* as compared to the cultivated ones. All these highlighted studies show that, it is very necessary to consider the genotype of the strawberry, and this is because genetic modifications produce differently enriched fruits. The use of wild strawberry species as a source of novel trait is valued by strawberry breeders especially for abiotic stress tolerance, pest resistance as well as the important role to improve the nutritional quality of the strawberry fruit (Di Vittori *et al.*, 2018). The wild and the cultivated strawberry species have their differences which of a peculiar interest. Comparing the two strawberry species, the wild have higher nutritional qualities than the cultivated ones. On the contrary, the wild strawberry species may lack some quality traits like firmness, and the fruit size. The use of wild germplasm serves as an important genetic source of enhancing the nutritional quality of fruit (Wang and lewers 2007; Diamanti *et al*., 2014). Also, there is the possibility to obtain different manipulations through the traditional breeding programs as well as the use advanced biotechnology or genetic manipulation (Diamanti *et al*., 2012). These are some of the powerful tools that could be used to modifying antioxidant fruit patterns and contents.

2.4.4 Environmental and climatic factors

Environmental factors in which the plants grow play major role in the development of food crops because these factors largely have influence on the micronutrients and the phytochemical composition of the product. Also, the climatic changes that takes place during the ripening stage of the strawberry development have influence on the composition of the fruit as well. Different cultural practices must be taken into consideration when assessing the TAC and the polyphenols of strawberry fruits. According to the study reported by Wang and Miller (2009), it was inferred that, comparing fruits grown in compost socks and those grown in matter row system, fruits from plants grown in compost socks had significantly higher flavonoid, anthocyanin and oxygen radical absorbance capacity and phenolic content and vice versa. More so, these authors found also that vinegar treatment in culture practice increased the total phenolic, total anthocyanin and TAC values.

Notwithstanding, fruits grown in matter row system showed low antioxidant and micronutrient as content as compared to those fruits grown in the hill plasticulture system (Wang *et al.*, 2002). Furthermore, Jin and his co-authors (2011) reported on the study, the effect of cultural system and storage temperature on antioxidant capacity and phenolic compounds in strawberries. From their study, two cultivation methods were used namely the conventional and organic cultivation; the findings showed that the strawberry cultivated in organic culture had higher activities in antioxidant enzymes. In addition, the level of phytonutrients contained were significantly higher for strawberries produced from organic than those produced from conventional culture. It is interesting to note that study reported by Häkkinen and Törrönen (2000), showed contrary results that organic cultivation had no consistent effects on the level of phenolic compounds in strawberry.

2.5 Overview and bioaccessibility of vitamin C

Vitamins are organic substances that are needed in small quantities for the development of cellular metabolism and normal body functioning. They are indispensable for the vital functions and growth. Vitamins are not biosynthesized by the organism and for that matter, they are acquired from other source such as in food into the body (Yaman *et al*., 2021). Studies show that there are 13 vitamins found in human nutrition and they are classified in two major groups in terms of their solubility, namely fat soluble and water-soluble vitamins. Fat soluble vitamins are stored in the body in fat tissues and liver while water soluble vitamins are absorbed and metabolized in the body but are excreted from the body through the urine when excess unlike fat soluble vitamins (Stevens, 2021).

Water soluble vitamins are composed of B vitamins such as (B1, B2, B3, B5, B6, B7, B9, B12) and vitamin C. There are several important roles of water-soluble vitamins in human metabolism and some of them are they act as coenzymes that are partake in biochemical reactions, also involved in DNA synthesis. They are also involved in biosynthesis and metabolism activities such as amino acid metabolism, energy metabolism, biosynthesis of pentose sugars, and fatty acids (Yaman *et al*., 2021). Vitamins are essential so when they are deficient in the human body system, will results in diseases such as beriberi, scurvy, rickets, pellagra etc.

Vitamin C **(fig 9)** is one of the most important nutritional molecules in many horticultural crops. It is an essential vitamin for human nutrition and more than 90 % is supplied by fruits and vegetables (Lee and Kader, 2000). The best source of vitamin C is found in fruit and vegetables such as cherry, mango, papaya, brussel sprouts, tomato, kiwi, watermelon, cauliflower, red and green peppers and melon, broccoli, strawberry. The daily requirement for male and female adults is 90 mg and 75 mg respectively (Yaman *et al.*, 2021). There are some pre- and post-harvest factors that cause significant loss of vitamin C content in crops, and they include presence metal ions such as Cu^{2+}, Fe^{3+} and Ag^{+}, exposure to light and oxygen, higher temperatures, prolonged storage times. Also, physical damage such as cutting, chopping, crushing, washing, grinding, cooking, canning can reduce its content (El-Ishaq and Obirinakem, 2015). In addition, cultural practices such as pruning, thinning, the use of pesticides, the use of excessive nitrogenous fertilizers, the use of growth regulators, irrigation procedures, can affect vitamin C amount in fruits.

The two most important biological active form of vitamin C are L-ascorbic acid and L-dehydroascorbic acid. They exhibit biological activity and are vital for metabolic functions. The L-ascorbic acid is the main active form of vitamin C whereas the L-dehydroascorbic acid is the oxidation product of L-ascorbic acid at neutral or alkaline pH. The reaction of L-dehydroascorbic acid can be reduced back to L-ascorbic acid and also non-reversibly oxidizes to the 2,3-diketogulonic acid form. (Travica *et al.*, 2017). Vitamin C plays a vital role for bone health, growth and non-viral infections. Recent study has shown that vitamin C levels decrease in some viral infections, and supplementation of 6 g/day vitamin C improves immune response by reducing the symptoms associated with the common cold. It is a good reducing agent that is capable of donating electrons, turning into dehydroascorbic acid; it can be reduced by dependent glutathione reductase and reused. It plays biological roles in the body such as synthesis of bile acid, carnitine, steroid hormones etc. Clinical studies show that, there is an established link between some vitamins and the physiological and biochemical functions in the body. For instance, vitamin C acts as a coenzyme for lysine and proline hydroxylase enzyme that induces collagen gene expression as well as stabilize the tertiary structure of collagen molecule (Pullar *et al.*, 2017). In addition to the biological functions, vitamin C is involved in the absorption of inorganic ion, and inhibition of the formation of nitrosamine (Lee and Kader, 2000).

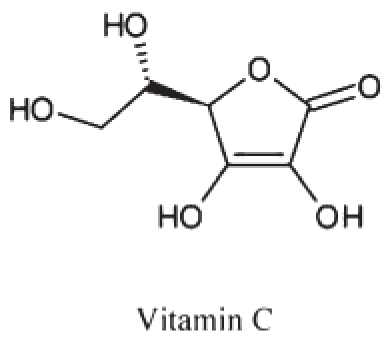


Figure 9: Structure of vitamin C

Vitamin C is also thought to play an important role as an immune system booster to counteract the effect of the current deadly virus called the SARS-CoV-2 infection. The deficiency of vitamin C results in the disruption of collagen synthesis and the delay of wound healing. It acts as antioxidants that can reduce the incidence of cardiovascular diseases due to the prevention of low-density lipoprotein oxidation. Furthermore, it also prevents the oxidation of folate thereby reducing folate deficiency (Honarbakhsh, and Schachter, 2008). Moreover, it is necessitated in the prevention of gum and blood vessel, scurvy, maintenance of skin health etc.

2.6 Overview and bioaccessibility of Folate/Folic acid (Vitamin B9)

Folate, also known as vitamin B9, is a water-soluble vitamin, and the name of folate usually outlines a class of compounds with chemical structures as folic acid (FA, vitamin M, B9 or B11) (Deconinck *et al*, 2011). Folates are naturally available in the form of pterin associated with methylene bridges to p-aminobenzoic acid, which binds to glutamic acid(s) by peptide bonds. All folates are mainly in the form of polyglutamate which is composed of five to seven glutamate residues with a peptide linkage. Folic acid (**fig 10**) is also known as pteroylglutamic acid: is a synthetic form of folates which is not naturally found in food and contains only one glutamic acid unlike their natural form (Combs and McClung, 2016). Folates in their natural form are found in food and are bound to polysaccharides and proteins and are predominantly present in the form of tetrahydrofolate (THF), 5-methyl-THF, and 10-formyl-THF.

Comparing folates to folic acid, the latter is highly stable at 100 ºC and pH of 5-12 and more bioavailable, thus mostly used in the fortification of food (Eitenmiller et al., 2016). The reason for the less stability and bioavailability of folates can be attributed to the high electronegativity of polyglutamates structure making it difficult to enter the cells. Hence in order to reverse this reaction, it is needful to deconjugate all folates to monoglutamate forms by the brush-border conjugase folylpoly-γ-glutamyl carboxypeptidase (EC 3.4.12.10). After deconjugation of polyglutamate to monoglutamyl form, about up to 50 to 70 % of the polyglutamate can be absorbed in the body. Regardless the form of the folates, all are susceptible to photochemical oxidation and some reducing agents such as ascorbic acid and dithiothrietol, stabilize folates. On the other hand, folate can be lost in the presence of Fe^{2+} also with an increasing temperature and decreasing pH.

Moreover, foods such as orange juice extract and tomatoes, and organic acids can reduce folate bioavailability in human diet and as well as inhibit conjugase enzyme activity. Furthermore, every enzyme works best in an optimum pH and temperature and a cofactor, for conjugase enzyme, it works best in pH between 6.7 to 7.0 and is activated by Zn^{2+}. The study of Ball (2006), highlighted that there is an enzyme called dihydrofolate reductase (DHFR) in the liver that is capable of converting 5-methyl-THF found in plasma to THF which is the active form of folate.

The principal function of 5- methyl-THF is to transfer methyl groups to homocysteine which intends forms methionine by the enzyme methionine synthase. Moreover, about 10 to 20 % of reduced folate is taken by the liver and the remaining is transferred to the extrahepatic tissues. Folate plays essential roles in the human body including the role of DNA synthesis, serine and glycine metabolism. Folate deficiency give rise to megaloblastic anemia, certain types of cancer and neural tube defects (Yaman *et al*., 2021). Clinal studies show that elevated levels of homocysteine in the plasma can results into cardiovascular diseases (Ball, 2004).

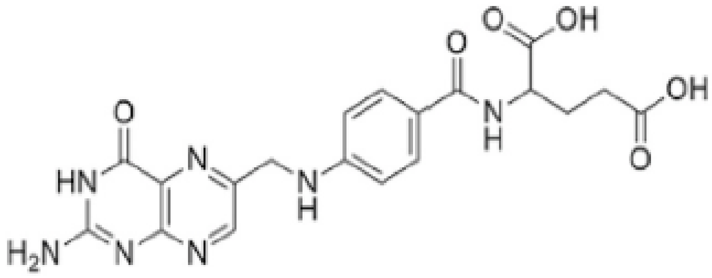


Figure 10: structure of folic acid

The RDA for both males and females for folate is 300-400 µg; however, the requirement increases during pregnancy and lactation to about 600 µg and more. Naturally, folate is mostly found in plants and vegetables such as dried beans, lentils, chickpeas, broccoli, okra, brussels sprouts, nuts and seeds, cauliflowers, beets, corn, celery, carrots and squash, spinach (Cheung *et al*., 2009). Furthermore, eggs, breakfast cereals, meat products such as chicken, turkey, lamb, beef and pork liver are also good source of folate (USDA, 2020).

2.7 Overview of breeding of strawberry

Fruit breeding is the process of improving the genetics of fruit crops (Janick, 2012). Until now, the main focus of strawberry breeding programs has been geared towards the improvement the commercial and agronomic traits (Diamanti *et al*., 2012). However, in these recent times, there is a shift of focus towards sensorial and the nutritional qualities of strawberry fruit in major breeding targets (Zorrilla-Fontanesi *et al*., 2011). In particular, research article reported by Diamanti et al. (2012) were aimed in increasing the sensorial and nutritional parameters of strawberry and as well compared different breeding approaches that would help increase the narrow genetic base of the cultivated strawberry. To further explain their study, the wild octoploid strawberry (*Fragaria virginiana ssp. Glauca*) were tested in inter-species crosses and through subsequent back-cross cycles. Also, the procedure for the inter-specific backcross was through crossing of a *Fragaria* × *ananassa* (F×a) genotype with FVG, the donor of the trait of interest. The following first filial generation (F1) was then crossed back with an F×a genotype leading to the backcross 1 (BC1) generation. The progenies of this generation that display the trait of interest deriving from FVG were again crossed back with F×a resulting in the backcross 2 (BC2) generation. This method of selection and crossing was repeated a third time to generate the backcross 3 (BC3). The goal was to obtain a genotype that is as similar to the F×a genotype with the additional nutritional traits of FVG (Diamanti *et al.* 2012). It was concluded that, the two-breeding approach could be used for the improvement of strawberry nutritional quality.

In 2017, statistics have shown that the annual global production and cultivation of strawberries has exceeded 9 million tons, making it the most widely cultivated fruit crops in the world. Integration of breeding programs in strawberry cultivation is economically important and have several merits among improving the fruit quality and increasing the yield performance, disease resistance capacity, etc. (Gezan *et al*., 2017).

To date, many breeding programs have yielded a better result, particularly, in the strawberry breeding program. The reason is that, strawberry has peculiar characteristics that make it more phenotypically variable, able to easily interact with its surrounding environment, also, its high ploidy value (eight-ploidy) (Bucci *et al.*, 2010). Furthermore, cultivated strawberry (*Fragaria x ananassa*) also known as modern strawberry is derived from interspecific hybridization between *Fragaria virginiana* and *Fragaria chiloensis* which is an octoploid (2n = 8x = 56) species (Yamamoto *et al*., 2021). However, there were few cultivars obtained from this hybridization that produce overall varieties but thanks to crossbreeding, strawberries are highly adaptable to different climatic and cultivation conditions in many environments with high yield. There are some similar objectives shared by current breeding programs such as good balance between resistance to biotic stress, perfect flower. Besides the aforementioned, more specifically are the parameters such as the size constituency, period of ripening, the nutritional quality of the fruit (https://agronotizie.imagelinenetwork.com/vivaismo-e-sementi/2008/03/10/l-evoluzione-varietale-della-fragola/5006).

The breeding program of the Department of Agricultural, Food and Environmental Sciences of the Università Politecnica delle Marche (UNIVPM) begun in 1993 with the aim of creating new cultivars; with an improved fruit quality, with high adaptability to heavy-chalky soils, that are resistance to soil bound diseases and late ripening. As part of the contribution to the wellbeing of the stakeholders in the community, researchers in the D3A Department of UVIVPM have developed and still developing breeding programs with the objective to obtain new genotypes with high-production levels and high-quality fruits, and adaptability to resilient conditions.

Moreover, there has been increase in the knowledge and awareness of the relationship between diet and consumer health which has heightened the demand for fruits that are not only nutritious, and high quality but also fruit that could help fight against cardiovascular diseases. Thanks to the high levels of BAC in strawberry fruit, breeding to create new genotypes rich these compounds become easier. In the last decade, the market has driven breeders to produce cultivars with high nutritional and sensorial traits. On the contrary, the correlation between high production and high-quality fruit is difficult to obtain and sometimes this challenge cause inefficiency in breeding programs. To explain further, for example, high production of strawberry fruit does not necessarily mean that the quality such as the size, aroma, taste, sugar content will be intact.

For that matter, the attention of the current UNIVPM-D3A breeding program is also pinned on the nutritional properties of new strawberry genotypes, through the improvement of the concentration of vitamin C vitamin B9 and polyphenols. Furthermore, depending on the genotype, the nutritional composition of strawberries could vary (Singh *et al*., 2010). Besides, there are some posts and pre factors (Pineli *et al*., 2011; Josuttis *et al*., 2013) that have been discussed already in this chapter which also have an impact on the chemical and nutritional composition of strawberry fruit.

In addition to the aforementioned, thanks to constant implementation of breeding programs in UNIVPM-D3A, it has led to the release of eight (8) registered new commercial varieties namely; ("Adria", "Cristina", Romina", "Sveva", "Lauretta", "Dina", "Silvia", and "Francesca") and hundreds of new selections currently evaluated for their high commercial value.

More, so, the UNIVPM-D3A breeding program addresses the genetic variability of the chemical composition. Notwithstanding, there are still some gaps on how nutritional quality traits of strawberries can be modified by breeding (Capocasa *et al*. 2008). On the one hand, biotechnological approaches could be also be possible to determine biosynthesis pathways of nutritional compounds through genetically modification. On the other hand, traditional breeding techniques can be applied as well (Mazzoni *et al*. 2016).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Plant materials, collection and preparation of the samples

Forty-two (42) new selections of strawberry and twelve cultivars (12) from the UPM-D3A breeding program were planted in 2018 while 86 new selections and 13 cultivars were planted in 2019 in non-fumigated soil in "P. Rosati" experimental farm of Università Politecnica delle Marche, and were used for the study **(Table 1)**. The farm is situated in Agugliano (Ancona, Italy), and the strawberries were 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. The strawberries were harvested at fully red stage by handpicking in bulk, at the second, third and fourth seasonal pickings. Each genotype was labeled and stored in the laboratory refrigerator at -20°C until further analysis. Ten (10) ripe strawberry fruits from each genotype were randomly sampled and for each fruit, the opposite sides (head and tail) were cut and chopped into tiny pieces and evenly mixed. For vitamin C and folate extraction procedure, about 1 g and 2 g respectively as a representative sample from the whole chopped 10 fruits sample were weighed in a falcon tube and labelled and stored at -20 °C prior to the extraction of the components (vitamin C and vitamin B9). The same procedure was repeated for each genotype.

Figure 11: P. Rosati experimental farm of UNIVPM

Table 1: List of the 2019 and 2020 cultivars and selections of strawberry fruit for the study

2019			2020		
CULTIVARS	SELECTIONS		CULTIVARS	SELECTIONS	
JANISS	AN06,164,52		AUREA	AN00,239,55	AN15,07,53
DINA	AN07,105,53		CRISTINA	AN06,164,52	AN15,07,57
MONTEREY	AN10,04,51		DINA	AN11,05,53	AN15,08,51
FRANCESCA	AN11,32,55		FRANCESCA	AN11,05,58	AN15,09,57
ROMINA	AN12,05,54		JANISS	AN12,05,54	AN15,13,53
GALLETTA	AN12,13,58		LAURETTA	AN12,13,58	AN15,19,55
SIBILLA	AN12,20,51		MONTEREY	AN12,20,51	AN16,04,52

SILVIA	AN12,20,53		ROMINA	AN12,20,53	AN16,04,53
LAETITIA	AN12,23,53		SCALA	AN12,23,53	AN16,22,52
CRISTINA	AN12,23,58		SIBILLA	AN12,23,58	AN16,22,53
LAURETTA	AN12,23,66		SILVIA	AN12,23,66	AN16,22,56
TEA	AN12,24,52		TALIA	AN12,24,52	AN16,27,52
	AN12,29,54		ALBA	AN12,29,54	AN16,27,53
	AN12,29,60			AN12,29,60	AN16,27,54
	AN12,35,52			AN12,29,62	AN16,32,51
	AN12,44,51			AN12,44,51	AN16,32,53
	AN12,44,60			AN12,44,60	AN16,32,55
	AN12,45,53			AN12,45,53	AN16,37,51
	AN13,13,55			AN12,48,54	AN16,37,52
	AN13,13,62			AN12,49,53	AN16,37,53
	AN13,16,51			AN12,49,65	AN16,37,57
	AN13,16,56			AN12,50,52	AN16,37,58
	AN13,16,59			AN12,51,56	AN16,37,60
	AN13,21,56			AN13,13,55	AN17,07,51
	AN14,12,58			AN13,13,62	AN17,07,52
	AN14,16,62			AN13,15,57	AN17,07,53
	AN14,17,51			AN13,16,57	AN17,07,54
	AN14,20,51			AN13,20,52	AN17,07,55
	AN14,21,55			AN13,20,58	AN17,12,51
	AN14,21,62			AN13,44,52	AN17,12,52
	AN14,27,62			AN14,01,52	AN17,12,53
	AN15,08,51			AN14,08,55	AN17,12,54
	H106			AN14,12,58	AN17,12,55
	H107			AN14,16,62	AN17,19,51
	H18			AN14,20,51	AN17,19,52
	H2			AN14,21,55	AN17,19,53
	H33			AN14,21,56	AN17,19,54
	H38			AN14,21,62	AN17,19,55
	H41			AN15,01,57	AN17,19,56
	H71			AN15,01,60	AN17,19,57
	H81			AN15,04,54	BL13,9,5 FB5 (Bianca)
	H97			AN15,07,51	FVG
					H71
					H97

The highlighted green means the same selections and cultivars appears in both 2019 and 2020 whereas yellow color means the cultivars and the selections differs for both years

3.2 Determination of vitamin C

3.2.1 **Preparation** *of the vitamin C extract solution*

Fifty gram (50 g) of MPA and 0.393 g of DTPA were weighed and dissolved in 1 L of deionized water and stirred with the aid of a magnetic stirrer (Argrlab M2-A) under cold condition.

3.2.1.2 Extraction and Quantification of Vitamin C

Method cited by Mezzetti *et* al., (2016) with slight modification was used. Four milliliters (4 mL) of the extract solution were added to 1 g of the frozen strawberry samples labeled falcon tube for vitamin C and homogenized with the aid of Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Denmark) at medium-high speed for 30 sec. Afterwards, the homogenized sample was sonicated using the transonic bath 460 (Elma) for 5 min and the principle behind this equipment is that waves is created in order to facilitate the extraction of vitamin C from the strawberry sample into the extract solution. After a centrifugation at 4000 rpm for 15 min at 4 °C, the supernatant was filtered with the aid of 0.45 µm NY (nylon) filter into 1.8 mL HPLC vials and were analyzed. The same procedure was repeated for all the genotypes. The HPLC system was composed of Jasco PU-2089 Plus controller, a Jasco UV-2070 Plus ultraviolet (UV) (Jasco Inc., Easton, MD, USA) detector which was set at absorbance of 260 nm, and an autosampler AS-4050 (Jasco, Easton, MD, USA). The HPLC column used was a Ascentis Express C18 150 × 4.6 mm (Sigma-Aldrich Corp., St. Louis, MO, USA). The elution was isocratic with 50 mM of KH_2PO in MilliQ (MQ) water, which was led to pH 3.2 (below the pKa of the ascorbic acid), through orthophosphoric acid. The analysis was run for 10-min and afterwards, the column was cleaned with 50 % acetonitrile. Finally, the quantification of the vit C content was carried out through a calibration curve prepared by running standard concentrations of vit C. Results were expressed as mg vit C per 100 g FW (Fresh weight).

3.3 Determination of Folate Content (Vitamin B9)

3.1 Preparation of Folate extract solution

Phosphate buffer was prepared by dissolving 13.6 g of KH_2PO_4 and 17.4 g of K_2HPO_4 in 1L deionized water, stirred on a magnetic stirrer and was checked for pH around 6.4. Afterwards, 10 g of L (+)-ascorbic acid as well as 1 mL of 2 mercaptoethanol (v/v) was weighed into the extract solution.

3.3.1.2 Extraction of vitamin B9 (Folate)

Method cited by Mezzetti *et* al., (2016) with slight modification was used. Eight milliliters (8 mL) of the extract solution were pipetted in 2 g of the frozen strawberry samples labeled falcon tubes for folate and was homogenized using the Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Denmark) at medium-high speed for 30 sec. The falcon tube was loosely capped and was boiled on a heating plate for 12 min at 180 °C (the purpose was to degrade the other molecules in the strawberry sample and to free the folate molecule into the extraction solution) and rapidly cooled in the freezer for 10 min. Hog kidney folate conjugase from rat serum was prepared and about 1.5 mL of the enzyme was added to the cooled solution (the purpose is for deconjugation of polyglutamylated folates) and incubated on a shaking oven at 37 °C for 3 h. Afterwards, the enzyme was inactivated by boiling on a heat plate for 5 min followed by cooling for 10 minutes in the freezer. The samples were then centrifuged at 4500× g for 30 min at 4 °C, the supernatant was transferred into a new labelled falcon tube. Moreover, addition of another 8 mL of the extract solution was added to the pellet and was centrifuge again for 30 min at 4 °C at 4500 × g, followed by the transfer of the supernatant to the new falcon tube containing the previous supernatant to make 16 mL mark. Also, the extraction solution was used to top up till 25 mL mark. 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 Isolute cartridges as described by Iniesta et al., 2009.

3.3.1.3 Solid Phase Extraction

This is the second phase of folate extraction procedure. This purification procedure using the SPE, helps to separate and purify the folate from unwanted compounds. Eluting solution was prepared with the components: 0.1 mol/l sodium acetate containing 10% (w/v) sodium chloride, 1% (w/v) ascorbic acid 0.1% 2-mercaptoethanol. The SPE cartridges were conditioned (activated and made more effective) using 2.5ml of methanol which was run twice through the cartridge and later equilibrated with same volume of water to ensure the interaction of the SPE material with the sample. Aliquots of samples were then loaded to the preconditioned cartridges and allowed to flow under vacuum through the SPE material. 0.7ml of eluting solution, was then used to wash away unwanted materials from the cartridges and finally the desirable portion attached to the cartridge was collected into 15 mL labeled falcon tubes by adding 4 mL of the eluting solution, and stored at -20 °C until further analysis.

3.3.1.4 Quantification of Folate using HPLC

The folates were quantified using the HPLC as cited by Stralsjo et al., (2003) with slight modification. The HPLC system comprised of a pump model PU- 2089 (Jasco, Easton, MD, USA), a Fluorescence detector (FLD) FP-2020 Plus (Jasco, Easton, MD, USA) set at wavelengths of 290 nm excitation and 360 nm emission, and an autosampler AS-4050 (Jasco, Easton, MD, USA). The analytical column was a Luna C18, 250×4.6, 5 µm (Phenomenex, Torrance, California, USA). The mobile phase consisted of 30 mmol/l phosphate buffer, pH 2.3, using a gradient with acetonitrile starting at 6 %, a lag time of 5 min and rising linearly to 25 % within 20 min. The total run time was 33 min. Retention time of 10 min was used for peak identification, and quantification of folates content was determined through a calibration curve prepared by running standard concentrations of 5-methyl- tetrahydrofolic acid (5-CH3-H4folate). Results are expressed as µg 5-CH3-H4folate per 100g of fresh weight of strawberry (µg 5-CH3-H4folate/100g FW). In order to accurate quantify and characterize the folate, standard concentrations were prepared and ran using 5-methyl-tetrahydrofolic acid (5-CH3-H4folate) and a calibration chromatogram was obtained. Folate concentration of samples was quantified by comparing their peaks to the standard. All the genotype samples were analyzed using the same procedure.

3.4 Statistical analysis

Statistical analyses were performed using the software "Statistica 7" (Stasoft, Tibco Software, Palo Alto, California, USA). The data were analysed using one-way analysis of variance (ANOVA), while means were compared using the student- Neumann-Keuls (SNK) test, with $p<0.05$. Results were expressed as mean ± standard error (SE).

CHAPTER 4

4.0 RESULTS AND DISCUSSION

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 of fruit, having an improved nutritional quality is strictly linked to the health of the consumer (Capocasa *et al*., 2008). Furthermore, to identify the commercial exploitation of new released cultivar and proper selection of strawberry, it is essential to evaluate its nutritional quality and per this study folate and vitamin C were evaluated.

More so, this research study portrays the variation of vitamin C and B9 content of strawberry fruits. In the study, fruits from 12 commercial cultivars and 42 new selections, and 13 commercial cultivars and 86 new selections were harvested in the years 2019 and 2020, respectively, for vitamin C evaluation. Whereas 12 commercial cultivars and 42 new selections harvested in 2019 were assessed for the vitamin B9 content. In the study for vitamin C evaluation, there were 6 UNIVPM registered cultivars found in both 2019 and 2020 and other seven (7) commercial cultivars distributed among 2019 and 2020. Likewise for vitamin B9, the same 6 UNIVPM registered cultivars were found in the 2019 strawberry samples. The similar cultivars in both 2019 and 2020 were nine (9), they were; **"Romina, Monterey, Dina, Sibilla, Silvia, Lauretta, Janiss, Francesca, Cristina".** Seven (7) cultivars differ in both years, the cultivars namely; **"Galletta, Laetitia and Tea"** was found in 2019 while **"Aurea, Alba, Scala and Talia"** cultivars were found in 2020.

Moreover, there were **twenty-two (22) same new selections** that appeared in both 2019 and 2020 strawberry samples whereas **eighty-four (84) different new selections** (20 different selections for 2019 and 64 different selections for 2020) were found for both years for the vitamin C assessment. In the last years, when a quality parameter of a fruit is mentioned, the antioxidant property of the fruit is mostly highlighted. This parameter is closely related to vitamin C and other bioactive compounds, and they synergistically act as antioxidants and protective agent against sources of wounds and damages in plants (Schijlen *et al*., 2006). Furthermore, in human aspect, these compounds play important role by scavenging free radicals that occurs through oxidation reactions (Mazzoni *et al*., 2016). Moreover, the folate (vitamin B9) as a water-soluble B vitamin play an important role in the biosynthesis and metabolism of amino acids, nucleotides, and vitamin B5. Also, in the human body, folate is essential for the red blood cell formation, healthy growth and as well as provides methyl units to methyltransferases, which use a broad range of substrates, such as hormones, DNA, proteins, and lipids, as part of the methyl cycle (Mazzoni *et al*., 2021).

VITAMIN C DISCUSSION

2019 commercial cultivars of strawberry

The general trend could be seen in the ascending order of vitamin C content. The cultivar Janiss had the lowest vitamin C content of all the cultivars with 1.67 mg 100 g-1 FW. However, the highest amount of vitamin C was found in Tea with 21.98 mg 100 g-1 FW followed by Lauretta and Cristina with (19.64 and 19.41 mg 100 g-1 FW) respectively (**Figure 12**). It could be seen from the figure that, there were no much significant differences statistically (P>0.05) among the 12 cultivars. Also, there were no significant differences (P> 0.05) from "Galletta" to "Tea" (from left to right)

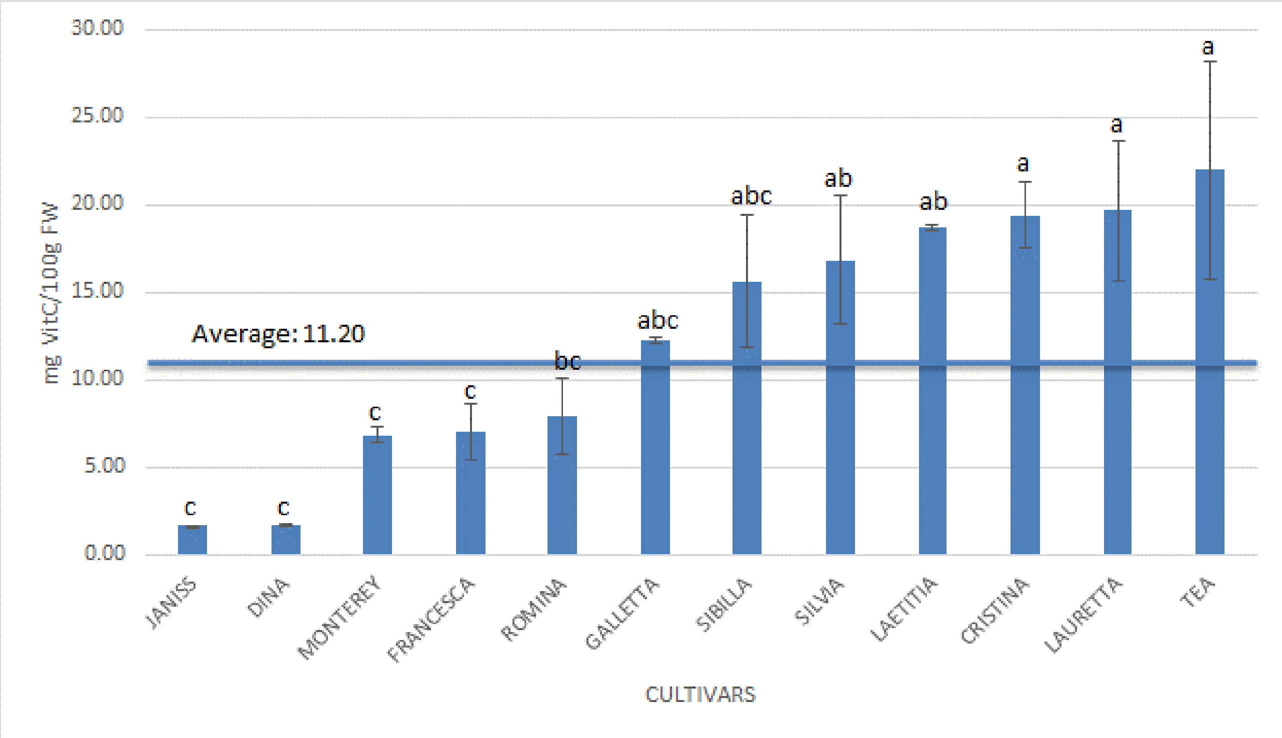


Figure 12: vitamin C content for 12 commercial strawberry cultivars for 2019 expressed as milligrams of vitamin C per 100 g Fresh weight. Values are express as means ± standard errors. Different letters indicate statistical difference (LSD test, p≤0.05).

Furthermore, out of the 6 registered UNIVPM commercial cultivars for this study, Lauretta had the highest vitamin C, followed by Cristina, Silvia, Romina, Francesca and Dina, which had the lowest with 1.74 mg 100 g-1 FW. The average value for the vitamin C content was 11.20 mg 100 g-1 FW, which is generally very low content. This average value of vitamin C represents less than the 50 % of daily recommended amount of vitamin C suggested by the US National Institutes of Health. Also, the recommended daily intake of vitamin C which is in the range of 40 to 120 mg/day, depending on age and gender (Abdullah *et al*., 2021) is far from the average vitamin C content of the strawberry for all the cultivars. These low values were affected by many factors different from the genetic factor, as the long storage time (fruits were harvested in 2019 and analyzed in 2021) due to the pandemic situation, and to some electric shocks which happened during this period and interrupted the cold storage of the sample. Anyway, the obtained differences could be used to underline which are the best genotypes among the analyzed cultivars.

2019 new selections of strawberry

Moreover, the vitamin C content of 42 new strawberry selections for 2019 has been reported **(figure 13)**. Generally, there was an increasing trend from the lowest "H41" with 0.99 mg 100 g-1 FW, to highest "AN,13,16,51" selection with 59.97 mg 100 g-1 FW. There was significant difference among them ($P<0.05$). The amount is not generally high, for the same issue highlighted for the cultivars analysis: in particular the average vitamin C for all the selections was highlighted as 12.85 mg 100 g-1 FW. However, the top three (3) selections with high content of vitamin C in ascending order were AN,13,21,56, AN,13,16,56 and AN,13,16,51, with 44.40, 46.23 and 59.97 mg 100 g-1 FW respectively. These individual selections are within the recommended daily intake (40-120 mg). However, the overall average vitamin C content (12.85 mg 100 g-1 FW) for the selections is far below the RDI stated and this is not quite interesting.

Cultivars versus Selections for 2019 strawberry

Generally, it could be observed that the average vitamin C content in both the cultivar and the selections (11.20 and 12.85 mg 100 g-1 FW respectively) were far below the RDI for vitamin C. This low vitamin C value exhibited in both genotypes could be attributed to the genetic factors, post-harvest factors such as long-term storage as well as external factors such as power fluctuations, as previously explained. The 2019 strawberry samples were kept in the refrigerator for 2 years prior to the analysis. So, due to the long-term storage, it could be said that, most of the vitamin C content were degraded since it is highly perishable and sensitive compound. Vitamin C is water soluble vitamins and a powerful antioxidant essential for the human body. Generally, fruits with low pH such as citrus fruits are capable of retaining most of the vitamin C unlike the fruits with soft consistency like strawberry are highly affected by eternal influence (Pavlovska *et al*., 2015). The vitamin C loss of strawberries is caused by the oxidation of ascorbic acid to dehydroascorbic acid but there will be still some vitamin properties. On the contrary, long storage of strawberry results in the oxidation of dehydroascorbic acid to dicetogluconic acid, thus when such happens, there is no vitamin properties exhibited by the fruit (Pavlovska and Tanevska, 2013). Pavlovska et al. (2015) evaluated the influence of temperature and time of 3 different storage on the vitamin C content in strawberries. From that study, it was noted that, the amount of vitamin C in the fresh strawberry after 11 days were reduced from 60.85 mg/100 g to 0.55 mg/100 g, 28.21 mg/100 g and 37.92 mg/100 g for room temperature, frozen temperature (-18 °C) and cool temperature (4 °C) respectively. From the study, there was degradation of vitamin C content in strawberry when the storage condition was prolonged, however, same explanation could be said for this study as well.

Though, the general trend for both genotypes were poor, however, comparing the cultivars and selections for the year 2019, it could be said that, the results for the selections were more interesting than the results for the cultivars. The top three cultivars such as "**Tea", "Lauretta", and "Cristina"** were quite good but they were below the RDI for vitamin C. But most importantly, some of the stated individual selections were higher than the individual cultivars. This means that the selections are ameliorating and most importantly these three selections namely **AN,13,21,56, AN,13,16,56 and AN,13,16,51** are of high interest and could be served as potential parents for new crossing as far as increasing vitamin C is concerned. Moreover, it could be suggested that when all the other quality parameters evaluated for these selections are high, they could be potential registered cultivar available on the market.

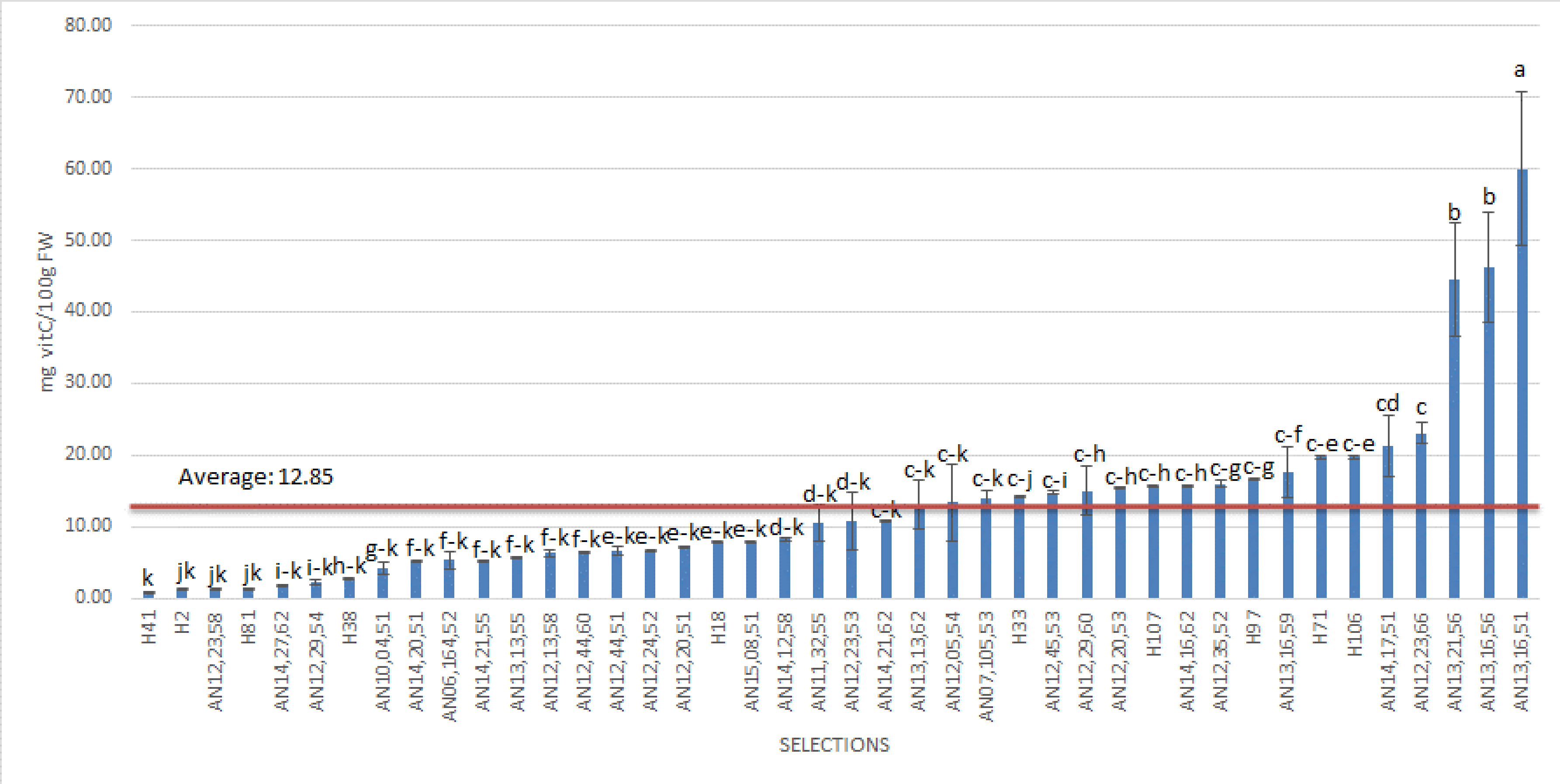


Figure 13: vitamin C content of the 42 new selections of strawberry for 2019 expressed as milligrams of vitamin C per 100 g Fresh weight. Values are express as means ± standard errors. Different letters indicate statistical difference (LSD test, $p \leq 0.05$).

2020 commercial cultivars of strawberry

The general trend shows increasing order of vitamin C content. The cultivar “Monterey” had the lowest vitamin C content (14.58 mg 100 g-1 FW). The highest amount of vitamin C was found in cultivar **“Alba”** with 61.63 mg 100 g-1 FW, followed by **“Aurea”** and **“Cristina”** with 46.48 and 40.15 mg 100 g-1 FW respectively **(Figure 14).** These top three cultivars highlighted were above the RDI of vitamin C. Also, there were not so much significant differences among the values obtained (P>0.05).

Comparing the registered commercial cultivar of UNIVPM, it could be seen that **“Cristina”** (40.15 mg 100 g-1 FW), **“Francesca”** (39.81 mg 100 g-1 FW), and **“Romina”** (32.19 mg 100 g-1 FW) were the top three against “Lauretta” (28.18 mg 100 g-1 FW, “Silvia” (26.05 mg 100 g-1 FW) and “Dina” (21.76 mg 100 g-1 FW). The average value for the vitamin C content was 34.07 mg 100 g-1 FW, which is quite high amount. The average value of the vitamin C obtained were near to the stated recommended daily intake of vitamin C but still below the recommended value.

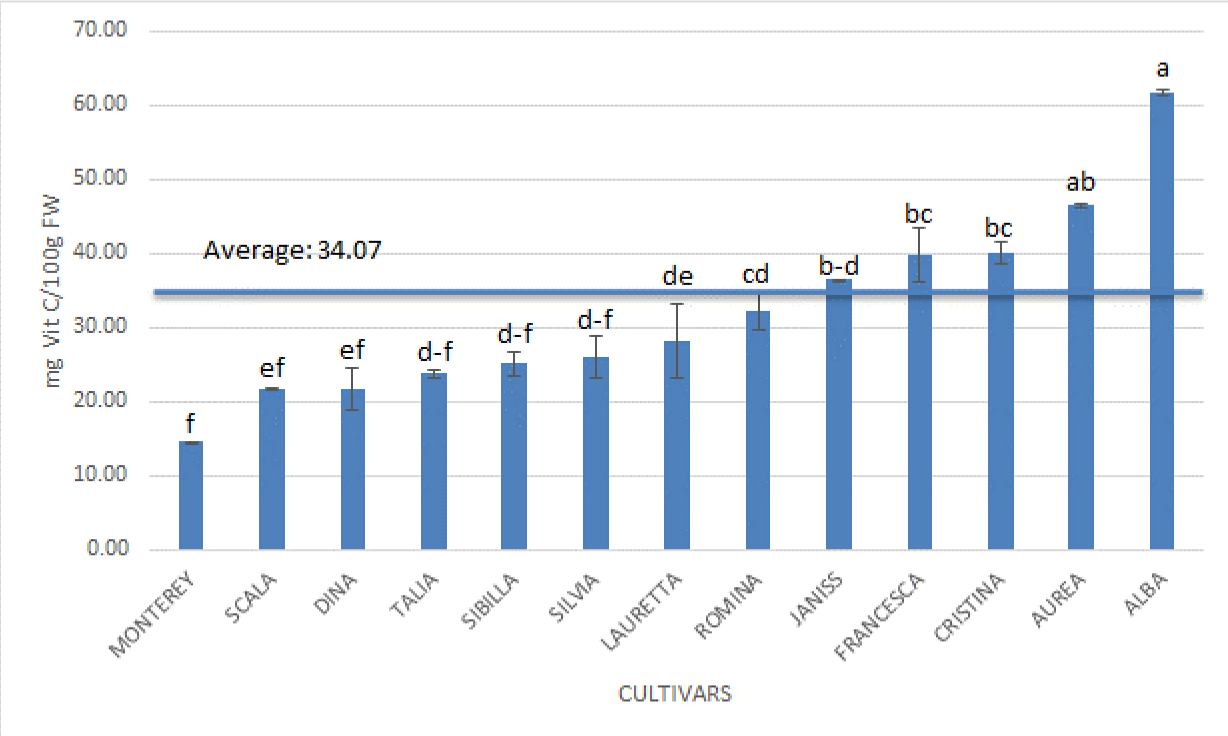


Figure 14: vitamin C content of strawberry commercial cultivars for 2020 expressed as milligrams of vitamin C per 100 g Fresh weight. Values are express as means ± standard errors. Different letters indicate statistical difference (LSD test, p≤0.05).

2020 new selections of strawberry

Figure 15 below shows the vitamin C content of the 86 new strawberry selections for 2020 including 1 wild germplasm. Generally, there was an increasing trend from the lowest “FVG” which is the only wild germplasm with 4.02 mg 100 g-1 FW, to highest **“AN,13,20,52”** selection with 144.38 mg 100 g-1 FW. Generally, the trend shown is quite good, in particular the average of all the selections which was highlighted as 35.95 mg 100 g-1 FW and this average value is closer to the RDI for vitamin C (40-120 mg). Most of the vitamin C values obtained for the 2020 selections were in the range between 30 -144 mg 100 g-1 FW and in percentage-wise is more than 60 %. The vitamin C values ranging from 38-52.5 mg 100 g-1 FW reported in the study of Zhong et al. (2017) was in the range of this current study but slightly lower. Similar findings reported by Capocasa et al. (2016) on the previous study on UPM-D3A breeding program showed a range between 38.5 to 46.7 mg 100 g-1 FW of the amount of vitamin C in different genotypes.

In fact, the 2020 selections for vitamin C are generally higher than the previous study done by our group The top three (3) selections with high content of vitamin C in decreasing order were: **"AN,13,20,52, "AN,17,12,55" and "AN,13,15,57"** with 144.38, 72.92 and 70.15 mg 100 g-1 FW respectively. Interestingly, most of the selections were within the recommended daily intake for vitamin C. (Fig.15).

<u>2020 strawberry cultivars versus selections</u>

Generally, it could be observed that the average vitamin C content for both 2020 cultivars and selections (34.07 and 35.95 mg 100 g-1 FW respectively) were closer to the RDI for vitamin C. The results obtained could have been better as provided the analysis were done in the same year harvested. Factors related to genetic factors, power fluctuations as well as long-term storage may be some of the reason for the vitamin C degradation.

More so, the general trend for both genotypes (selections and cultivars) were good, however, comparing both cultivars and selections for the year 2020, it could be said that, the trend for the selections were better than the cultivars. Also, both the top 3 cultivars (**Alba, Cristina, Aurea,**) and the selections **(AN,13,20,52, AN,17,12,55, and AN,13,15,57)** were within the RDI for vitamin C. Notwithstanding, comparing the individual selections against the cultivars, it could be seen that, the selections are improving. These top 3 selections could be of high interest and would as well as have potential in the near future. In particular, the selection **'AN,13,20,52'** which had an excessive content of vitamin C. This inference shows that, the top 3 selections could be served as utmost importance for generating new cultivars for high vitamin C. Moreover, it could be suggested that when all the other quality parameters evaluated for these selections are high, they could be potential cultivar available on the market.

<u>General remarks of 2020 and 2019 strawberry cultivars and selections for vitamin C</u>

Generally, the trend for both cultivars and selections for 2019 strawberry samples were lower as compared to the 2020 samples. This could be attributed to the fact that the 2019 strawberry were kept long in the refrigerator and this impact had consequently affected the vitamin C content as already discussed.

There isn't any great recommendation from the cultivars of 2019 strawberry sample since they were generally low and were below the RDI for vitamin C. However, there could be some recommendation for the cultivars in 2020 such as **"Cristina, Aurea, and Alba''**. Furthermore, for the UNIVPM registered commercial cultivars, namely **"Cristina and Francesca",** performed well for the 2020 strawberry samples, whereas all the registered commercials cultivars for UNIVPM for the 2019 strawberry samples underperformed. Furthermore, in the previously published study done by our group, the Alba cultivar was highlighted for the highest vitamin C content of 55.96 mg 100 g-1 FW (Mazzoni *et al.,* 2021), whereas the vitamin C content of this cultivar in 2020 for this study is 61.3 mg 100 g-1 FW. Thus, the amount of vitamin C obtained by the best cultivar in our 2020 study for instance is higher than those obtained in previous studies.

In addition, 2019 strawberry new selections that performed well were **AN,13,21,56, AN,13,16,56 and AN,13,16,51**(fig 13) **whereas** 2020 strawberry new selections that also performed well were; **"AN,13,20,52, "AN,17,12,55" and "AN,13,15,57"** (fig15).

In the previous study reported by Mazzoni *et al*. (2021) the highest amount of vitamin C was found in the selection ('AN10,39,51') with 64.1 mg 100 g-1 FW. However, the three best selections of 2020 from the UNIVPM breeding program ("AN,13,20,52, "AN,17,12,55" and "AN,13,15,57"), with 144.38 mg 100 g-1 FW, 72.92 mg 100 g-1 FW, and 70.15 mg 100 g-1 FW respectively, exhibited elevated levels but the highest value obtained for "AN,13,20,52 doubled as compared to 'AN10,39,51' in the previous study by our group.

Moreover, it could be said that, comparing the cultivars and selections of 2019 to 2020, generally, the vitamin C content for the 2020 cultivars and selections were 50 % and 47 % respectively higher than the 2019 strawberry samples. Specifically, comparison between the nine cultivars (**"Romina, Monterey, Dina, Sibilla, Silvia, Lauretta, Janiss, Francesca, Cristina")** found in both years showed that, out of the 9 same cultivars, the 2020 cultivars showed 47 % increment of vitamin C retention over the 2019. This clearly shows that, there is massive vitamin C reduction that could be attributed to several factors already explained.

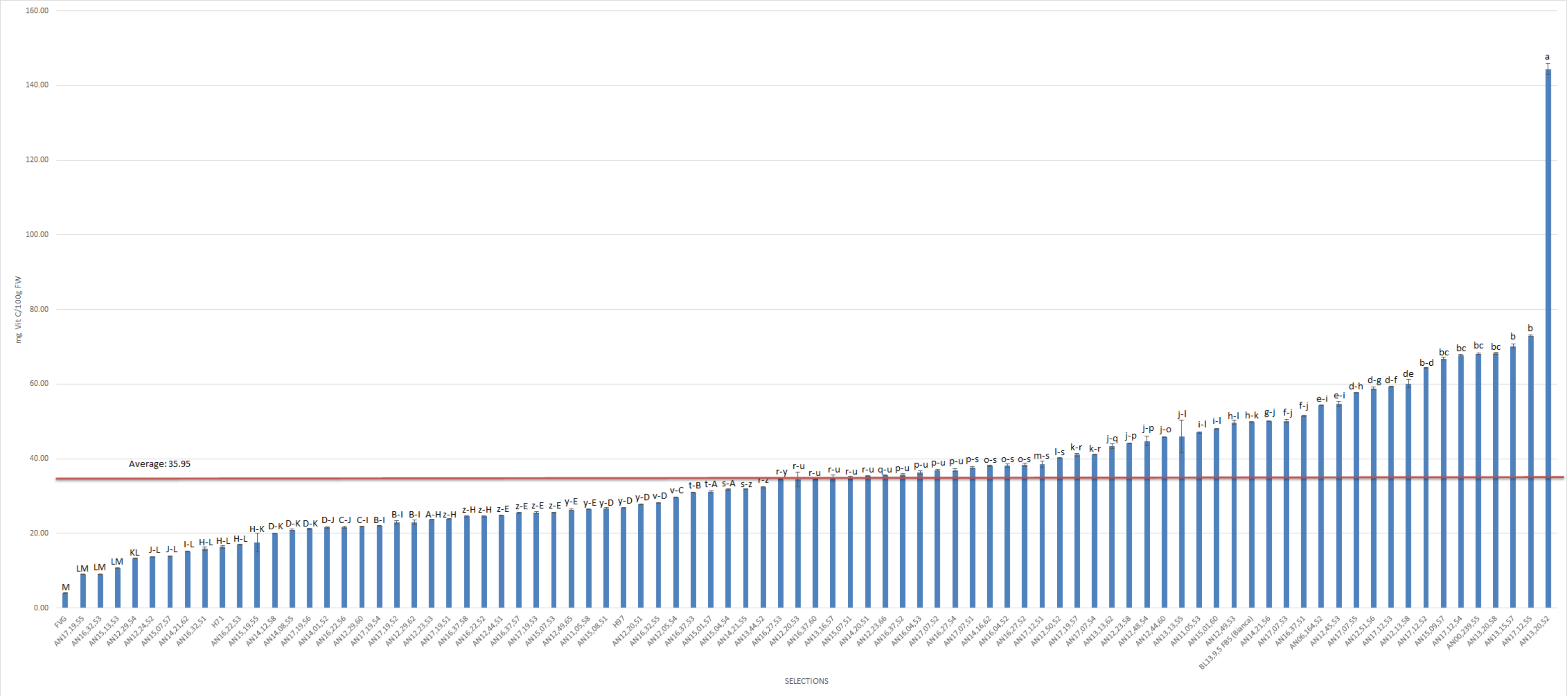


Figure 15: vitamin C content of strawberry new selections for 2020 expressed as milligrams of vitamin C per 100 g Fresh weight. Values are express as means ± standard errors. Different letters indicate statistical difference (LSD test, p≤0.05).

Furthermore, both the highlighted 2019 and 2020 new selections could be used as parents crop to generate new cross for other selections, or they could be released as new cultivar if commercial and sensorial quality is confirmed by more researches.

FOLATE DISCUSSION

2019 commercial cultivars of strawberry

The vitamin B9 measured in this study ranged from 10 -15 µg 100 g-1 FW. The general trend shows increasing order of vitamin B9 content. The cultivar "Laetitia" registered the lowest vitamin B9 content (10.08 µg 100 g-1 FW). There were not so much significant differences among the values obtained (P>0.05). However, it is possible to highlight the top 3 cultivars with high vitamin B9, in a decreasing order: **"Francesca"** with 14.98 µg 100 g-1 FW, followed by **"Dina"** and **"Galletta"** with 13.88 and 12.96 µg 100 g-1 FW respectively **(Figure 16).** The vitamin B9 content for the commercial cultivars reported by Mazzoni et al. (2021) was in the range of 14 to 23 µg 100 g-1 FW and the results obtained for this study is not much different.

The cultivars derived from the UNIVPM-D3A breeding program showed statistically similar values for this compound, it could be seen that **"Francesca"** (14.98 µg 100 g-1 FW), **"Dina"** (13.88 µg 100 g-1 FW), and **"Lauretta"** (12.16 µg 100 g-1 FW) were the top three against "Cristina" (11.93 µg 100 g-1 FW, "Silvia" (11.70 µg 100 g-1 FW) and "Romina" (11.66 mg 100 g-1 FW). The average value for the vitamin B9 content for the cultivars 2019 was 12.39 µg 100 g-1 FW, which is quite normal.

Interestingly, comparing the 2019 individual commercial cultivars of vitamin C to the vitamin B9, it was noted that, the top 3 cultivars that appeared in both vitamins (C and B9) were not repeated in each of the top 3 cultivars. Notwithstanding, the cultivar "Janiss" that had the lowest vitamin C 2019 content, registered enough amount of folate content. More so, **"Lauretta "**was the only cultivar that appeared to be among the top cultivars derived from the UNIVPM -D3A breeding program for both vitamins (C and B9) in the 2019 analyzed strawberry samples.

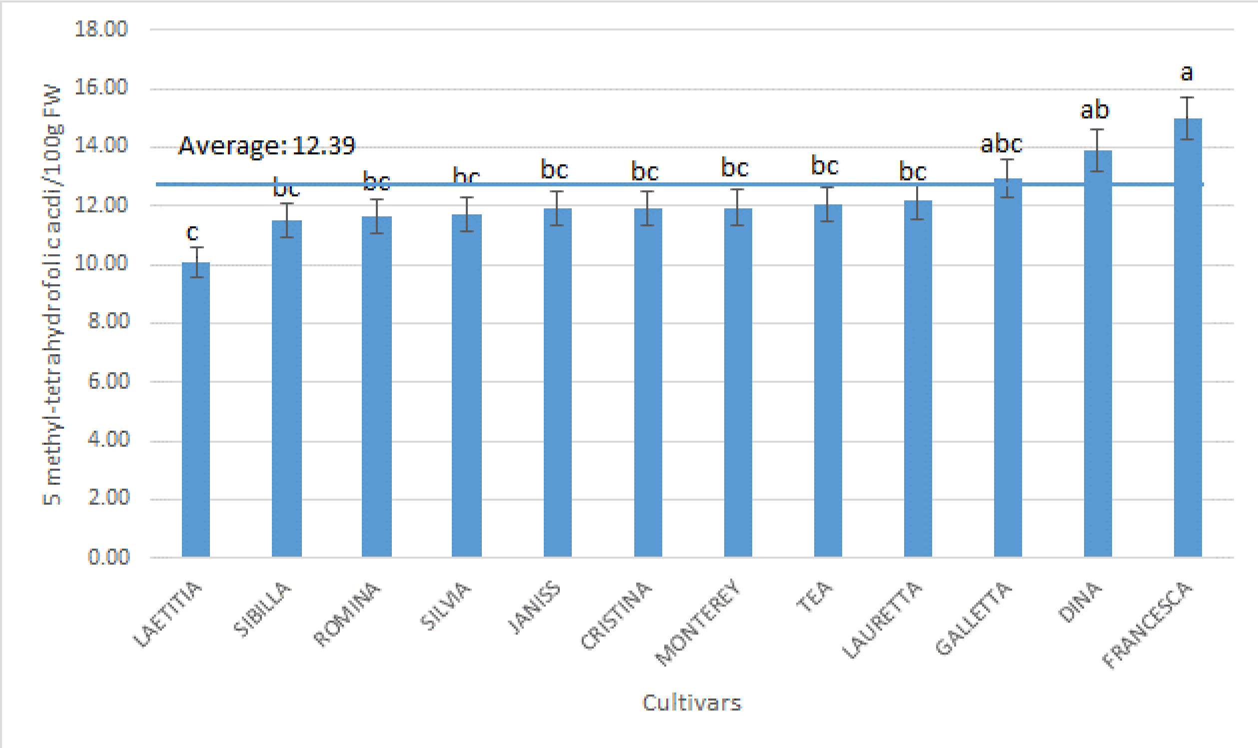


Figure 16: Vitamin B9 content of strawberry commercial cultivars for 2019 expressed as ug 5methyltetrahydrofolic acid/100 g Fresh weight. Values are express as means ± standard errors. Different letters indicate statistical difference (LSD test, p≤0.05).

2019 strawberry new selections

Furthermore, the vitamin B9 content of 42 new strawberry selections for 2019 has been reported **(figure 17)**. Generally, there was an increasing trend from the lowest "H71" with 10.74 µg 100 g-1 FW, to highest **"AN,12,29,54"** selection with 17.95 µg 100 g-1 FW. Generally, there was significant difference among them (P<0.05) but the difference was not much. The average vitamin B9 for all the selections was highlighted as 13.35 µg 100 g-1 FW. The top three (3) selections with high content of vitamin B9 in descending order were: AN,12,29,54, AN,13,16,56 and AN,12,13,58, with 17.95, 17.51 and 16.36 µg 100 g-1 FW respectively. In previous study done by our group, Mazzoni et al. (2021), reported the vitamin B 9 content in the range between 15 to 25 µg 100 g-1 FW. From that, it can be said that the top 3 selections registered in this current study is within the reported value.

Interestingly, this single selection **"AN,13,16,56"** was registered in the top 3 of each of the vitamins in the 2019 strawberry analyzed samples. This shows that, this particular selection could be interesting as well as useful for other crossings. In addition, the selection" H41" that registered the lowest vitamin C 2019 sample performed well in the vitamin B9 (folate).

2019 strawberry cultivars versus selections

Generally, the average vitamin B9 content registered for both 2019 cultivars and selections (12.39 and 13.25 µg 100 g-1 FW respectively) were almost the same. The results obtained could have been better as provided the analysis were done in the same year harvested. More so, the general trend for both genotypes (selections and cultivars) were good. Even though the strawberries harvested were not analyzed the same year, the results are encouraging as compared to some other literature study. Moreover, it could be seen that, the 2020 cultivars and selections will do well when analyzed for vitamin B9.

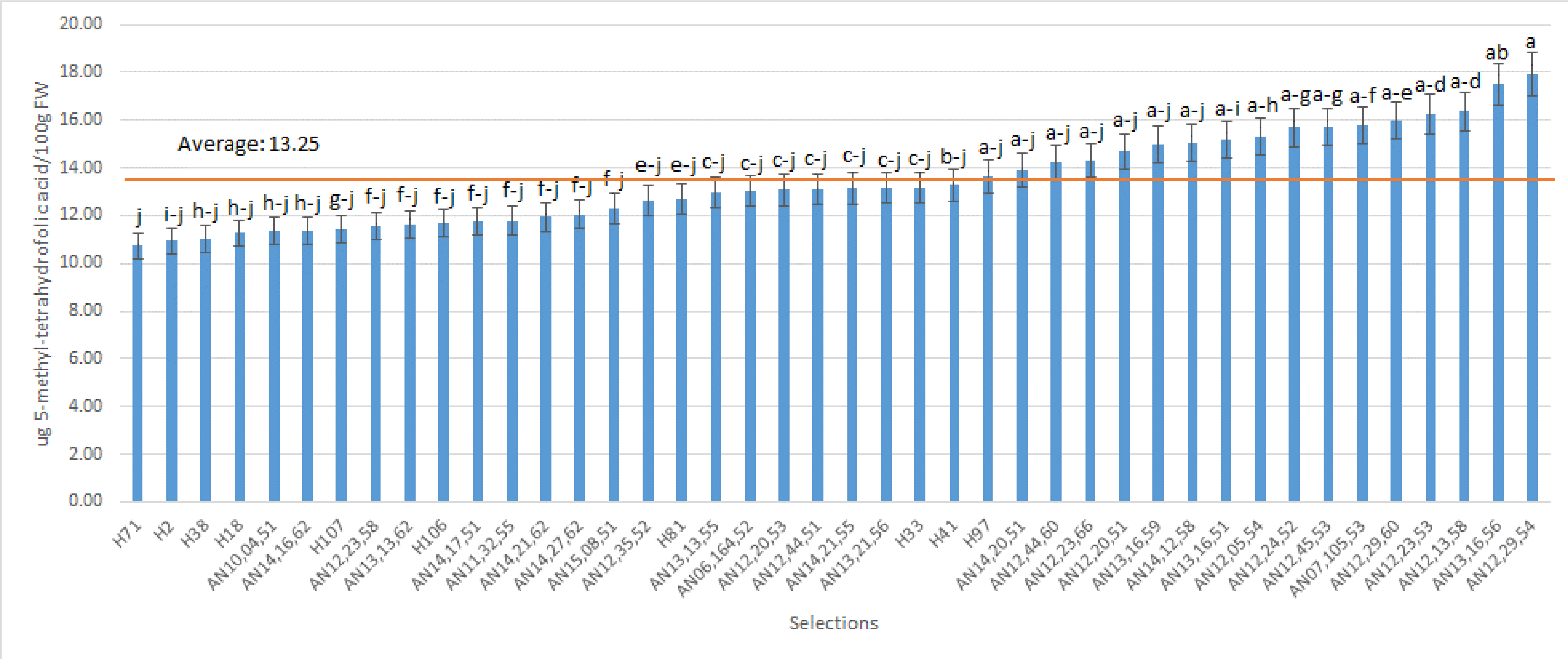


Figure 17: Vitamin B9 content of strawberry new selections for 2019 expressed as ug 5methyltetrahydrofolic acid/100 g Fresh weight. Values are express as means ± standard errors. Different letters indicate statistical difference (LSD test, p≤0.05)

Based on all what have been discussed, the best cultivars and selections for vitamin C and folate have been summarized **(Table2)**

Table 2: Grouping the three best cultivars and new selections from both years (2019&2020)

YEAR	**VITAMIN C**		**FOLATE**	
	Best Cultivars	**Best new selections**	**Best Cultivars**	**Best new selections**
2019	Cristina- **(19.41 mg 100 g-1 FW)**	AN,13,21,56- **(44.40 mg 100 g-1 FW)**	Lauretta- (12.96 µg 100 g-1 FW)	AN,12,13,58- (16.36 µg 100 g-1 FW)
	Lauretta- **(19.64 mg 100 g-1 FW)**	AN,13,16,56- **(46.23 mg 100 g-1 FW)**	Dina- (13.88 µg 100 g-1 FW)	AN,13,16,56- (17.51 µg 100 g-1 FW)
	Tea- **(21.98 mg 100 g-1 FW)**	AN,13,16,51- **(59.97 mg 100 g-1 FW)**	Francesca- **(14.98 µg 100 g-1 FW)**	AN,12,29,54- **(17.95 µg 100 g-1 FW**
2020	Cristina- **(40.15 mg 100 g-1 FW)**	AN,13,15,57- **(70.15 mg 100 g-1 FW)**		
	Aurea- **(46.48 mg 100 g-1 FW)**	AN,17,12,55- **(72.92 mg 100 g-1 FW)**		
	Alba- **(61.63 mg 100 g-1 FW)**	AN,13,20,52- **(144.38 mg 100 g-1 FW)**		

CONCLUSION & RECOMMENDATION

Strawberries belong to fruits with a high content of bioactive compounds. Among cultivars for 2020, **"Cristina, Aurea, and Alba''** showed very interesting result for vitamin C, whereas the results for the cultivars for 2019 were not quite interesting. Moreover, 2019 strawberry new selections that performed well were; **"AN,13,21,56", "AN,13,16,56" and "AN,13,16,51"** while for the 2020 strawberry new selections **"AN,13,20,52, "AN,17,12,55" and "AN,13,15,57"** performed well with interesting results. Moreover, the best selections for the 2019 strawberry sample for vitamin B9 (folate) were **"AN,12,29,54", "AN,13,16,56" and "AN,12,13,58"** while for the 2019, the best cultivars for folate were **"Francesca", "Dina" and "Lauretta".** It is to keep in mind that data on vitamin C obtained from fruits harvested in 2019 are negatively affected by storage length and conditions, so that these data are less precise than 2020 data. The highlighted selections and cultivars 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. Among cultivars that were derived from the UNIVPM breeding program, '**"Cristina and Francesca"** are the best-performing 2020 strawberry samples, followed by 'Lauretta', 'Silvia', and 'Dina'. It is noted that the breeding program was successful to create new genotype with increased vitamin C, and folate contents. As a matter of fact, releasing new cultivar could serve as both starting and end point for breeding programs for increasing the nutritional quality of strawberry fruit.

REFERENCES

1. Aaby, K., Ekeberg, D. and Skrede, G., 2007. Characterization of phenolic compounds in strawberry (Fragaria× ananassa) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. *Journal of agricultural and food chemistry*, *55*(11), pp.4395-4406.
2. Aaby, K., Skrede, G. and Wrolstad, R.E., 2005. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (Fragaria ananassa). *Journal of Agricultural and Food chemistry*, *53*(10), pp.4032-4040.
3. Abdullah, M., Jamil, R.T. and Attia, F.N., 2021. Vitamin C (ascorbic acid). *StatPearls [Internet]*.
4. Alvarez-Suarez, J.M., Mazzoni, L., Forbes-Hernandez, T.Y., Gasparrini, M., Sabbadini, S. and Giampieri, F., 2014. The effects of pre-harvest and post-harvest factors on the nutritional quality of strawberry fruits: A review. *Journal of Berry Research*, *4*(1), pp.1-10.
5. Ayala-Zavala, J.F., Wang, S.Y., Wang, C.Y. and González-Aguilar, G.A., 2004. Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *LWT-Food Science and Technology*, *37*(7), pp.687-695.
6. Ball, G. F. M., 2006. *Analysis, bioavailability, and stability* (2nd ed.). Boca Raton, FL: CRC Press Taylor and Francis Group.
7. Ball, G.F.M., 2004. Vitamins: Their Role in the Human Body. [sl] Blackwell Publishing Ltd.
8. Bucci A., Faedi W., and Baruzzi G., 2010. *Origine ed evoluzione. La fragola*, Collana Colturaandcultura, Ed. Script, Bologna.
9. Capocasa, F., Balducci, F., Di Vittori, L., Mazzoni, L., Stewart, D., Williams, S., Hargreaves, R., Bernardini, D., Danesi, L., Zhong, C.F., and Mezzetti, B., 2016. Romina and Cristina: two new strawberry cultivars with high sensorial and nutritional values. Int. J. Fruit Sci. *16* (*sup1*), pp. 207–219 https://doi.org/10.1080/15538362.2016.1219292.
10. Capocasa, F., Scalzo, J., Mezzetti, B. and Battino, M., 2008. Combining quality and antioxidant attributes in the strawberry: The role of genotype. *Food Chemistry*, *111*(4), pp.872-878.
11. Casto, B.C., Knobloch, T.J., Galioto, R.L., Yu, Z., Accurso, B.T. and Warner, B.M., 2013. Chemoprevention of oral cancer by lyophilized strawberries. *Anticancer research*, *33*(11), pp.4757-4766.
12. CBI, 2019. https://www.cbi.eu/market-information/fresh-fruit-vegetables/fresh-strawberries. Accessed on; 19/08/2021.
13. Cheung, R.H.F., Hughes, J.G., Marriott, P.J. and Small, D.M., 2009. Investigation of folic acid stability in fortified instant Asian noodles by use of capillary electrophoresis. *Food chemistry*, *112*(2), pp.507-514.
14. Combs Jr, G.F. and McClung, J.P., 2016. *The vitamins: fundamental aspects in nutrition and health*. Academic press.
15. da Silva, F.L., Escribano-Bailón, M.T., Alonso, J.J.P., Rivas-Gonzalo, J.C. and Santos-Buelga, C., 2007. Anthocyanin pigments in strawberry. *LWT-Food Science and Technology*, *40*(2), pp.374-382.
16. Deconinck, E., Crevits, S., Baten, P., Courselle, P. and De Beer, J., 2011. A validated ultra-high pressure liquid chromatographic method for qualification and quantification of folic acid in pharmaceutical preparations. *Journal of pharmaceutical and biomedical analysis*, *54*(5), pp.995-1000.
17. DellaPenna, D., 2001. Plant metabolic engineering. *Plant Physiology*, *125*(1), pp.160-163.
18. Di Vittori, L., Mazzoni, L., Battino, M. and Mezzetti, B., 2018. Pre-harvest factors influencing the quality of berries. *Scientia Horticulturae*, *233*, pp.310-322.

19. Diamanti, J., Capocasa, F., Balducci, F., Battino, M., Hancock, J. and Mezzetti, B., 2012. Increasing strawberry fruit sensorial and nutritional quality using wild and cultivated germplasm.
20. Diamanti, J., Mazzoni, L., Balducci, F., Cappelletti, R., Capocasa, F., Battino, M., Dobson, G., Stewart, D. and Mezzetti, B., 2014. Use of wild genotypes in breeding program increases strawberry fruit sensorial and nutritional quality. *Journal of agricultural and food chemistry*, *62*(18), pp.3944-3953.
21. Đilas, S.M., Tepić, A.N., Savatović, S.M., Šumić, Z.M., Čanadanović-Brunet, J.M., Ćetković, G.S. and Vulić, J.J., 2011. Chemical composition and antioxidant activity of two strawberry cultivars. *Acta periodica technologica*, (42), pp.33-44.
22. Eitenmiller, R.R., Landen Jr, W.O. andYe, L., 2016. *Vitamin analysis for the health and food sciences*. CRC press.
23. El-Ishaq, A., and Obirinakem, S. 2015. Effect of temperature and storage on vitamin C content in fruits juice. *International Journal of Chemical and Biomolecular Science, 1*, pp.17–21.
24. Franke, A.A., Custer, L.J., Arakaki, C. andMurphy, S.P., 2004. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. *Journal of Food Composition and Analysis*, *17*(1), pp.1-35.
25. Gezan, S.A., Osorio, L.F., Verma, S. and Whitaker, V.M., 2017. An experimental validation of genomic selection in octoploid strawberry. *Horticulture research*, *4*(1), pp.1-9.
26. Giampieri, F., Forbes-Hernandez, T.Y., Gasparrini, M., Alvarez-Suarez, J.M., Afrin, S., Bompadre, S., Quiles, J.L., Mezzetti, B. and Battino, M., 2015. Strawberry as a health promoter: an evidence-based review. *Food and function*, *6*(5), pp.1386-1398.
27. Giampieri, F., Tulipani, S., Alvarez-Suarez, J.M., Quiles, J.L., Mezzetti, B. and Battino, M., 2012. The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition*, *28*(1), pp.9-19.
28. Gil, M.I., Aguayo, E. and Kader, A.A., 2006. Quality changes and nutrient retention in fresh cut versus whole fruits during storage. *Journal of Agricultural and Food chemistry*, *54*(12), pp.4284-4296.
29. Giné Bordonaba, J., Chope, G.A. and Terry, L.A., 2010. Maximising blackcurrant anthocyanins: temporal changes during ripening and storage in different genotypes. *Journal of Berry Research*, *1*(2), pp.73-80.
30. Goni, R. 2011. Strawberry boom is drain on Spain. BBC, online edition. < http://www.bbc.com/news/world-europe-13546715>
31. Häkkinen, S.H. and Törrönen, A.R., 2000. Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique. *Food research international*, *33*(6), pp.517-524.
32. Hartmann, A., Patz, C.D., Andlauer, W., Dietrich, H. and Ludwig, M., 2008. Influence of processing on quality parameters of strawberries. *Journal of Agricultural and Food Chemistry*, *56*(20), pp.9484-9489.
33. Honarbakhsh, S., and Schachter, M. 2008.Vitamins and cardiovascular disease. *British Journal of Nutrition, 101*(8), pp.1113–1131.
34. Iniesta, M.D., D. Perez-Conesa, J. Garcia-Alonso, G. Ros, and M.J. Periago. 2009. Folate content in tomato (Lycopersicon esculentum): Influence of cultivar, ripeness, year of harvest, and pasteurization and storage temperatures. J. Agr. Food Chem. 57, pp.4739–4745.
35. Janick, J.U.L.E.S., 2012, October. Fruit breeding: Past, Present, and Future. In *XXII Congresso Brasilerio de. Fruiticultura, Bento Goncalves-RS, 22a* (Vol. 26, pp. 1-22).

36. Jin, P., Wang, S.Y., Wang, C.Y. and Zheng, Y., 2011. Effect of cultural system and storage temperature on antioxidant capacity and phenolic compounds in strawberries. *Food chemistry*, *124*(1), pp.262-270.
37. Josuttis, M., Verrall, S., Stewart, D., Krüger, E. and McDougall, G.J., 2013. Genetic and environmental effects on tannin composition in strawberry (Fragaria× ananassa) cultivars grown in different European locations. *Journal of agricultural and food chemistry*, *61*(4), pp.790-800.
38. Kalt, W., Forney, C.F., Martin, A. and Prior, R.L., 1999. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *Journal of agricultural and food chemistry*, *47*(11), pp.4638-4644.
39. Kelebek, H. and Selli, S., 2011. Characterization of phenolic compounds in strawberry fruits by RP-HPLC-DAD and investigation of their antioxidant capacity. *Journal of Liquid Chromatography and Related Technologies*, *34*(20), pp.2495-2504.
40. Klopotek, Y., Otto, K. and Böhm, V., 2005. Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry*, *53*(14), pp.5640-5646.
41. Kunwar, R.M., Shrestha, K.P. and Bussmann, R.W., 2010. Traditional herbal medicine in Far-west Nepal: a pharmacological appraisal. *Journal of Ethnobiology and Ethnomedicine*, *6*(1), pp.1-18.
42. Lee, S.K. and Kader, A.A., 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest biology and technology*, *20*(3), pp.207-220.
43. Mattila, P. and Kumpulainen, J., 2002. Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. *Journal of agricultural and food chemistry*, *50*(13), pp.3660-3667.
44. Mazzoni, L., Balducci, F., Marcellini,M., Pergolott, V., Capocasa,F and Mezzetti, B. 2021. Evaluation of strawberry nutritional quality. In *IX International Strawberry Symposium 1311* (PP. 47-54)
45. Mazzoni, L., Perez-Lopez, P., Giampieri, F., Alvarez-Suarez, J.M., Gasparrini, M., Forbes-Hernandez, T.Y., Quiles, J.L., Mezzetti, B. and Battino, M., 2016. The genetic aspects of berries: from field to health. *Journal of the Science of Food and Agriculture*, *96*(2), pp.365-371.
46. Mazzoni, L., Qaderi, R., Marcellini, M., Mezzetti, B. and Capocasa, F., 2021, May. Variation of polyphenol and vitamin C fruit content induced by strawberry breeding. In *IX International Strawberry Symposium 1309* (pp. 1017-1024).
47. Mezzetti, B., Balducci, F., Capocasa, F., Zhong, C.F., Cappelletti, R., Di Vittori, L., Mazzoni, L., Giampieri, F. and Battino, M., 2016. Breeding strawberry for higher phytochemicals content and claim it: is it possible?. *International Journal of Fruit Science*, *16*(sup1), pp.194-206.
48. Mezzetti, B., Giampieri, F., Zhang, Y.T. and Zhong, C.F., 2018. Status of strawberry breeding programs and cultivation systems in Europe and the rest of the world. *Journal of Berry Research*, *8*(3), pp.205-221.
49. Mezzetti, B., Mazzoni, L., Qaderi, R., Balducci, F., Marcellini, M. and Capocasa, F., 2021, May. Generating novel strawberry pre-breeding material from a Fragaria× ananassa backcrossing program with F. virginiana subsp. glauca inter-specific hybrids. In *IX International Strawberry Symposium 1309* (pp. 197-204)
50. Musilová, J., Trebichalský, P., Timoracká, M. and Bystrická, J., 2013. Cultivar as one of the factors affecting the anthocyanin content and antioxidant activity in strawberry fruits. *Journal of Microbiology, Biotechnology and Food Sciences*, *2021*, pp.1765-1775.
51. Olsson, M.E., Ekvall, J., Gustavsson, K.E., Nilsson, J., Pillai, D., Sjöholm, I., Svensson, U., Åkesson, B. and Nyman, M.G., 2004. Antioxidants, low molecular weight carbohydrates, and

total antioxidant capacity in strawberries (Fragaria× ananassa): effects of cultivar, ripening, and storage. *Journal of Agricultural and Food Chemistry*, *52*(9), pp.2490-2498.

52. Oszmiański, J. and Wojdyło, A., 2009. Comparative study of phenolic content and antioxidant activity of strawberry puree, clear, and cloudy juices. *European Food Research and Technology*, *228*(4), pp.623-631.
53. Pavlovska, G. and Tanevska, S., 2013. Influence of temperature and humidity on the degradation process of ascorbic acid in vitamin C chewable tablets. Journal of thermal analysis and calorimetry, 111(3), pp.1971-1977.
54. Pavlovska, G., Dukovska, E., Knights, V.A. and Jankuloska, V., 2015. Influence of temperature and time of storage on amount of vitamin C in strawberries. Journal of Hygienic Engineering and Design, 11, pp.15-19.
55. Piljac-Žegarac, J.and Šamec, D., 2011. Antioxidant stability of small fruits in postharvest storage at room and refrigerator temperatures. *Food Research International*, *44*(1), pp.345-350.
56. Pineli, L.D.L.D.O., Moretti, C.L., dos Santos, M.S., Campos, A.B., Brasileiro, A.V., Córdova, A.C. and Chiarello, M.D., 2011. Antioxidants and other chemical and physical characteristics of two strawberry cultivars at different ripeness stages. *Journal of Food Composition and Analysis*, *24*(1), pp.11-16.
57. Prasath, G.S. and Subramanian, S.P., 2014. Antihyperlipidemic Effect of Fisetin, a Bioflavonoid of Strawberries, Studied in Streptozotocin-Induced Diabetic Rats. *Journal of biochemical and molecular toxicology*, *28*(10), pp.442-449.
58. Pullar, J. M., Carr, A. C., and Vissers, M. 2017. The roles of vitamin C in skin health. *Nutrients, 9*(8), pp.866
59. Šamec, D. and Piljac-Žegarac, J., 2015. Fluctuations in the levels of antioxidant compounds and antioxidant capacity of ten small fruits during one year of frozen storage. *International Journal of Food Properties*, *18*(1), pp.21-32.
60. Šamec, D., Maretić, M., Lugarić, I., Mešić, A., Salopek-Sondi, B., and Duralija, B., 2016. Assessment of the differences in the physical, chemical and phytochemical properties of four strawberry cultivars using principal component analysis. *Food chemistry*, *194*, 828–834. https://doi.org/10.1016/j.foodchem.2015.08.095
61. Santos-Buelga, C. and Scalbert, A., 2000. Proanthocyanidins and tannin-like compounds–nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture*, *80*(7), pp.1094-1117.
62. Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B. and Battino, M., 2005. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition*, *21*(2), pp.207-213.
63. Schijlen, E., Ric de Vos, C.H., Jonker, H., van den Broeck, H., Molthoff, J., van Tunen, A., Martens, S., and Bovy, A. (2006). Pathway engineering for healthy phytochemicals leading to the production of novel flavonoids in tomato fruit. *Plant Biotechnol J* 4 (4), 433–444 https://doi.org/10.1111/j.1467-7652.2006.00192.x. PubMed
64. Singh, A., Singh, B.K., Deka, B.C., Sanwal, S.K., Patel, R.K. and Verma, M.R., 2011. The genetic variability, inheritance and inter-relationships of ascorbic acid, β-carotene, phenol and anthocyanin content in strawberry (Fragaria× ananassa Duch.). *Scientia horticulturae*, *129*(1), pp.86-90.
65. Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T.and Sochor, J., 2015. Bioactive compounds and antioxidant activity in different types of berries. International journal of molecular sciences, 16(10), pp.24673-24706.
66. Stevens, S.L., 2021. Fat-Soluble Vitamins. Nursing Clinics, 56(1), pp.33-45.

67. Strålsjö, L., Åhlin, H., Witthöft, C.M. and Jastrebova, J., 2003. Folate determination in Swedish berries by radioprotein-binding assay (RPBA) and high-performance liquid chromatography (HPLC). *European Food Research and Technology*, *216*(3), pp.264-269.
68. Travica, N., Ried, K., Sali, A., Scholey, A., Hudson, I., and Pipingas, A. 2017. Vitamin C status and cognitive function: A systematic review. *Nutrients, 9*(9), pp.960.
69. Tulipani, S., Marzban, G., Herndl, A., Laimer, M., Mezzetti, B. and Battino, M., 2011. Influence of environmental and genetic factors on health-related compounds in strawberry. *Food Chemistry*, *124*(3), pp.906-913.
70. Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., De Vos, C.R., Capanoglu, E., Bovy, A. and Battino, M., 2008. Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *Journal of Agricultural and Food chemistry*, *56*(3), pp.696-704.
71. Tulipani, S., Romandini, S., Battino, M., Bompadre, S., Capocasa, F. and Mezzetti, B., 2008, March. Variation in strawberry micronutrients, phytochemical and antioxidant profiles: the combined effect of genotype and storage. In *VI International Strawberry Symposium 842* (pp. 867-872).
72. USDA. (2020). United States department of agriculture. *USDA Food Composition Databases*. https://ndb.nal.usda.gov/ndb/, 2020. (Accessed 1 September 2021).
73. Wang, S.Y. and Lewers, K.S., 2007. Antioxidant capacity and flavonoid content in wild strawberries. *Journal of the American Society for Horticultural Science*, *132*(5), pp.629-637.
74. Wang, S.Y. and Millner, P., 2009. Effect of different cultural systems on antioxidant capacity, phenolic content, and fruit quality of strawberries (fragaria× aranassa duch.). *Journal of agricultural and food chemistry*, *57*(20), pp.9651-9657.
75. Wang, S.Y., Zheng, W. and Galletta, G.J., 2002. Cultural system affects fruit quality and antioxidant capacity in strawberries. *Journal of Agricultural and Food Chemistry*, *50*(22), pp.6534-6542.
76. Wang, Z., Cang, T., Qi, P., Zhao, X., Xu, H., Wang, X., Zhang, H. andWang, X., 2015. Dissipation of four fungicides on greenhouse strawberries and an assessment of their risks. *Food Control*, *55*, pp.215-220.
77. Yamamoto, E., Kataoka, S., Shirasawa, K., Noguchi, Y. and Isobe, S., 2021. Genomic Selection for F1 Hybrid Breeding in Strawberry (Fragaria× ananassa). *Frontiers in Plant Science*, *12*, p.308.
78. Yaman, M., Çatak, J., Uğur, H., Gürbüz, M., Belli, İ., Tanyıldız, S.N., Yıldırım, H., Cengiz, S., Yavuz, B.B., Kişmiroğlu, C. and Özgür, B., 2021. The bioaccessibility of water-soluble vitamins: A review. Trends in Food Science and Technology. 109, pp.552–563.
79. Zhong, C.F., Mazzoni, L., Balducci, F., Di Vittori, L., Capocasa, F., Giampieri, F. and Mezzetti, B., 2016, August. Evaluation of vitamin C content in fruit and leaves of different strawberry genotypes. In *VIII International Strawberry Symposium 1156* (pp. 371-378).
80. Zorrilla-Fontanesi, Y., Cabeza, A., Domínguez, P., Medina, J.J., Valpuesta, V., Denoyes-Rothan, B., Sánchez-Sevilla, J.F. and Amaya, I., 2011. Quantitative trait loci and underlying candidate genes controlling agronomical and fruit quality traits in octoploid strawberry (Fragaria× ananassa). *Theoretical and applied genetics*, *123*(5), pp.755-778.