DEPARTMENT OF AGRICULTURAL, FOOD, AND ENVIRONMENTAL SCIENCES

DEGREE COURSE: FOOD AND BEVERAGE INNOVATION AND MANAGEMENT

Vitamin B9 and Vitamin C Content Variation in Advanced Strawberry Breeding Selections

TYPE OF DISSERTATION: RESEARCH

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THE ACADEMIC YEAR 2021/2022

DEDICATION

This research is dedicated to my lovely parents Dr. Joseph Ofei Darko and the late Mrs. Gladys Ofei Darko as well as my five amazing sisters Michelle, Sophia, Josephine, Christine, and Edith.

ACKNOWLEDGEMENTS

I have taken efforts in this final year experience and project work. However, it would not have been possible without the kind support and assistance of many people and organizations. I would like to extend my sincere thanks to all of them. It is my special pleasure to acknowledge the Almighty God for bringing me this far and providing me with everything I need to complete my project. I am highly indebted to my supervisor and mentor, Prof. Bruno Mezzetti, and my Assistant Supervisor, Dr. Luca Mazzoni for their invaluable guidance, constant encouragement, constructive comments, sympathetic attitude, and immense motivation, which have sustained my efforts at all stages of this project work. I would like to express my gratitude towards my parents and sisters for their kind cooperation and encouragement which helped me in the completion of my project. I would like to express my special gratitude and thanks to Davide Raffaelli and all laboratory persons for giving me such attention and time. My thanks and appreciation also go to my course mates and the people who willingly helped me out with their abilities.

ABSTRACT

One of the berry fruits that people consume the most globally is the strawberry. It contains significant amounts of vitamins C (ascorbic acid) and B9 (folates), as well as bioactive compounds like beta-carotene, phenolic acids, flavonoids, and anthocyanins. The year-round high level of consumer demand has led to an expansion of the strawberry market in Europe. Numerous EU and non-EU businesses have worked hard to expand their production regions in various climates and growing systems by employing the right cultivars to meet this demand. Due to the commercial significance of cultivated strawberries, breeding programs are being conducted to enhance fruit quality in terms of nutritional and sensorial quality, disease resistance, and yield performance. The UNIVPM-D3A breeding program promotes the nutritional properties of novel strawberry genotypes by increasing the concentration of vitamins C, B9, and polyphenols. 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, strawberry cultivars were compared with new selections derived from the D3A breeding program for the content of vitamin C and B9, through the HPLC-UV-FLD analysis. The results showed that many new selections showed higher content of both vitamins than cultivars, indicating the effectiveness of the breeding program to create new selections with improved vitamin content. 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-FLD

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ACRONYMS AND ABBREVIATIONS

HPLC- High Pressure Liquid Chromatography

UV-FLD- Ultraviolet-Fluorescence light detector

DTPA- Diethylenetriaminepentaacetic acid

KH2PO- Potassium phosphate

K2HPO4- Potassium phosphate dibasic.

KH2PO4- Potassium Phosphate monobasic

MPA- Meta-phosphoric acid

BAC- Bioactive Compounds

HBA- Hydroxybenzoic acid

HCA-Hydroxycinnamic acid

TAC- Total Antioxidant Capacity

NQ- Nutritional Quality

THF- Tetrahydrofolate

CVD-cardiovascular disease

CHD- coronary heart disease

UNIVPM- Università Politecnica delle Marche

RDI- Recommended Daily Intake

RDA- Recommended Daily Allowance

DFE- Dietary Folate Equivalent

NTD-Neural Tube Defect

CHAPTER 1

INTRODUCTION

1.1. Overview of Strawberry, Production, Import, and Export

The strawberry plant is a member of the *Rosaceae* family and the genus *Fragaria*, with the most grown species being *Fragaria x ananassa*, also known colloquially as the garden strawberry. There are, however, several species, countless hybrids, and numerous cultivar variants. Strawberry species are classified as diploids, tetraploids, hexaploids, octaploids, or decaploids based on the number of chromosomal pairs (Strawberry Plants.org, 2022). Strawberries (*Fragaria x ananassa*, *Duch*.) are widely consumed and extensively researched for their nutritional and medicinal potential (Mazzoni *et al*., 2021). It is high in bioactive substances such as beta-carotene, phenolic acids, flavonoids, and anthocyanins, as well as vitamins C (ascorbic acid) and B9 (folates) (Mezzetti *et al*., 2016). They are economically and commercially significant, and they are frequently consumed either fresh or in processed forms such as jellies, jams, and juices. As a result, they also happen to be the most researched berry in terms of agronomy and genomics (Giampieri *et al*., 2012). Extracts from berries such as strawberries are increasingly being used as ingredients in functional foods and dietary supplements, often in combination with other fruits, vegetables, and herbal extracts (Nile & Park, 2014).

Strawberry Cultivars are based on their ability to produce fruits during the year and are classified as Short- Day cultivars/ Floricanes (June Bearing), Day- Neutral cultivars/ Remontant, and Long-Day cultivars/ Remontant (Ever Bearing). They can also be distinguished by their ripening times, and they can be categorized into Early- ripening cultivars, Intermediate- ripening cultivars, Late-ripening cultivars, and very late-ripening cultivars (Faedi & Baruzzi, 2016).

The European strawberry market has expanded due to high customer demand throughout the year. To meet this need, numerous EU and non-EU enterprises have made significant efforts to extend production areas in various climates and cultivation systems by using appropriate cultivars. Fresh strawberries are now available on the market practically all year throughout Europe. Southern areas are typically allocated to winter production from November to March. The southern region of Spain is regarded as the major production location, accounting for over 70% of total EU demand. Other countries that contribute to this need include Italy, Greece, Turkey, Morocco, Egypt, and Tunisia (Mezzetti *et al*., 2018a).

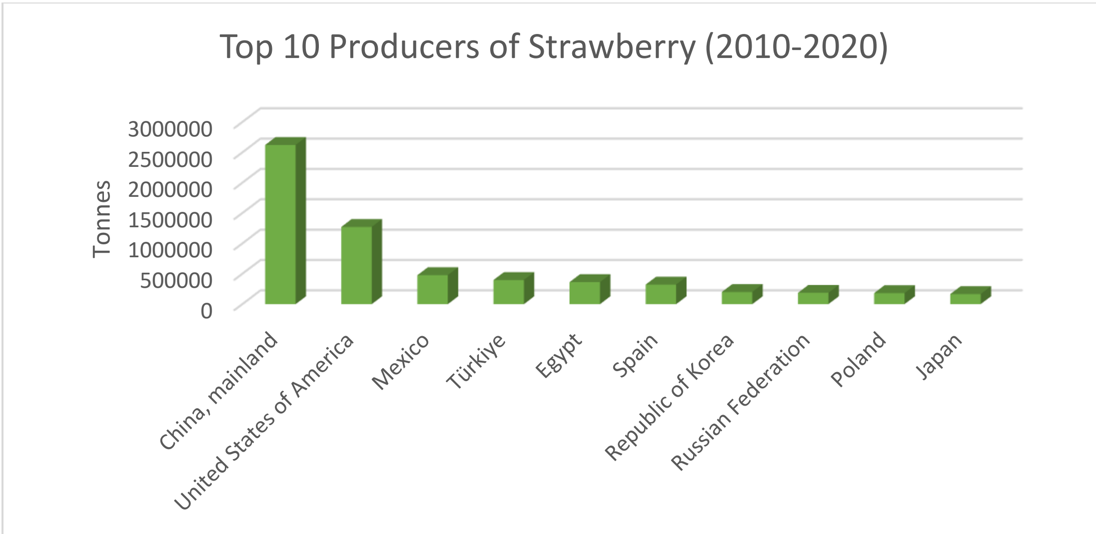

Figure 1*: Top 10 Producers of strawberry* (FAOSTAT, 2020)

Figure 1 is a graphical illustration of the top ten producers of strawberries in the world. The top three leading producers as of 2020 happens to be China, USA and Mexico followed by Turkey, Egypt, Spain, Korea, Russia, Poland, and Japan. The only African representation in the top ten is Egypt. Though other north African countries are also into the production of strawberries, it is important to note that land and climatic issues make the cultivation of strawberries almost impossible in most parts of Africa. Concerning EU representation, Spain is the leading producer of strawberries followed by Russia and Poland.

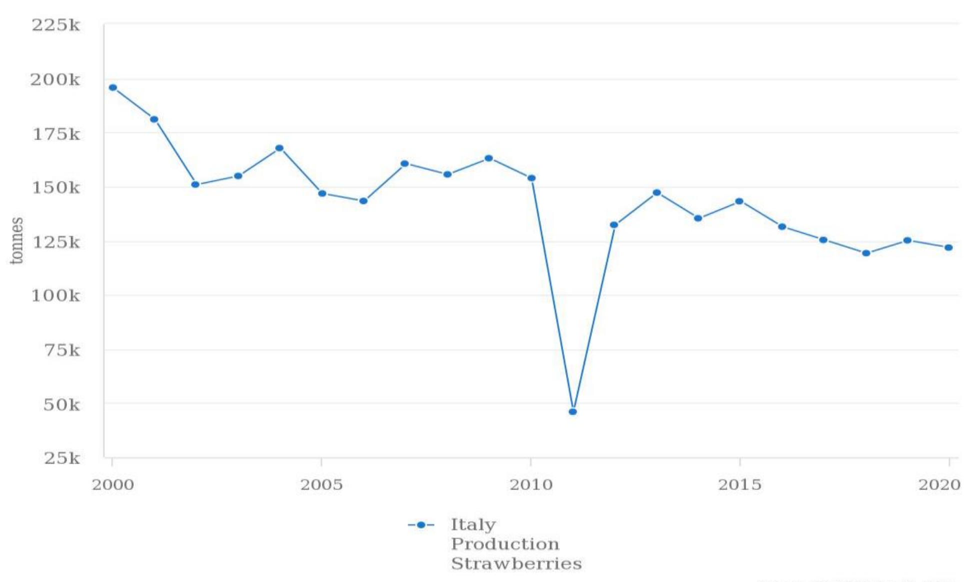

Figure 2*: Production of strawberries in Italy* (FAOSTAT, 2022b)

Figure 2 is a graphical illustration of the gross production of strawberries in Italy from 2000 to 2020. The highest amount of production for the past 20 years was in 2000 after which a decline occurred. After 2001, there was an increase in production in 2004 after which there was a major decline in 2011 which could be related to the economic crises endured by the country. There was a sharp increase in 2012 after which gross production plateaued.

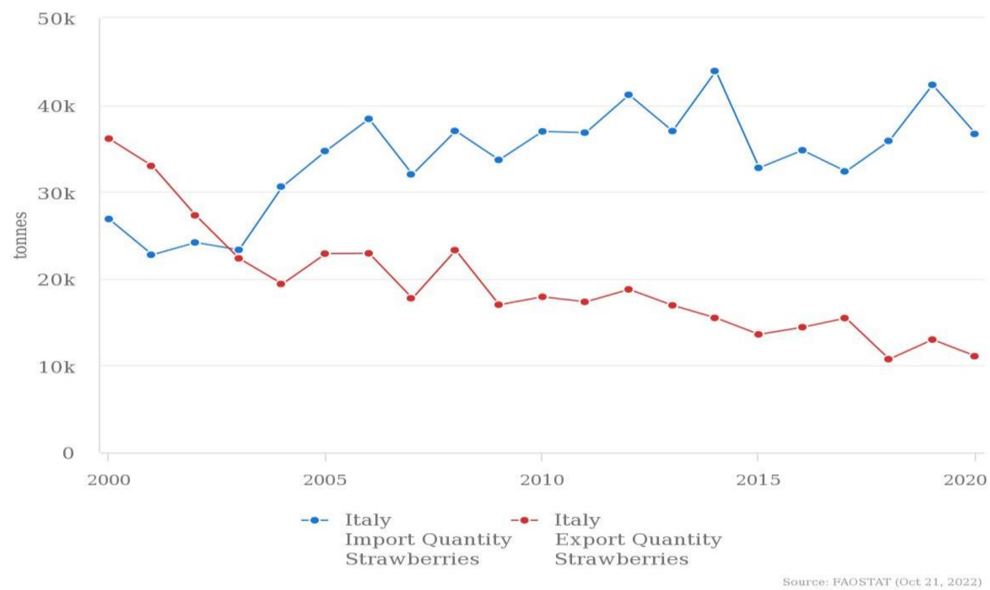

Figure 3*: Import and Export Quantity of strawberries in Italy* (FAOSTAT, 2022a)

Figure 3 represents the import and export quantity of strawberries from 2000 to 2020. From this illustration, it can be said that, from 2003, the import quantity is higher than the export quantity which is probably due to the increased demand for strawberries in the country. More so, the export quantity has dwindled every year which also shows that a greater portion of strawberries produced is retained in the country to meet the demand. Notwithstanding, Italy is a strawberry fruit exporter to other countries. Germany, Austria, Switzerland, the Czech Republic, and Slovenia are Italy's top strawberry export markets. Strawberry fruits shipped from Italy are divided into four categories. They are exported as fresh strawberries, strawberries temporarily preserved by sulfur dioxide gas, brine, sulfur water, or other preservative solutions but unfit for immediate consumption, strawberries prepared or preserved, whether containing added sugar, sweeteners or spirits, or frozen strawberries uncooked or cooked by steaming or boiling in water, whether sweetened or not (Wamucii, 2022).

1.2. Breeding Program of strawberry

Fruit breeding is the intentional genetic enhancement of fruit crops using a variety of approaches, such as selection, hybridization, mutation, induction, and molecular methods. This method is employed to choose genotypes with the most desirable traits (Janick, 2012). When it comes to strawberry breeding, the major goals are to increase agronomical qualities, fulfill nutritional quality criteria, and meet aesthetic standards. Since sensory quality satisfies consumers' hedonistic preferences, it is also addressed. Strawberry fruit genotype has a strong influence on the quantity and quality of bioactive chemicals.

The following distinct market acceptance requirements for new genotypes must be considered when establishing a breeding program for strawberries with an emphasis on acquiring innovative genotypes with greater nutritional quality.

- To achieve general farmer approval, crop productivity (yield) must be maintained or enhanced.
- The levels of micronutrient enrichment attained must significantly impact human health.
- All edaphic habitats and climate zones must have similar micronutrient enrichment properties.
- To make sure they increase the micronutrient status of individuals who prepare and eat them traditionally in typical family settings, the bioavailability of micronutrients in enriched lines must be studied in humans.
- To achieve the greatest influence on nutritional health, consumer approval must be evaluated, as well as taste quality acceptance.

The availability of new fruits with high sensory and nutritional qualities is the only option for encouraging higher fruit consumption with increased health benefits, with the primary goal of enhancing consumer health (Mezzetti, 2013). *Fragaria* x *ananassa*, an interspecific hybrid of *Fragaria virginiana* and *Fragaria chiloensis*, is an allo-octoploid (2n = 8x = 56) species. It is the most extensively strawberry fruit crop in the world, whose 2017 global yearly production exceeded 9 million tons. Due to the commercial significance of cultivated strawberries, breeding projects are being conducted to enhance fruit quality in terms of nutritional and sensorial quality, disease resistance, and yield performance(Yamamoto *et al*., 2021).

The need to improve strawberry characteristics and strawberry farming practices has prompted institutions and individuals to launch significant breeding programs that identify new varieties. North America (35 programs, 13 of which are private), the EU (34 programs, 16 of which are private), non-EU European countries (17 programs, 2 of which are private), and Asia (19 programs, 1 of which is private) have the most breeding programs. There are 79 public and 32 private programs in total (AgroNotizie, 2008).

There are many strawberry germplasm collections in Europe, and these collections are related to taxonomic and phylogenetic research as well as breeding efforts. This aids in promoting connections and exchanges among current germplasm collections to develop an efficient system (unrestricted access databases) for retrieving information such as pedigree, phenotypic data, regional adaptation, genotype, and breeding value. This will help to maximize the use of germplasm collections for breeding programs.

Using cutting-edge molecular tools, a European project on berry genetic resources (Geneberry) funded by EUDG Agriculture has worked to increase and improve the amount of strawberry genetic material available in various European collections, characterize the available germplasm, and better identify materials with the greatest interest as a genetic source for crucial breeding traits like disease resistance and fruit nutritional value quality. Several research stations in several EU nations have been designated as part of this initiative that will oversee the gathering and conserving of strawberry germplasm(Mezzetti *et al*., 2018a).

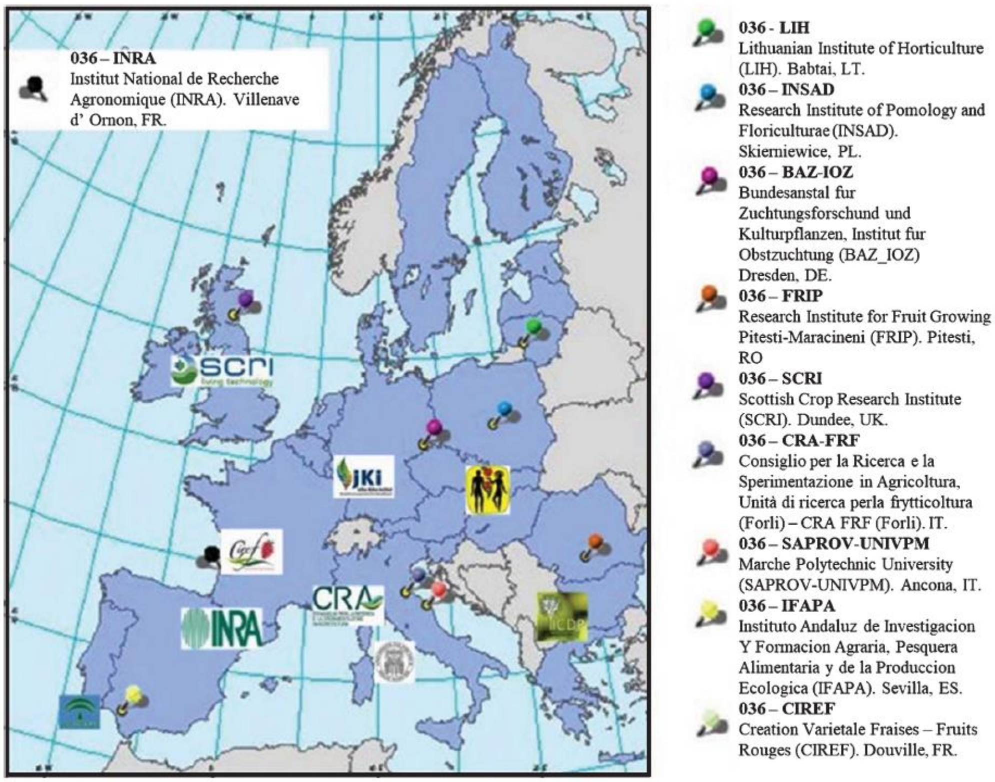

***Figure 4**: list and map of locations of the EU strawberry germplasm repository* (Mezzetti *et al*., 2018a)

Figure 4 is a pictorial illustration of a list and map of locations of the European Union strawberry germplasm repository. In keeping with the topic of breeding programs, the Department of Agricultural, Food, and Environmental Sciences at the Università Politecnica delle Marche (UNIVPM) launched a breeding program in 1993 to develop new cultivars with enhanced fruit quality, high adaptability to heavy-chalky soils, resistance to soil-bound diseases, and late ripening. UNIVPM researchers in the D3A Department have created and are still creating breeding programs to obtain new genotypes with high production levels, high-quality fruits, and adaptability to resilient conditions. This is part of their contribution to the well-being of community stakeholders. By boosting the concentration of vitamin C, vitamin B9, and polyphenols, the UNIVPM-D3A breeding program is also focused on enhancing the nutritional qualities of novel strawberry genotypes. The consistent application of this breeding program has led to the release of eight (8) new commercial varieties that have been registered, namely "Adria", "Cristina," Romina," "Sveva," "Lauretta," "Dina," "Silvia," and "Francesca," as well as hundreds of new selections that are currently being assessed for their high commercial value.

1.3. Nutritional composition of strawberry

Strawberry is a nutritious food choice. First of all, due to their extraordinarily high vitamin C content, strawberries have grown in popularity and are now a significant source of this vitamin for human nutrition. Together with vitamin C, folate is crucial in highlighting the strawberry's micronutrient profile because it is one of the fruit's finest natural sources of this critical component, with an estimated value of 20 to 25 µg/100 g Fresh Weight (FW) (Giampieri *et al*., 2012). They contain potassium, which is essential in many bodily functions such as regulating blood pressure as well as manganese, and lesser amounts of several other vitamins and minerals like vitamin A, vitamin B6, and vitamin B12 just to mention a few (Bjarnadottir, 2019). By slowing down digestion, its dietary fiber and fructose content may assist to regulate blood sugar levels. Additionally, because fiber has a satiating effect, it may also aid to reduce calorie intake. Because strawberry seed oil is rich in unsaturated fatty acids, strawberries are a source of beneficial, essential fatty acids to a lower level (approximately 72 percent polyunsaturated fatty acids) (Giampieri *et al*., 2012; Dragišić Maksimović *et al*., 2015).

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 5**: Nutritional Composition of Strawberry* (Giampieri *et al*., 2012)

1.3.1. Phytochemical Composition of Strawberries

In the diet, strawberries are one of the richest sources of phytochemicals. Although phytochemicals are secondary metabolites created by plants, the term is frequently used to refer to substances derived from plants that may have health benefits but are not considered to be traditional nutrients. They carry out a range of biological tasks, such as defense, development, and plant growth. They also act as pigments, antibacterial and antifungal agents, insect repellents, UV radiation shields, poisonous heavy metal chelators, and antioxidants that quench free radicals produced during photosynthesis (Alvarez-Suarez *et al*., 2014a). The phenolic components found in strawberries are phenolic acids (*hydroxybenzoic acids and hydroxycinnamic acids*), hydrolyzable tannins (*ellagitannins and gallotannins*), flavonoids (*primarily anthocyanins, with flavonols and flavanols playing a small role*), and condensed tannins (*proanthocyanidins*).

Many fruits and vegetables contain flavonoids, which are naturally occurring substances. They help the body rid itself of toxins and contain antioxidant capabilities. Our bodies can preserve their health by consuming foods rich in flavonoids, such as strawberries, in our diet. Flavonoid subtypes include flavanols, flavan-3-ols, flavones, flavanones, isoflavones, and anthocyanins (Watson, 2019). Strawberry is high in anthocyanins, which are the most abundant type of water-soluble pigment in plants. In some plant cultivars, anthocyanins in plant tissues produce pigments with blue, black, red, and purple hues (Tulipani *et al*., 2008). The predominant anthocyanins in cultivated strawberry fruit are pelargonidin-based anthocyanins such as pelargonidin 3-glucoside, pelargonidin 3-rutinoside, and pelargonidin 3-glucoside-succinate. Strawberry fruit contains significantly fewer cyanidin-based anthocyanins, such as cyanidin 3-glucoside and cyanidin 3-glucoside-succinate, than pelargonidin-based anthocyanins (Wang & Lewers, 2007).

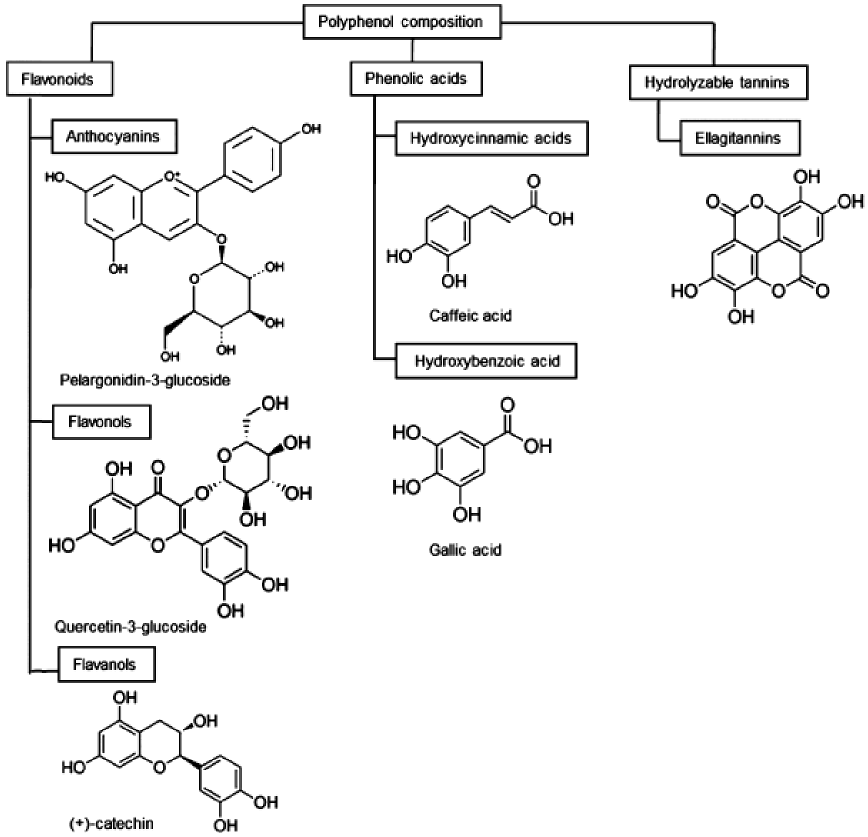

Figure 6: Chemical structures of the main classes of strawberry polyphenols

Pomegranates, strawberries, raspberries, and blackberries all contain ellagic acid (C14H6O8). It has antifibrotic, antioxidant, and anticarcinogenic properties. Several cancers, including cutaneous, esophagus, and colon cancers, have shown that ellagic acid has an anticarcinogenic effect (Edderkaoui *et al*., 2008; Larrosa *et al*., 2006; Stoner & Gupta, 2001). Ellagitannins and ellagic acid glucosides, which break down to pure ellagic acid, are two phenolic acids found in strawberries. Ellagic acid is advantageous to human health because it has antimutagenic and anticarcinogenic properties against malignancies brought on by chemicals. Ellagitannins are mostly present in the achenes of strawberries. Ellagic acid, glucosides, and pure ellagic acid are all present in the red, fleshy receptacle. In contrast to ellagitannins and ellagic acid glucosides, which are easily absorbed in mammalian systems, ellagic acid in its pure form is weakly absorbed and mostly physiologically inaccessible. Strawberry cultivars contain more ellagic acid glucoside than ellagic acid (Okuda *et al*., 1989; Cerdá *et al*., 2005;Wang & Lewers, 2007).

Benzoic acid derivatives and cinnamic acid derivatives are the two distinct kinds of phenolic acids. The hydroxybenzoic acid content in edible plants is typically relatively low, apart from some red fruits, which can have concentrations of several tens of milligrams per kilogram fresh weight. Moreover, hydrolyzable tannins like gallotannins in mangoes and ellagitannins in red fruit like strawberries, raspberries, and blackberries contain complicated structures made of hydroxybenzoic acids (Proestos *et al*., 2011). Because hydroxybenzoic acids, both free and esterified, are only present in a small number of plants used by humans, they have not been extensively studied and are not regarded to be of major nutritional interest. Hydroxybenzoic acids are less frequent than hydroxycinnamic acids (Manach *et al*., 2004).

Class	Group	Compound
Flavonoids	Anthocyanins	Cyanidin-3-glucoside
		Cyanidin-3-rutinoside
		Cyanidin-3-malonylglucoside
		Cyanidin-3-malonylglucosyl-5-glucoside
		Pelargonidin-3-galactoside
		Pelargonidin-3-glucoside
		Pelargonidin-3-rutinoside
		Pelargonidin-3-arabinoside
		Pelargonidin-3,5-diglucoside
		Pelargonidin-3-malylglucoside
		Pelargonidin-3-malonylglucoside
		Pelargonidin-3-acetylglucoside
		Pelargonidin-dissacharide (hexose + pentose) acylated with acetic acid
		5-pyranopelargonidin-3-glucoside
	Flavonols	Quercetin-3-glucuronide
		Quercetin-3-malonyglucoside
		Quercetin-rutinoside
		Quercetin-glucoside
		Quercetin-glucuronide
		Kaempferol-3-glucoside
		Kaempferol-3-malonyglucoside
		Kaempferol-coumaroyl-glucoside
		Kaempferol-glucunoride
	Flavanols	Proanthocyanidin B1 (EC-4,8-C)
		Proanthocyanidin trimer (EC-4,8-EC-4,8-C)
		Proanthocyanidin B3 (C-4,8-C)
		(+)-catechin
Phenolic acids	Hydroxycinnamic acids	*p*-coumaroyl hexose
Hydrolyzable tannins	Ellagitannins	Ellagitannin
		Bis-HHDP-glucose
		Galloyl-HHDP-glucose
		HHDP-galloyl-glucose
		Galloyl-bis-HHDP-glucose
		Dimer of galloyl-bis-HHDP
		Sanguiin H-6
		Methyl-EA-pentose conjugates
		Ellagic acid pentoside
		Ellagic acid

EA, ellagic acid; HHDP, galloylbis-hexahydroxydiphenoyl

***Figure** 7: Polyphenol composition reported in strawberries* (Giampieri *et al*., 2012)

1.4. Health Benefits of strawberry

According to several studies, phytochemical composition and antioxidant activity of fruits and vegetables help them fight off chronic and degenerative diseases. To prevent damage to lipids, proteins, and nucleic acids as well as eventual cellular damage and death, these natural antioxidants either scavenge free radicals or suppress the production of reactive species during normal cell metabolism (Meyers *et al*., 2003). Berries have been investigated for their biological activity *in vitro* and in animal models for an exceptionally long time, but there is increasing evidence from human epidemiologic and interventional investigations. Inflammation, oxidative stress, cardiovascular disease (CVD), as well as some cancers, type 2 diabetes, obesity, and neurodegeneration have all been associated with strawberry consumption.(Giampieri *et al*., 2012; Tulipani *et al*., 2009; Zafra-Stone *et al*., 2007).

The primary global cause of illness and mortality is cardiovascular diseases. Approximately in the next ten years, it is anticipated that the number of fatalities from cardiovascular diseases—which currently account for over 17 million deaths annually—will increase by 30 percent. However, most CVDs may be avoided. Observational studies have shown a relationship between fruit consumption and a reduced risk of total CVD, CHD, and stroke (Aune *et al*., 2017). A popular fruit known for lowering the risk of such diseases is strawberries. It has been demonstrated that strawberry polyphenols possess antibacterial, anti-allergy, and antihypertensive characteristics. They have also been found to block the actions of several physiological enzymes and receptors and to offer protection from oxidative stress-related disorders (Gao *et al*., 2020).

Evidence suggests that berry fruits may be helpful in the treatment of several human malignancies. According to Seeram N. (2008), the abundance of bioactive phytochemicals present in berry fruits such as polyphenols, stilbenoids, lignans, and triterpenoids, has been connected to the anticancer potential of berries, at least in part. According to studies, anticancer properties of berry bioactives are partially mediated by their capacity to mitigate, lessen, and repair harm brought on by oxidative stress and inflammation. Berry bio-actives also affect the enzymes that break down xenobiotics and carcinogens. Consuming berry fruit may offer a defense against radiotherapy-related harm.

Human interventional studies have shown that strawberries can reduce lipids, lipid oxidation, postprandial hyperglycemia, hyperlipidemia, and inflammatory responses in both healthy and CVD-risk participants, such as those with metabolic syndrome (Basu & Lyons, 2012). In cell and animal models of diabetes and obesity, strawberry fruits, extracts, or purified anthocyanins have been demonstrated to have the following therapeutic effects: activation of the endothelial nitric oxide synthase (eNOS), inhibition of glucose uptake and transport, normalization of blood sugar levels, inhibition of the digestive enzymes that break down carbohydrates and lipids, particularly -glucosidase and -amylase, and pancreatic lipase activity, and inhibition of the angiotensin I-converting enzyme (ACE), which may be related to the therapeutic management of hyperglycemia and hypertension (Lazzè *et al*., 2006; Manzano & Williamson, 2010;Roy *et al*., 2008;McDougall *et al*., 2005;Cheplick *et al*., 2010).

1.5. Vitamin C Bio-Accessibility

The bio-accessibility of micronutrients from dietary matrixes in the human digestive tract and subsequent bioavailability is critical for their positive effects. Bio-accessibility is the quantity of a bioactive substance that is released from a solid food matrix into the gastrointestinal tract and may be able to penetrate the intestinal barrier. Enzymatic breakdown, which includes digestion in the upper portions of the human digestive tract and additional breakdown of the residual solids by gut bacteria in the colon, releases micronutrients from food matrixes (Balasooriya *et al*., 2020; Mullen *et al*., 2008).

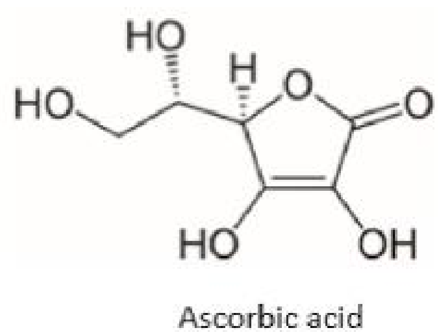

Figure 8*: Ascorbic acid chemical structure*

Vitamin C exists in two biologically active forms, L-ascorbic acid, and L-dehydroascorbic acid, and is required for a variety of metabolic functions. L-ascorbic acid oxidizes easily to L-dehydroascorbic acid, which can then be reduced back to L-ascorbic acid (Smirnoff, 2018). In many biochemical reactions, vitamin C acts as a reducing agent or antioxidant (Yaman *et al*., 2021). Vitamin C is an essential nutrient that cannot be synthesized by humans due to the loss of a key enzyme in the biosynthetic pathway. Scurvy, a potentially fatal disease, is caused by severe vitamin C deficiency. Scurvy is characterized by the weakening of collagenous structures, which results in poor wound healing and impaired immunity (Carr & Maggini, 2017).

There are several dietary sources of vitamin C such as guava, kiwi, orange, grapefruit, mango, and strawberry. The effectiveness of how well vitamin C is absorbed depends largely on how much is ingested. At modest doses (20 mg), absorption can approach 100%, but at high doses (12 g), absorption drops to 16%. One dose of 100 mg/d is sufficient to saturate the tissue; however, greater intakes (>500 mg/d) are necessary to saturate the plasma and enhance antioxidant protection (Johnston & Cox, 2001). Single dosages of more than 1000 mg/d might result in nausea, osmotic diarrhea, and digestive upset as the body works to rid itself of the high intraluminal concentration of vitamin C. The probability of osmotic diarrhea and other gastrointestinal disorders led to the development of the tolerated upper intake level (UL) for vitamin C, which is 2000 mg per day (Schlueter & Johnston, 2011). According to Yaman *et al*., (2021), vitamin C is the most susceptible water-soluble vitamin to degradation. Significant vitamin C losses can be caused by oxygen, light, crushing, cutting, chopping, washing, cooking, canning, and the presence of metal ions such as $Cu2+$ and $Fe3+$. However, its bioaccessibility improves when antioxidants and some binding proteins are present. In a study comparing the bioaccessibility of fortified vitamin C in dietary supplements, infant formula, and fortified foods to multi-supplements (0.1–44%), it was discovered that mono-supplements (49–99%), infant formulas (0.3–1.4%), and fortified foods (0.4-6%) had higher vitamin C bioaccessibility than other vitamin supplements.

The authors hypothesized that additional food ingredients and encapsulation may interfere with vitamin C bioaccessibility in the gastrointestinal system, leading to reduced bioaccessibility in multi-supplements and other fortified foods (Brandon *et al*., 2014). Consumption of vitamin C from foods or supplements has a positive influence on many disease states. Vitamin C is thought to shorten and lessen the severity of common cold symptoms by boosting immune responses and acting as an antihistamine. High vitamin C intake has been linked to a lower risk of certain cancers, particularly cancers of the pharynx, oral cavity, esophagus, lung, and stomach (Schlueter & Johnston, 2011).

1.6. Vitamin B9 (Folate) Bio-Accessibility

Folates are found in the structure of pterin as methylene bridges to p-aminobenzoic acid, which binds to glutamic acid(s) via peptide bonds. All folates are found primarily in polyglutamate forms, which contain five to seven glutamate residues linked by a peptide link. Folic acid, also known as pteroylglutamic acid, is not found naturally in foods and, unlike natural forms, contains only one glutamic acid. Natural forms of folates are abundant in foods, primarily in the form of tetrahydrofolate (THF), 5-methyl-THF, and 10-formyl-THF (Yaman *et al*., 2021).

Folate is a crucial vitamin for expectant mothers and women who are breastfeeding because it protects against neural tube abnormalities, which can cause congenital malformations like spina bifida and/or anencephaly, a condition in which a sizable portion of the brain is missing. Additionally, folate may aid in the prevention of several malignancies as well as neuropsychiatric and neurological conditions like Alzheimer's, dementia, and depression (Kronenberg *et al*., 2009; Oakley, 2002; Rampersaud *et al*., 2002). It is necessary for human metabolism since it plays a role in cell division, DNA replication, methylation, nucleotide biosynthesis, and amino acid metabolism (Bationo *et al*., 2020).

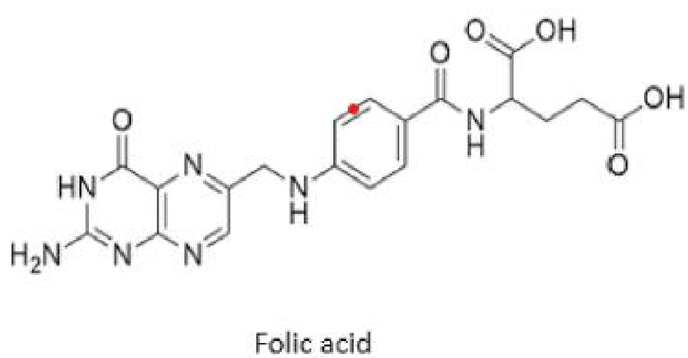

***Figure 9**: Folic acid chemical structure*

Folic acid, a synthetic form of the vitamin, is found in supplements as well as fortified foods and exists as a monoglutamate. The term "polyglutamate" refers to the main chemical form of folate that may be found in nature. This is because the polyglutamate form has a side chain of conjugated glutamic acid molecules that must be cleaved to the monoglutamate form for absorption to take place. On the other hand, folic acid already exists as a monoglutamate. The observed disparities in bioavailability between synthetic and dietary folate are due to their structural differences, with synthetic folate being more easily absorbed (Rampersaud *et al*., 2002).

The dynamic gastrointestinal model is the gastrointestinal model for measuring folate bioaccessibility (TIM). Verwei *et al*., (2003) used this model to investigate the bioaccessibility of folate and folic acid from foods. It was discovered that folate binding proteins (FBPs) added to milk samples have different folic acid and 5-CH3-H4-folate binding properties. A large fraction of folic acid remains bound to FBPs during gastric passage, whereas a large fraction of 5-CH3-H4-folate dissociates from the FBP, increasing the vitamin's bioaccessibility. It was concluded in that study that the fortification of milk with 5-CH3-H4-folate yielded a higher folate bioaccessibility than that fortified with folic acid.

1.7. Pre- and Post-harvest factors affecting the composition of strawberries.

Many preharvest and postharvest conditions are known to affect the concentrations of micronutrients and phenolic compounds in strawberries. Genetics and degree of maturation are two preharvest factors, and processing conditions and storage time are two postharvest factors (Alvarez-Suarez *et al*., 2014a). In terms of the degree of ripening or maturity, according to a study conducted by Tulipani *et al*. (2011) to evaluate the ripening and seasonal influence on the fruit's nutritional and non-nutritional traits, the total antioxidant capacity (TAC) of the strawberries gradually decreased during ripening, and in parallel, a decrease in the total content of phenolics and flavonoids was observed. However, anthocyanins accumulated in the fruit, and caused its red color, at the fruit's last phases of maturity. The sharp decline in tannins that occurs during fruit ripening has been proven to be the sole cause of the fall in antioxidant capabilities. These dynamics prove that the polyphenolic composition of strawberries is susceptible to change. The polyphenolic composition of unripe fruits is always higher than ripe fruits. Since there was a minor but insignificant rise in vitamin C concentration, it appeared that the vitamin C levels were not impacted by the fruit's developmental stage (Olsson *et al*., 2004).

Genetic factors are known to affect the polyphenolic composition of strawberries in general. It plays a vital role in how nutritional components are exhibited in these fruits since the micronutrients found in these fruits may differ from one genotype or cultivar to the other. According to Alvarez-Suarez *et al*., (2014b) and Wang & Lin (2000), a comparison of total phenolic contents among several genotypes showed a wide range of differences.

Concerning environmental factors, scientists found that fruits planted in compost sacks had much higher phenolic content, flavonoid, anthocyanin, and oxygen radical absorption capability than fruits produced in a matted row method. Additionally, these scientists found that adding vinegar to culture practices significantly changed the levels of total phenolic, total anthocyanin, and TAC (Wang & Millner, 2009). It is safe to deduce that the substantial variation in micronutrient and phytochemical content is a result of the environmental factors that affect plant growth, including climate changes that take place during the strawberry ripening season.

Strawberries generally have a short postharvest life due to their high-water content, high metabolic activity, and susceptibility to microbial spoilage (Ayala-Zavala *et al*., 2004). Storage temperature happens to be one of the most principal factors that affect the overall quality index of strawberries in terms of nutritional and aesthetic quality. A study conducted to determine the effects of different temperatures (10°C, 5°C, 0°C) on total phenolics, anthocyanins, antioxidant capacity as well as overall fruit quality resulted in a continuously increasing overall quality loss of strawberries stored at 10°C at a higher rate than in those stored at 5°C and 0°C. A storage temperature of 0°C was the most effective in maintaining the highest overall quality of strawberry fruit during the storage period. Strawberries at 5°C maintained an acceptable quality for up to 7 days. The total phenolic compounds also increased continuously in berries stored at 10°C and 5°C but remained constant at 0°C. Overall quality is better maintained at 0°C but a storage temperature of 10°C enhances the antioxidant capacity (Ayala-Zavala *et al*., 2004).

1.8. Breeding Techniques for enhancing nutritional quality of strawberries.

It has become evident that strawberries and fruits in general contain great amounts of micronutrients, macronutrients and antinutrients. To enhance the desirable nutrients and suppress unwanted ones, several techniques have been adopted and this has brought about many health benefits to the consumer (Sabbadini *et al*., 2021). Techniques that have been accepted and are practiced for this form of nutrient enhancement or biofortification are agronomic practices, conventional breeding, and biotechnological approaches (Garg *et al*., 2018). But the most accepted technique is the conventional breeding technique. Conventional breeding, also combined with mutagenesis (considered to be traditional breeding) is deemed as a more cost-effective and sustainable system. It has been used to address nutritional improvement, commercial and agronomic traits such as yield, architecture, resistance against biotic, abiotic stresses and physiological disorders in staple foods with very little attention paid to the nutritional quality of fruits (Ray, 2002). This is because conventional breeding is particularly longer in fruits due to the long juvenile phase of some fruit species as well as the unexpected or undesirable traits of progeny obtained. These reasons among others have propelled the establishment of new and improved technologies that improve variability, shorten the breeding process, cause the expression of traits of interest in new progeny and overall fruit quality improvement (Karanjalker & Begane, 2016).

When choosing a successful strawberry cultivar, many traits must be taken into account, including disease resistance, fruit firmness and vulnerability, productivity, and of course, taste. However, breeding for strawberry improvement is challenging because *Fragaria* x *ananassa* exhibits very little genetic variation, which is an important requirement for advancement in conventional breeding. Additionally, the strawberry's octoploid hybrid status, which resulted from a relatively recent cross between two wild octoploid *Fragaria* species, *F. virginiana* and *F. chiloensis,* complicates breeding because of the strawberry's complex genetic makeup (Devold Kjellsen *et al*., 2016).

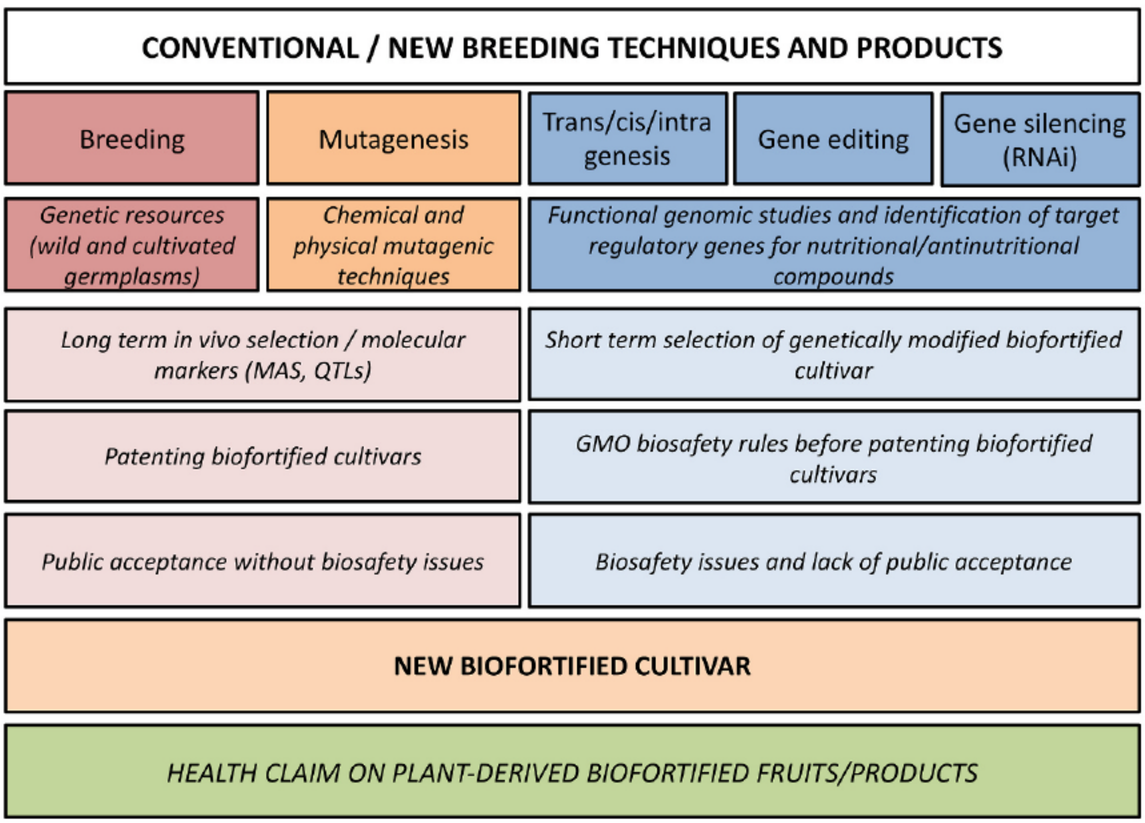

Figure 10*: Conventional and New Breeding Techniques.* (Sabbadini *et al*., 2021)

Figure 10 is a diagram that illustrates the conventional and new breeding techniques that have been adopted for strawberry improvement. The strawberry fruit has eventually been improved through the application of new breeding techniques including intragenesis and cisgenesis, which use genes and regulatory elements from the strawberry species' own DNA or from cross-compatible species. A gene of interest, as well as its own introns, 5′ - and 3′ -untranslated sections, and regulatory elements (promoter and terminator) are all present in the newly imported DNA in cisgenesis (Schouten *et al*., 2006). In contrast to cisgenesis, intragenesis creates new genes by merging functional genetic elements such as promoters, coding regions (with or without introns), and terminators of several natural genes. This new chimeric gene is then inserted into the genome (Rommens, 2004). Contrarily, transgenesis is the genetic alteration of a recipient plant with one or more genes from any non-plant organism or from a donor plant that is sexually incompatible with the recipient plant (Schouten *et al*., 2006).

Widespread use of plant genome editing has transformed agricultural improvement. Due to precise DNA manipulation, genome-editing technologies like CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated systems), TALENs (Transcription Activator Like Effector Nucleases), and others offer an unprecedented development in genome engineering (Nadakuduti *et al*., 2018). CRISPR-based gene-editing is a revolutionary scientific tool that is being rapidly utilized in many crops. This technology can modify a gene of interest without altering the DNA of an elite variety. Gene editing enables direct repair of the genetic sequence in an already elite breeding selection or variety, eliminating the need for years of breeding to introduce a resistant gene from a wild strawberry into elite germplasm. Given that cultivated strawberries have complicated genetics, this is very helpful. Instead of rearranging the genetic cards in the hopes of creating a plant with all desired traits present, it would be far simpler to simply modify one trait (Lee, 2016).

RNA interference is yet another innovative breeding method. This is a unique strategy that has a great deal of promise to change gene expression in plants to improve nutritional value and quality attributes in various crops. This method has been utilized to produce parthenocarpy in fruits and to silence the genes that produce ethylene to regulate ripening in fruits, giving the majority of crops a high commercial value. It makes use of a sequence-specific gene-silencing mechanism that is started by the addition of double-stranded RNA (dsRNA), which causes the destruction of mRNA. Small interfering RNAs (siRNAs) are then produced from the dsRNA via the action of endonuclease Dicer. Based on sequence complementarities with the siRNA, the RNAi-induced silencing (RISC) complex then eliminates particular target mRNAs. This is a great method that has developed into a potent tool for silencing the expression of target genes (Schab *et al*., 2011; Younis *et al*., 2014). In both kinds of techniques, new biofortified cultivars are obtained. However, in conventional breeding techniques, in vivo selection/ Marker Assisted selection is long term whereas in the new breeding techniques, obtaining new cultivars do not take as long. On the other hand, new breeding techniques need to follow GMO biosafety rules before any form of patenting can take place. New cultivars obtained from these new techniques experience a lack of public acceptance as compared to cultivars obtained from classical breeding techniques (Sabbadini *et al*., 2021).

1.9. Background and objective of the Study

It has become critical for consumers to pay close attention to their health and diet. Because of the functional properties of fruits and vegetables, most individuals have established the habit of including more of them in their diets. They are high in antioxidants, which slow the beginning of aging and, more significantly, lower the risk of cardiovascular disease and cancer. Their ability to scavenge free reactive radicals within the body primarily manifests in their metabolic activity. Due to their capacity to deliver essential antioxidants and bioactive compounds for improved health, fruits and vegetables have seen an increase in consumption.

Berry fruits are widely consumed due to their appealing organoleptic qualities and elevated levels of natural antioxidants. They are rich in ascorbic acid, carotenoids, vitamin E, fiber, and phenolic compounds with ascorbic acid and phenolics being the most prevalent (Manganaris *et al*., 2014; Tulipani *et al*., 2008). One of the most significant sources of these beneficial compounds is strawberries. Other berries like raspberry (*Rubus species*), black raspberry (*Rubus accidentalis*), red raspberry (*Rubus idaeus*), blueberry (*Vaccinium corymbosum*), and cranberry (*Vaccinium macrocarpon*), are prominent among the colorful fruits (Nile & Park, 2014).

In recent times, the demand for strawberries has been on the increase. Biotechnology and breeding have been used to create new or improved varieties that have higher concentrations of vital bioactive components and antioxidants. This strategy supports a higher antioxidant intake even when fruit consumption is low (Mezzetti *et al*., 2018a). One of the main aims of breeding has been to boost plant yield by altering significant morphological or physiological traits ever since the advent of intensive agriculture. However, in recent years, the market for agricultural products has changed in favor of genotypes whose cultivation could lessen environmental effects, the creation and use of modern technology, and fruits with improved nutritional and organoleptic qualities. Crops are altered to have functional traits that would go well with new agronomic techniques as well as consumer demands. Nonetheless, there are still constraints in the use of the biotechnological approach in terms of commercial exploitation of new products due to public concern and biosafety regulations. Therefore, traditional breeding has since been embraced. However, it is important to note that the most useful genetic resources must be known and employed (Diamanti *et al*., 2011). As part of the UNIVPM-D3A breeding program, this study's main objective was to identify the variations in vitamin C and B9 concentration in well-defined strawberry cultivars and advanced strawberry breeding selections.

CHAPTER 2

MATERIALS AND METHODS

2.1. Planting materials, harvesting and Sample preparation

In the "P. Rosati" experimental farm of Università Politecnica delle Marche, fifty-two (52) new strawberry selections and seven (7) cultivars from the UNIVPM-D3A breeding program were planted in 2021 in non-fumigated soil and used for the study (Table 1) The strawberries were cultivated in open fields using the plastic hill culture method at the farm in Agugliano (Ancona, Italy). Each cultivar and selection were planted in a single plot of six plants, cultivated with the standard integrated pest management system, and harvested the following year (2022). At the second, third, and fourth seasonal pickings, the strawberries were handpicked in large quantities when they were fully red. Each genotype was given a label and kept in the lab refrigerator at -20°C pending further analysis. Ten (10) ripe strawberry fruits from each genotype were randomly sampled. The opposite (head and tail) sides of each fruit were cut into tiny pieces and uniformly combined. Prior to extracting Vitamin C and Folate, a representative sample of about 1 g and 2 g respectively, collected from the chopped fruits were weighed in a falcon tube, labeled, and stored at -20 °C. The same procedure was repeated for each genotype.

Table 1*: List of 2022 Cultivars and Selections of strawberry fruit under study*

2022			
CULTIVARS	SELECTIONS		
SIBILLA	AN17,09,59	AN14,20,57	AN17,30,53
FRANCESCA	AN17,09,57	AN18,47,53	AN16,16,53
DINA	AN16,34,55	AN16,12,59	AN14,16,62
TEA	AN14,25,54	AN17,43,113	AN12,29,60
SILVIA	AN17,31,54	AN13,13,62	AN14,21,56
ARIANNA	AN17,50,110	AN15,07,53	AN18,07,58
LAURETTA	AN12,24,52	AN18,40,56	AN17,50,02
	AN17,09,60	AN14,20,54	AN18,16,53
	AN15,25,53	AN12,20,53	AN13,16,57
	AN17,45,141	AN18,16,54	AN17,35,54
	AN18,19,53	AN17,35,53	AN15,19,55

	AN14,12,58	AN17,45,112	AN15,25,52
	AN12,13,58	AN12,44,60	AN14,22,51
	AN16,42,54	AN18,44,51	AN12,27,61
	AN13,13,55	AN12,23,53	AN15,09,57
	AN16,15,53	AN17,45,39	AN14,03,51
	AN17,03,54	AN14,08,55	AN13,16,56
	AN16,53,54		

2.2. Vitamin C extraction.

The method cited by Mezzetti *et al*. (2016) was applied with minor modifications. Four milliliters (4 mL) of the extract solution consisting of MilliQ water containing 5% meta-phosphoric acid and 1 mM diethylenetriaminepentaacetic acid (DTPA) was added to 1 g of frozen strawberry samples and homogenized for 30 seconds on medium-high speed with an Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Denmark). Following that, the homogenized sample was sonicated for 5 minutes using the transonic bath 460 (Elma). The theory behind this equipment is that waves are generated to promote the extraction of vitamin C from the strawberry sample into the extract solution. After centrifugation at 4500 rpm for 10 minutes at 4 °C, the supernatant was filtered into 1.8 mL HPLC vials using a 0.45 m NY (nylon) filter and analyzed. The same method was followed for each genotype.

2.2.1. Quantification of Vitamin C using HPLC

The HPLC system included a Jasco PU-2089 Plus controller, a Jasco UV-2070 Plus ultraviolet (UV) detector (Jasco Inc., Easton, MD, USA) set at an absorbance of 260 nm, and an autosampler AS-4050 (Jasco, Easton, MD, USA). The HPLC column utilized was an Ascentis Express C18 150 4.6 mm (Sigma-Aldrich Corp., St. Louis, MO, USA). The elution was isocratic with 50 mM of KH2PO in MilliQ (MQ) water that had been adjusted to pH 3.2 (below the pKa of the ascorbic acid) using orthophosphoric acid. After running the analysis for 10 minutes, the column was washed with 50% acetonitrile. Finally, the Vit C content was quantified using a calibration curve created by running standard concentrations of vitamin C. The results were given as mg Vit C per 100 g FW (Fresh weight).

2.3. Folate extraction.

The method cited by Mezzetti *et al*. (2016) was applied with minor modifications. Eight milliliters (8 mL) of the extract solution (Phosphate buffer made by dissolving 13.6 g of KH2PO4 and 17.4 g of K2HPO4 in 1L deionized water, stirring on a magnetic stirrer, and adjusting the pH to roughly 6.1 before adding 10 g of L (+)- ascorbic acid and 1 mL of 2 mercaptoethanol (v/v)) were pipetted into falcon tubes containing 2 g of frozen strawberry samples and homogenized for 30 seconds on medium-high speed with the Ultraturrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik, Staufen, Denmark). The falcon tubes were loosely closed and boiled for 12 minutes at 180 °C on a heating platform (the goal was to destroy the other molecules in the strawberry samples and free the folate molecule into the extraction solution) before being rapidly cooled in the freezer for 10 minutes. Hog kidney folate conjugase was taken, and 1.5 mL of the enzyme was added to the cooled solution (the aim is to deconjugate polyglutamylated folates), which was then incubated for 3 hours in a shaking oven at 37 °C. The enzyme was then inactivated by boiling for 5 minutes on a heat plate, followed by 10 minutes in the freezer. After centrifuging the samples at 4500 rpm for 30 minutes at 4 °C, the supernatant was transferred to a newly labeled falcon tube. 8 mL of the extract solution was added to the remaining pellet and centrifuged for 30 minutes at 4 °C at 4500 rpm before transferring the supernatant to a fresh falcon tube holding the previous supernatant to obtain the 16 mL mark. The extraction solution was then used to top up the container until it reached the 25 mL mark. The resulting supernatant of 25 mL was then filtered using 0.45-µm pore size, 25-mm inner diameter nylon disposable syringe filters, and the filtrates were purified using solid-phase extraction on strong anion-exchange Isolate cartridges as described by Iniesta et al. (2009).

2.3.1. Solid Phase Extraction

The second step of folate extraction is the Solid Phase Extraction. This process aids in the separation and purification of folates from undesirable molecules. The eluting solution was prepared for this process using the following components: 0.1 mol/l sodium acetate containing 10% (w/v) sodium chloride, 1% (w/v) ascorbic acid, and 0.1% 2-mercaptoethanol. To ensure the interaction of the SPE material with the sample, the SPE cartridges were conditioned (activated and made more effective) using 2.5ml of methanol that was run twice through the cartridge and then equilibrated with the same volume of water. Aliquots of samples were then placed into preconditioned cartridges and allowed to run through the SPE material under a vacuum. After washing away undesired components from the cartridges with 0.7ml of the eluting solution, the desirable fraction adhered to the cartridge was collected into 15 mL labeled falcon tubes with 4 mL of the eluting solution and stored at -20 °C until further analysis.

2.3.2. Quantification of Folate using HPLC

With minor modifications, the folates were determined using the HPLC as described by Strålsjö *et al*., (2003). The HPLC system included a pump model PU-2089 (Jasco, Easton, MD, USA), a Fluorescence detector (FLD) FP-2020 Plus (Jasco, Easton, MD, USA) with excitation and emission wavelengths of 290 nm and 360 nm, and an autosampler AS-4050 (Jasco, Easton, MD, USA). A Luna C18, 2504.6, 5 µm analytical column was used (Phenomenex, Torrance, California, USA). The mobile phase was 30 mmol/l phosphate buffer, pH 2.3, with an acetonitrile gradient commencing at 6%, a lag time of 5 minutes, and rising linearly to 25% within 20 minutes. The overall running time was 33 minutes. Peak identification was accomplished with a retention time of 10 minutes, and folate content was quantified using a calibration curve created by running standard quantities of 5-methyl-tetrahydrofolic acid (5-CH3-H4folate). The results are reported as µg 5-CH3-H4folate per 100g of fresh strawberry weight (µg 5-CH3-H4folate/100g FW). To accurately quantify and describe the folate, standard quantities of 5-methyl-tetrahydrofolic acid (5-CH3-H4folate) were prepared and run, and a calibration chromatogram was generated. The concentration of folate in the samples was determined by comparing their peaks to the standard. The same approach was used to evaluate all the genotype sampled.

2.4. Statistical analysis

Statistical analyses were carried out with the help of the software "Statistica 7" (Stasoft, Tibco Software, Palo Alto, California, USA). The data were analyzed using one-way ANOVA, and means were compared using the Tukey test with $p \leq 0.05$. The results were presented as mean standard error (SE).

CHAPTER 3

RESULTS AND DISCUSSION

Fruits' nutritional value has received a lot of attention from consumers in recent years, largely due to the positive effects they have on health (Alvarez-Suarez *et al*., 2014b). A standout among the many fruits sold on the international market for its distinct flavor and health advantages is the strawberry. Consumers of today are more informed, and they have higher expectations for superior plant produce that is safer and more nutritionally versatile (Bhat *et al*., 2015) to generally increase fruit consumption. Fruit consumption is directly influenced by several variables, including price, but primarily by consumer acceptance of quality. As a result, newly introduced varieties with improved fruit nutritional content are strictly tied to the improvement in consumer health. The assessment of strawberry fruit nutritional quality is a crucial task for quantifying the nutritional composition and determining the commercial viability of newly released cultivars (Capocasa *et al*., 2008). Thus, the need for this research.

3.1 VITAMIN C DISCUSSION

3.1.1. Commercial cultivars of strawberry

Figure 11 below illustrates the trend of the fruits of seven (7) commercial cultivars and their respective vitamin C contents in mg vit C/100 g FW of the fruit. The highest amount of vitamin C was recorded by the fruits of **Lauretta** with 57.69 mg vit C/100 g FW followed by the fruits of **Arianna** with 53.94 mg vit C/100 g FW, fruits of **Silvia** with 51.49 mg vit C/100 g FW, fruits of **Tea** with 43.44 mg vit C/100 g FW, fruits of **Dina** with 42.02 mg vit C/100 g FW, fruits of **Francesca** with 35.71 mg vit C/100 g FW and the lowest being fruits of **Sibilla** with 24.84 mg vit C/100 g FW. In a previous study conducted by Mazzoni *et al*., (2021), **Sibilla** performed much better than it did in this current study. This can possibly be attributed to pre, and postharvest factors as stated by (Alvarez-Suarez *et al*., 2014a). Among all seven (7) cultivars, minimal significant differences ($P \leq 0.05$) were observed. From the figure, it can be deduced that there are no significant differences between **Dina**, **Tea**, **Silvia**, **Arianna,** and **Lauretta**.

More so, no significant difference was observed between Francesca and Dina. The same can also be said for Sibilla and Francesca. However, there is a significant difference between Francesca and Silvia, Arianna, and Lauretta (from left to right). Sibilla is also significantly different from Dina, Tea, Silvia, Arianna, and Lauretta. Comparing the four cultivars obtained from the UNIVPM D3A breeding program (Lauretta, Silvia, Dina, and Francesca), the fruits of Lauretta, and Silvia were the top two with the highest fruit Vitamin C content followed by that of Dina, and Francesca. The average vitamin C content across all cultivars was pegged at 44.16 mg vit C/100 g FW. Comparing to the recommended dietary allowance of 90 mg/day for men and 75 mg/day for women as stated by (US National Institutes of Health, 2021) and (Jacob & Sotoudeh, 2002), the average amount recorded is sufficient since it represents about 50% of the recommended amount required per day for both men and women.

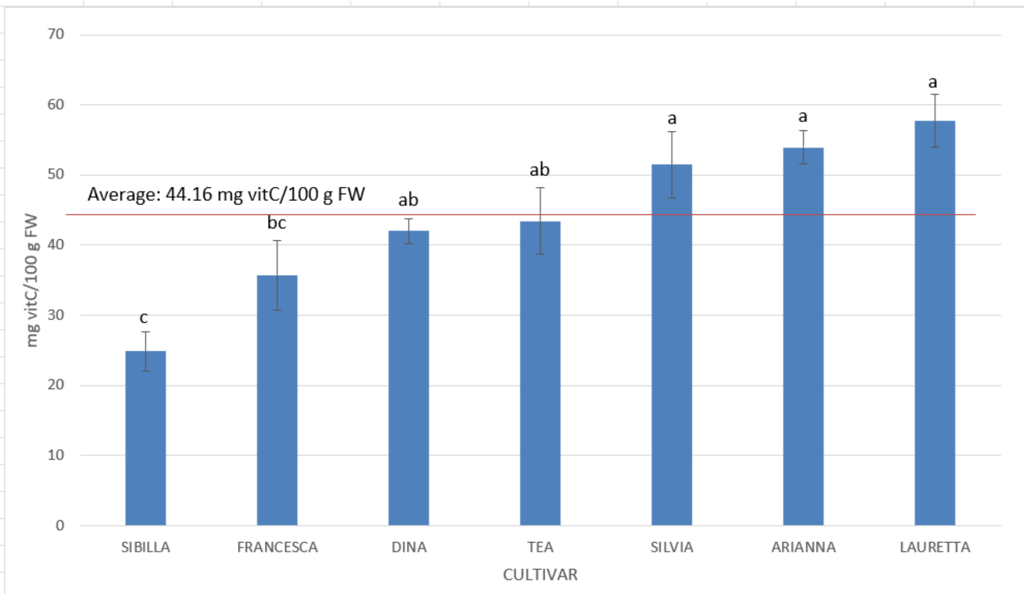

***Figure 11**: Vitamin C content for 7 commercial strawberry cultivars for 2022 expressed as milligrams of vitamin C per 100 g Fresh weight. Values are expressed as means ± standard errors. Different letters indicate statistical difference (Tukey test, p≤0.05).*

3.1.2 New Breeding Selections of Strawberry

Figure 12 below illustrates the trend of the fruits of fifty-two (52) new breeding selections and their respective vitamin C contents in mg vit C/100g FW of the fruit. According to this illustration, the best three (3) new selections with recorded fruit vitamin C contents of 106.29 mg vit C/100 g FW, 96.80 mg vit C/100 g FW and 94.15 mg vit C/100 g FW are **AN13,16,56**, **AN14,03,51** and **AN15,09,57** respectively. It is interesting to note that the fruits of these new selections extremely exceed the recommended dietary allowance and can be considered as potential genotypes for onward breeding programs. New selections with the lowest amount of fruit vitamin C recorded in decreasing order are **AN16,34,55**, **AN17,09,57**, and **AN17,09,59**. Among all 52 new selections, minimal significant differences were also observed as is the case in the cultivars. However, the average fruit vitamin C content across all new selections is pegged at 49.72 mg vit C/100 g (the fruits of 24 new selections recorded vitamin C contents higher than the average). The average content recorded is slightly higher than what was recorded for the cultivars. This happens to also fall within the recommended daily intake range of 45-120 mg/day as stated by (Snyder, 2019). Looking at this range, one can satisfy their daily need of vitamin C by consuming 100g of strawberry.

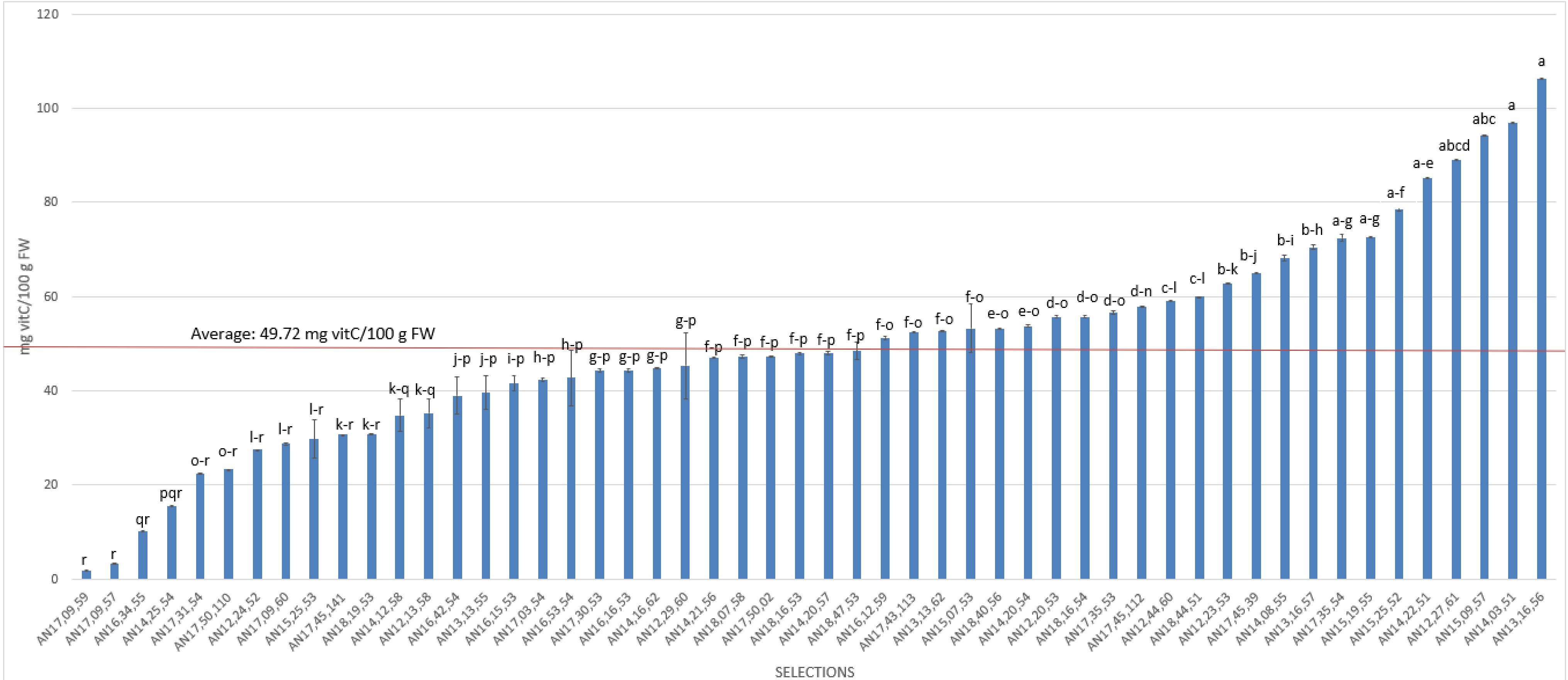

__Figure 12__: Vitamin C content for 52 new strawberry selections for 2022 expressed as milligrams of vitamin C per 100 g Fresh weight. Values are expressed as means ± standard errors. Different letters indicate statistical difference (Tukey test, p≤0.05).

3.1.3. General remarks on cultivars and new selections

Comparing the cultivars and new selections in terms of vitamin C content, it is safe to say that the new selections are relatively better in terms of nutritional composition. They recorded extremely high individual fruit vitamin C contents and a relatively high average amount of vitamin C across all selections (the fruit of more than 20 selections had a vitamin C content higher than the average, of which 5 selections had a double content). This is however not meant to discredit the cultivars since their fruits also contain reasonably sufficient and beneficial amounts of vitamin C on average. In terms of their eligibility (both cultivars and new selections) to meet health claims, they may qualify to be classified as a good source of vitamin C if they contain significant amounts of the nutrient in question. In this case, the term "significant amount" is defined as containing at least 15% of the recommended daily allowance in 100 g serving with the recommended daily intake value pegged at 80 mg/day according to the European Commission directive 2008/100/EC meaning that fresh strawberries must presumably contain at least 12 mg/100 g (Mezzetti *et al*., 2018b). Therefore, all the cultivars, and new selections with vitamin C contents above 12 mg/100 g may qualify as a good source and can be selected as genotypes for functional claim applications. This situation can particularly be adopted for the selections having contents of vitamin C higher than the average value observed for the cultivars.

3.2 FOLATE DISCUSSION

Folate is typically one of the vitamins whose availability is most constrained in human nutritional requirements (L. M. Strålsjö *et al*., 2003). According to the US National Institute of Health, (2022), the U.S. Food and Drug Administration (FDA) started requiring manufacturers to include 140 µg of folic acid per 100 grams in enriched breads, cereals, flours, cornmeals, pastas, rice, and other grain products to lower the risk of neural tube abnormalities (NTDs). Since cereals and grains are widely consumed in the United States, they have emerged as significant sources of folic acid in the diet of Americans. The fortification effort raised the average daily intake of folic acid in the United States by around 190 µg. The optional addition of up to 154 µg of folic acid per 100 grams of corn masa flour was allowed by the FDA in April 2016. The Canadian government has also mandated that several grains, such as enriched pasta, cornmeal, and white flour, be supplemented with 150 µg of folic acid per 100 g (Crider *et al*., 2011). Numerous other nations have likewise implemented compulsory folic acid fortification programs. The demand for more folate has also served as a driving force behind current breeding initiatives like the UNIVPM D3A breeding program to introduce genotypes with higher folate content in strawberries.

3.2.1. Commercial Cultivars of strawberry

Figure 13 below illustrates the trend of 7 commercial cultivars and their respective fruit vitamin B9 contents expressed in µg 5-methyltetrahydrofolic acid/100 g FW of the fruit. From the trend, it can be observed that the highest folate containing cultivar was **Arianna** with its fruits having a folate content of 74.83 µg/100 g FW followed by fruits of **Lauretta** with 62.50 µg/100 g FW closely followed by fruits of **Tea** with 62.19 µg/100 g FW, fruits of **Dina** with 57.08 µg/100 g FW, the fruits of **Silvia** with 50.47 µg/100 g FW, fruits of **Francesca** with 48.18 µg/100 g FW and finally fruits of **Sibilla** with the lowest folate content of 46.46 µg/100 g FW. A previous study conducted by Mazzoni *et al*., (2021) who also analysed the vitamin B9 content of Sibilla recorded about 18-20 µg/100 g FW. There has therefore been a major improvement in the folate content of Sibilla in this current study. Comparing the four cultivars obtained from the UNIVPM D3A breeding program (Lauretta, Silvia, Dina, and Francesca), fruits of Lauretta and Dina were the top two with the highest Vitamin B9 content followed by that of Silvia and Francesca. Among all seven (7) cultivars, no significant differences ($P \leq 0.05$) were observed. This supports the notion that there is high variability among the groups of cultivars being studied. The average folate content of the fruits across all studied cultivars was pegged at 57.39 µg/100 g FW.

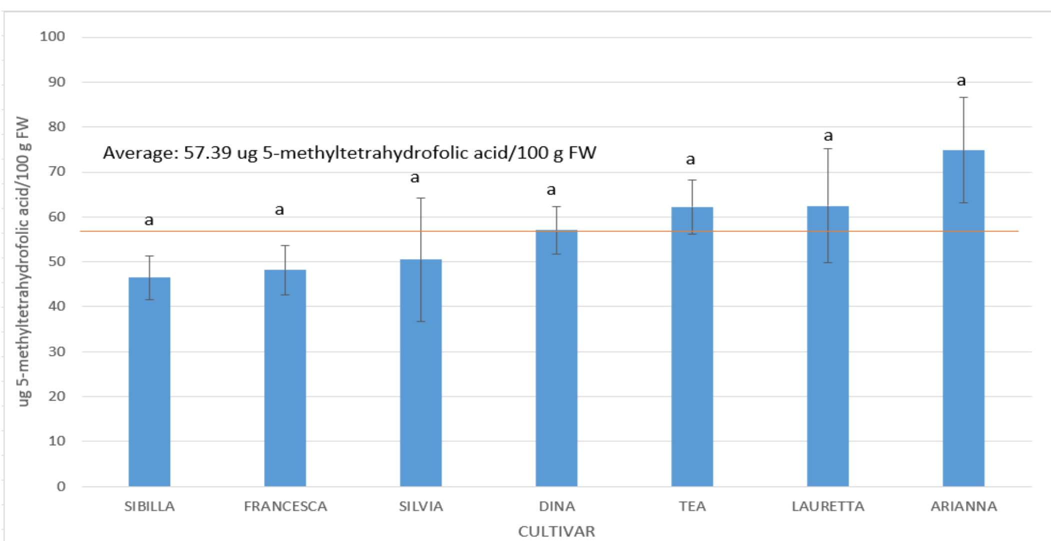

Figure 13*: Vitamin B9 (folate) content for 7 commercial strawberry cultivars for 2022 expressed as micrograms 5-methyltetrahydrofolic acid per 100 g Fresh weight. Values are expressed as means ± standard errors. Different letters indicate statistical difference (Tukey test, p≤0.05).*

With respect to the recommended dietary allowance of folates, the European Food Safety Authority (2014) states that based on findings from one controlled trial demonstrating that an intake of 205-257 µg DFE/day for seven weeks after a depletion phase sustains blood folate concentrations above the cut-off for folate adequacy, an average requirement (AR) of 250 µg DFE/day is advocated for healthy adult men and women. These results are highly congruent with two further controlled trials, which found that daily folate intakes of 200–300 µg may be adequate to maintain serum and red blood cell folate concentrations at ≥10 and ≥340 nmol/L, respectively. All though the average folate content recorded may not be as high as the average requirement stated, strawberries are still deemed as a rich source of dietary folate due to its successful application in the increment of natural folate content in milk products as conducted by (Holasova *et al*., 2005). Hence these cultivars can sufficiently contribute to meeting the average requirement of dietary folates.

3.2.2. New Selections of strawberry

Figure 14 below is an illustration of the trend of 49 new breeding selections expressed in µg 5-methyltetrahydrofolic acid/100 g FW of the fruit. From the trend, the three best performing selections with the highest fruit folate content arranged in decreasing order are **AN15,09,57** with a fruit folate content of 116.70 µg/100 g FW, **AN13,16,56** with 109.32 µg/100 g FW and **AN13,16,57** with 93.04 µg/100 g FW. The fruits of these three selections recorded folate contents high enough for them to be considered as good contributors to aiding consumers to meet their daily recommended intake of 200-300 µg (European Food Safety Authority, 2014). The three least performing cultivars with fruit folate contents of 15.91, 14.56, and 12.97 µg/100 g FW are **AN18,44,51**, **AN12,27,61**, and **AN18,47,53** respectively. Among all 49 new selections, the average folate content was pegged at 48.86 µg/100 g FW and minimal significant differences were observed.

3.2.3. General remarks on cultivars and new selections

Comparing both cultivars and new selections in terms of their fruit vitamin B9 content, the new selections performed relatively better than the cultivars. The fruits of the new selections recorded very high folate contents Notwithstanding, fruits of the cultivars performed much better in this study as compared to cultivars in similar studies conducted previously. The fruits of twenty-three (23) selections had folate content values higher than the overall average. Fruits of two (2) selections (in comparison to the average value) had double the average value of the entire population of selections, hence reaching nearly 40-50% of the daily intake of folates as recommended by the (European Food Safety Authority, 2014). This is a novelty because until now, previous studies including the study conducted by (Mazzoni *et al*., 2021) only showed fruits of cultivars recording average folate content values that reached only about 15-20% of the recommended daily amount. This is an indication of the effectiveness of the breeding program to create new selections with improved vitamin content.

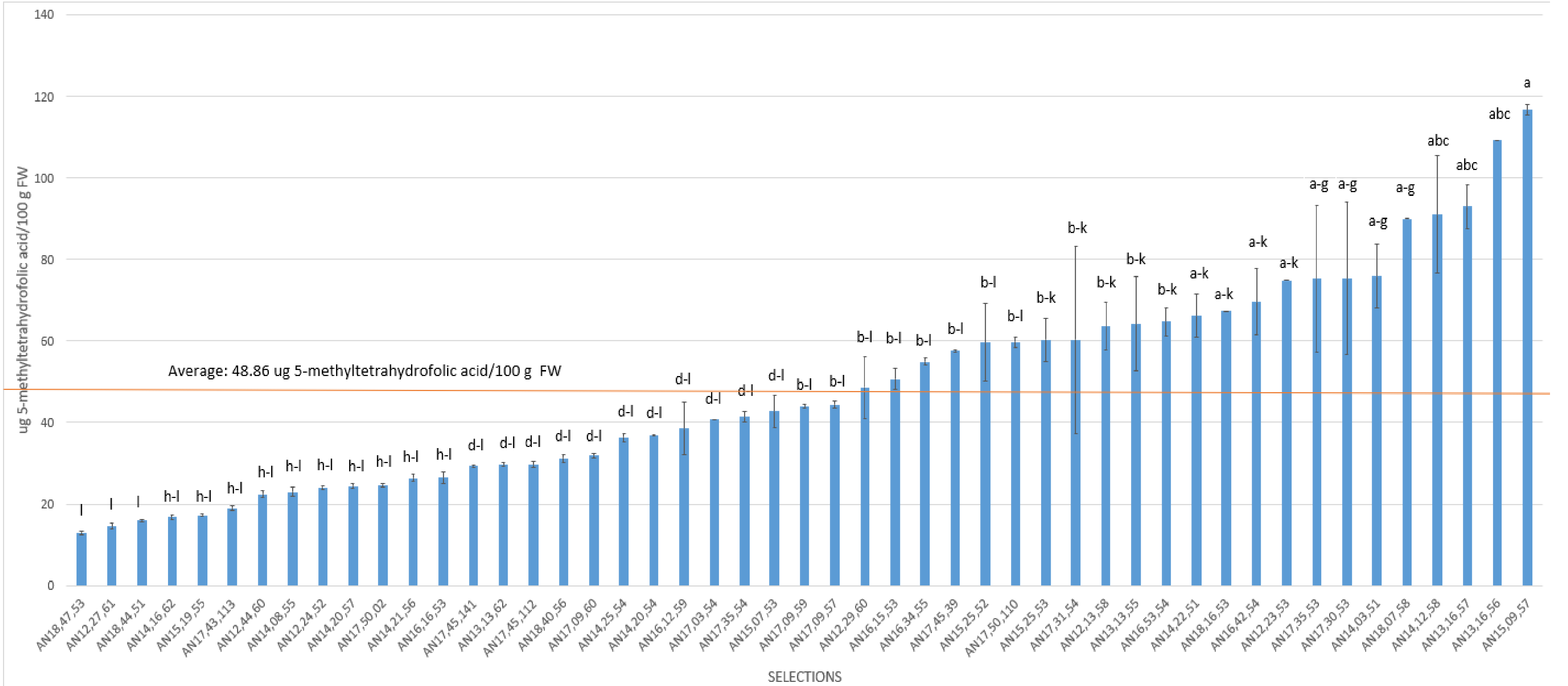

Figure 14*: Vitamin B9 (folate) content for 49 new strawberry selections for 2022 expressed as micrograms 5-methytetrahydrofolic acid per 100 g Fresh weight. Values are expressed as means ± standard errors. Different letters indicate statistical difference (Tukey test, p≤0.05).*

Considering all that has been discussed, the ideal cultivars and selections for vitamin C and folate have been outlined.

***Table 2**: Best cultivars and new selections*

VITAMIN C		FOLATE	
Best Cultivars	Best New Selections	Best Cultivars	Best New Selections
Lauretta 57.69 mg vit C/100 g FW	AN13,16,56 106.29 mg vit C/100 g FW	Arianna 74.83 µg/100 g FW	AN15,09,57 116.70 µg/100 g FW
Arianna 53.94 mg vit C/100 g FW	AN14,03,51 96.80 mg vit C/100 g FW	Lauretta 62.50 µg/100 g FW	AN13,16,56 109.32 µg/100 g FW
Silvia 51.49 mg vit C/100 g FW	AN15,09,57 94.15 mg vit C/100 g FW	Tea 62.19 µg/100 g FW	AN13,16,57 93.04 µg/100 g FW

3.2.4: General remarks on the ideal cultivars and selections

Table 2 above shows the best cultivars and selections with the highest fruit vitamin C and folate contents. It can be observed that two cultivars (**Lauretta** and **Arianna**) and two new selections (**AN13,16,56** and **AN15,09,57**) showed outstanding results in both parameters that were measured in this study. The fruits of **Silvia** recorded the third highest vitamin C content but did not emerge as one of the best in terms of fruit folate content. The same can be said for **Tea** since it performed relatively well with respect to fruit folate content but not in fruit vitamin C content. Correspondingly, the selection **AN14,03,51** which even had a higher fruit vitamin C content than **AN15,09,57** did not express a fruit folate content high enough to be part of the top three selections listed. In similar fashion, **AN13,16,57** listed among the top three selections with regards to fruit folate content did not appear in the top three selections for vitamin C. With reference to the cultivars derived from the D3A breeding program, the fruits of **Lauretta**, and **Silvia** both emerged as part of the top three cultivars, however, **Lauretta** is more interesting as a cultivar since it performed exceptionally well by expressing very high values for both vitamin C and B9 in its fruits.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

Two cultivars (**Lauretta** and **Arianna**) and two new selections (**AN13,16,56** and **AN15,09,57**) performed very well in both vitamin C and vitamin B9 contents. Between these two cultivars, **Lauretta** had the highest fruit vitamin C content, whereas **Arianna** had the highest fruit folate content. The same can be said for the new selections as **AN13,16,56** emerged as the best with respect to vitamin C content and **AN15,09,57** was the best with respect to folate content. It is also important to note that **Silvia** and **Tea** are also very good sources of fruit vitamin C and folate respectively. Likewise, **AN14,03,51** and **AN13,16,67** are acceptable choices. Among cultivars derived from the UNIVPM D3A breeding program, Lauretta and Silvia are the best performing cultivars. There is however the need to verify the agronomic data of the selections having fruits with higher vitamin C and folate contents to ascertain the possibility of releasing them as new cultivars and to be marketed for their health benefits to the consumer as well as to be used as parents for new breeding programs to improve nutritional content of progenies. It can therefore be concluded that the breeding program was successful in producing new genotypes with higher fruit vitamin C and folate levels. This further proves that breeding and even biotechnological approaches remain the best strategy to boost consumer interest and satisfaction by consistently producing berry fruits with excellent quality and exceptional nutritional status. The findings of this study are very helpful for the commercialization of new varieties, but more importantly, they can be used to choose new genotypes with superior fruit nutritional quality.

REFERENCES

AgroNotizie. (2008, October 3). *The varietal evolution of strawberries- Nursery and seeds*. https://agronotizie.imagelinenetwork.com/vivaismo-e-sementi/2008/03/10/l-evoluzione-varietaledella-fragola/5006

Alvarez-Suarez, J. M., Mazzoni, L., Forbes-Hernandez, T. Y., Gasparrini, M., Sabbadini, S., & Giampieri, F. (2014a). The effects of pre-harvest and post-harvest factors on the nutritional quality of strawberry fruits: A review. In *Journal of Berry Research* (Vol. 4, Issue 1, pp. 1–10). IOS Press. https://doi.org/10.3233/JBR-140068

Alvarez-Suarez, J. M., Mazzoni, L., Forbes-Hernandez, T. Y., Gasparrini, M., Sabbadini, S., & Giampieri, F. (2014b). The effects of pre-harvest and post-harvest factors on the nutritional quality of strawberry fruits: A review. In *Journal of Berry Research* (Vol. 4, Issue 1, pp. 1–10). IOS Press. https://doi.org/10.3233/JBR-140068

Aune, D., Giovannucci, E., Boffetta, P., Fadnes, L. T., Keum, N. N., Norat, T., Greenwood, D. C., Riboli, E., Vatten, L. J., & Tonstad, S. (2017). Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-A systematic review and dose-response meta-analysis of prospective studies. *International Journal of Epidemiology*, *46*(3), 1029–1056. https://doi.org/10.1093/ije/dyw319

Ayala-Zavala, J. F., Wang, S. Y., Wang, C. Y., & González-Aguilar, G. A. (2004). Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *LWT*, *37*(7), 687–695. https://doi.org/10.1016/j.lwt.2004.03.002

Balasooriya, H., Dasanayake, K., & Ajlouni, S. (2020). Bioaccessibility of micronutrients in fresh and frozen strawberry fruits grown under elevated carbon dioxide and temperature. *Food Chemistry*, *309*. https://doi.org/10.1016/j.foodchem.2019.125662

Basu, A., & Lyons, T. J. (2012). Strawberries, blueberries, and cranberries in the metabolic syndrome: Clinical perspectives. *Journal of Agricultural and Food Chemistry*, *60*(23), 5687–5692. https://doi.org/10.1021/jf203488k

Bationo, F., Humblot, C., Songré-Ouattara, L. T., Hama-Ba, F., le Merrer, M., Chapron, M., Kariluoto, S., & Hemery, Y. M. (2020). Total folate in West African cereal-based fermented foods: Bioaccessibility and influence of processing. *Journal of Food Composition and Analysis*, *85*. https://doi.org/10.1016/j.jfca.2019.103309

Bhat, R., Geppert, J., Funken, E., & Stamminger, R. (2015). Consumers Perceptions and Preference for Strawberries—A Case Study from Germany. *International Journal of Fruit Science*, *15*(4), 405–424. https://doi.org/10.1080/15538362.2015.1021408

Bjarnadottir, A. (2019, March 27). *Strawberries 101: Nutrition facts and Health benefits*. https://www.healthline.com/nutrition/foods/strawberries

Brandon, E. F. A., Bakker, M. I., Kramer, E., Bouwmeester, H., Zuidema, T., & Alewijn, M. (2014). Bioaccessibility of vitamin A, vitamin C and Folic acid from dietary supplements, fortified food and infant formula. *International Journal of Food Science and Nutrition*, *4*(65), 426–435.

Capocasa, F., Scalzo, J., Mezzetti, B., & Battino, M. (2008). Combining quality and antioxidant attributes in the strawberry: The role of genotype. *Food Chemistry*, *111*(4), 872–878. https://doi.org/10.1016/j.foodchem.2008.04.068

Carr, A. C., & Maggini, S. (2017). Vitamin C and immune function. In *Nutrients* (Vol. 9, Issue 11). MDPI AG. https://doi.org/10.3390/nu9111211

Cerdá, B., Tomás-Barberán, F. A., & Espín, J. C. (2005). Metabolism of antioxidant and chemopreventive ellagitannins from strawberries, raspberries, walnuts, and oak-aged wine in humans: Identification of biomarkers and individual variability. *Journal of Agricultural and Food Chemistry*, *53*(2), 227–235. https://doi.org/10.1021/jf049144d

Cheplick, S., Kwon, Y. I., Bhowmik, P., & Shetty, K. (2010). Phenolic-linked variation in strawberry cultivars for potential dietary management of hyperglycemia and related complications of hypertension. *Bioresource Technology*, *101*(1), 404–413. https://doi.org/10.1016/j.biortech.2009.07.068

Crider, K. S., Bailey, L. B., & Berry, R. J. (2011). Folic acid food fortification-its history, effect, concerns, and future directions. In *Nutrients* (Vol. 3, Issue 3, pp. 370–384). MDPI AG. https://doi.org/10.3390/nu3030370

Devold Kjellsen, T., Mehli, L., Henning Iversen, T., Schaart, J. G., Kjellsen, T. D., Heggem, R., Iversen, T.-H., Schouten, H. J., & Krens, F. A. (2016). *Towards the production of genetically modified strawberries which are acceptable to consumers*. https://www.researchgate.net/publication/254841437

Diamanti, J., Battino, M., & Mezzetti, B. (2011). Breeding for Fruit Nutritional and Nutraceutical Quality. In *Breeding for Fruit Quality* (pp. 61–79). John Wiley and Sons. https://doi.org/10.1002/9780470959350.ch3

Dragišić Maksimović, J., Poledica, M., Mutavdžić, D., Mojović, M., Radivojević, D., & Milivojević, J. (2015). Variation in Nutritional Quality and Chemical Composition of Fresh Strawberry Fruit: Combined Effect of Cultivar and Storage. *Plant Foods for Human Nutrition*, *70*(1), 77–84. https://doi.org/10.1007/s11130-014-0464-3

Edderkaoui, M., Odinokova, I., Ohno, I., Gukovsky, I., Go, V. L. W., Pandol, S. J., & Gukovskaya, A. S. (2008). Ellagic acid induces apoptosis through inhibition of nuclear factor κB in pancreatic cancer cells. *World Journal of Gastroenterology*, *14*(23), 3672–3680. https://doi.org/10.3748/wjg.14.3672

European Food Safety Authority. (2014). Scientific Opinion on Dietary Reference Values for folate. *EFSA Journal*, *12*(11). https://doi.org/10.2903/j.efsa.2014.3893

Faedi, W., & Baruzzi, G. (2016). Strawberry Breeding. In M. Husaini., N. D. Amjad (Ed.), *Strawberry: Growth, Development and Diseases* (pp. 26–40). CAB International.

FAOSTAT. (2020). *Production of Strawberries: Top ten producers.* https://www.fao.org/faostat/en/#data/QCL/visualize.

FAOSTAT. (2022a, October 21). *Production of Strawberries: Import and Export Quality of strawberries in Italy*. https://www.fao.org/faostat/en/#data/QCL/visualize

FAOSTAT. (2022b, October 21). *Production of Strawberries: Production of Strawberries in Italy*. https://www.fao.org/faostat/en/#data/QCL/visualize.

Gao, Q., Qin, L. Q., Arafa, A., Eshak, E. S., & Dong, J. Y. (2020). Effects of strawberry intervention on cardiovascular risk factors: A meta-analysis of randomised controlled trials. *British Journal of Nutrition*, *124*(3), 241–246. https://doi.org/10.1017/S000711452000121X

Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., & Arora, P. (2018). Biofortified Crops Generated by Breeding, Agronomy, and Transgenic Approaches Are Improving Lives of Millions of People around the World. In *Frontiers in Nutrition* (Vol. 5). Frontiers Media S.A. https://doi.org/10.3389/fnut.2018.00012

Giampieri, F., Tulipani, S., Alvarez-Suarez, J. M., Quiles, J. L., Mezzetti, B., & Battino, M. (2012). The strawberry: Composition, nutritional quality, and impact on human health. In *Nutrition* (Vol. 28, Issue 1, pp. 9–19). https://doi.org/10.1016/j.nut.2011.08.009

Holasova, M., Fiedlerova, V., Roubal, P., & Pechacova, M. (2005). Possibility of increasing natural folate content in fermented milk products by fermentation and fruit component addition. *Czech Journal of Food Science, 23*(5), 196–201.

Iniesta, M. D., Pérez-Conesa, D., García-Alonso, J., Ros, G., & Periago, M. J. (2009). Folate Content in Tomato (Lycopersicon esculentum). Influence of Cultivar, Ripeness, Year of Harvest, and Pasteurization and Storage Temperatures. *Journal of Agricultural and Food Chemistry*, *57*(11), 4739–4745. https://doi.org/10.1021/jf900363r

Jacob, R. A., & Sotoudeh, G. (2002). Vitamin C function and status in chronic disease. *Nutrition in Clinical Care: An Official Publication of Tufts University*, *5*(2), 66–74. https://doi.org/10.1046/j.1523-5408.2002.00005.x

Janick, J. U. L. E. S. (2012). Fruit breeding: past, present and future. *In XXII Congresso Brasilerio de Fruiticultura, Bento Goncalves-RS*, *26*, 1–22.

Johnston, C. S., & Cox, S. K. (2001). Plasma- saturating intakes of vitamin C confer maximal antioxidant protection to plasma. *Journal of the American College of Nutrition*, *6*(20), 623–627.

Karanjalker, G., & Begane, N. (2016). Verbesserung der Fruchtqualität von Obst durch Züchtung. *Erwerbs-Obstbau*, *58*(2), 119–126. https://doi.org/10.1007/s10341-015-0264-4

Kronenberg, G., Colla, M., & Endres, M. (2009). Folic acid, neurodegenerative and neuropsychiatric disease. *Current Molecular Medicine*, *3*(9), 315–323.

Larrosa, M., Tomás-Barberán, F. A., & Espín, J. C. (2006). The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway. *Journal of Nutritional Biochemistry*, *17*(9), 611–625. https://doi.org/10.1016/j.jnutbio.2005.09.004

Lazzè, M. C., Pizzala, R., Perucca, P., Cazzalini, O., Savio, M., Forti, L., Vannini, V., & Bianchi, L. (2006). Anthocyanidins decrease endothelin-1 production and increase endothelial nitric oxide synthase in human endothelial cells. *Molecular Nutrition and Food Research*, *50*(1), 44–51. https://doi.org/10.1002/mnfr.200500134

Lee, S. (2016). CRISPR Gene-Editing in Strawberry. In *FSREF Research Reports*.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. In *American Journal of Clinical Nutrition* (Vol. 79, Issue 5, pp. 727–747). American Society for Nutrition. https://doi.org/10.1093/ajcn/79.5.727

Manganaris, G. A., Goulas, V., Vicente, A. R., & Terry, L. A. (2014). Berry antioxidants: small fruits providing large benefits. In *Journal of the Science of Food and Agriculture* (Vol. 94, Issue 5, pp. 825–833). https://doi.org/10.1002/jsfa.6432

Manzano, S., & Williamson, G. (2010). Polyphenols and phenolic acids from strawberry and apple decrease glucose uptake and transport by human intestinal Caco-2 cells. *Molecular Nutrition and Food Research*, *54*(12), 1773–1780. https://doi.org/10.1002/mnfr.201000019

Mazzoni, L., Balducci, F., Marcellini, M., Pergolott, V., Capocasa, F., & Mezzetti, B. (2021). Evaluation of strawberry nutritional quality. *Acta Horticulturae*, *1311*, 47–54. https://doi.org/10.17660/ActaHortic.2021.1311.6

McDougall, G. J., Shpiro, F., Dobson, P., Smith, P., Blake, A., & Stewart, D. (2005). Different polyphenolic components of soft fruits inhibit α-amylase and α-glycosidase. *Journal of Agricultural and Food Chemistry*, *53*(7), 2760–2766. https://doi.org/10.1021/jf0489926

Meyers, K. J., Watkins, C. B., Pritts, M. P., & Liu, R. H. (2003). Antioxidant and Antiproliferative Activities of Strawberries. *Journal of Agricultural and Food Chemistry*, *51*(23), 6887–6892. https://doi.org/10.1021/jf034506n

Mezzetti, B. (2013). Breeding and biotechnology for improving the nutritional quality of strawberry. In *Journal of Berry Research* (Vol. 3, Issue 3, pp. 127–133). https://doi.org/10.3233/JBR-130053

Mezzetti, B., Balducci, F., Capocasa, F., Zhong, C. F., Cappelletti, R., di Vittori, L., Mazzoni, L., Giampieri, F., & Battino, M. (2016). Breeding Strawberry for Higher Phytochemicals Content and Claim It: Is It Possible? *International Journal of Fruit Science*, *16*, 194–206. https://doi.org/10.1080/15538362.2016.1250695

Mezzetti, B., Giampieri, F., Zhang, Y. T., & Zhong, C. F. (2018a). Status of strawberry breeding programs and cultivation systems in Europe and the rest of the world. *Journal of Berry Research*, *8*(3), 205–221. https://doi.org/10.3233/JBR-180314

Mezzetti, B., Giampieri, F., Zhang, Y. T., & Zhong, C. F. (2018b). Status of strawberry breeding programs and cultivation systems in Europe and the rest of the world. *Journal of Berry Research*, *8*(3), 205–221. https://doi.org/10.3233/JBR-180314

Mullen, W., Edwards, C. A., Serafini, M., & Crozier, A. (2008). Bioavailability of pelargonidin-3-O-glucoside and its metabolites in humans following the ingestion of strawberries with and without cream. *Journal of Agricultural and Food Chemistry*, *56*(3), 713–719. https://doi.org/10.1021/jf072000p

Nadakuduti, S. S., Buell, C. R., Voytas, D. F., Starker, C. G., & Douches, D. S. (2018). Genome editing for crop improvement – applications in clonally propagated polyploids with a focus on potato (solanum tuberosum L.). *Frontiers in Plant Science*, *871*. https://doi.org/10.3389/fpls.2018.01607

Nile, S. H., & Park, S. W. (2014). Edible berries: Bioactive components and their effect on human health. In *Nutrition* (Vol. 30, Issue 2, pp. 134–144). https://doi.org/10.1016/j.nut.2013.04.007

Oakley, Jr. G. P. (2002). Global prevention of all folic acid- Preventable spina bifida and anencephaly by 2010. *Public Health Genomics*, *1*(5), 70–77.

Okuda', T., Yoshida', T., & Hatano', T. (1989). *Review Ellagitannins as Active Constituents of Medicinal Plants*.

Olsson, M. E., Ekvall, J., Gustavsson, K. E., Nilsson, J., Pillai, D., Sjöholm, I., Svensson, U., Åkesson, B., & Nyman, M. G. L. (2004). Antioxidants, Low Molecular Weight Carbohydrates, and Total Antioxidant Capacity in Strawberries (Fragaria x ananassa): Effects of Cultivar, Ripening, and Storage. *Journal of Agricultural and Food Chemistry*, *52*(9), 2490–2498. https://doi.org/10.1021/jf030461e

Proestos, C., Koutelidakis, A. E., Kapsokefalou, M., & Komaitis, M. (2011). *Fruits and Vegetables: a rich source of phenolic acids. Phenolic acids: Composition, application, and health benefits*. 1–13.

Rampersaud, G. C. , Bailey, L. B., & Kauwell, G. P. A. (2002). Relationship of folate to colorectal and cervical cancer: review and recommendations for practitioners. *Journal of the American Dietetic Association*, *102*, 1273–1282.

Ray, P. K. (2002). *Breeding Tropical and Subtropical fruits*. Springer Science and Business Media.

Rommens, C. M. (2004). All-native DNA transformation: A new approach to plant genetic engineering. In *Trends in Plant Science* (Vol. 9, Issue 9, pp. 457–464). https://doi.org/10.1016/j.tplants.2004.07.001

Roy, M., Sen, S., & Chakraborti, A. S. (2008). Action of pelargonidin on hyperglycemia and oxidative damage in diabetic rats: Implication for glycation-induced hemoglobin modification. *Life Sciences*, *82*(21–22), 1102–1110. https://doi.org/10.1016/j.lfs.2008.03.011

Sabbadini, S., Capocasa, F., Battino, M., Mazzoni, L., & Mezzetti, B. (2021). Improved nutritional quality in fruit tree species through traditional and biotechnological approaches. In *Trends in*

Food Science and Technology (Vol. 117, pp. 125–138). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2021.01.083

Schab, W., Hoffmann, T., Kalinowski, G., & Preub, A. (2011). Genomics, Transgenics and Molecular Breeding and Biotechnology of Strawberry. In A. M. Husaini & Mercado Jose (Eds.), *Genes, Genomes and Genomics* (1st ed., Vol. 5, pp. 100–109). Global Science Books.

Schlueter, A. K., & Johnston, C. S. (2011). Vitamin C: Overview and update. In *Complementary Health Practice Review* (Vol. 16, Issue 1, pp. 49–57). https://doi.org/10.1177/1533210110392951

Schouten, H. J., Krens, F. A., & Jacobsen, E. (2006). Cisgenic plants are similar to traditionally bred plants: International regulations for genetically modified organisms should be altered to exempt cisgenesis. In *EMBO Reports* (Vol. 7, Issue 8, pp. 750–753). https://doi.org/10.1038/sj.embor.7400769

Seeram, N. (2008). Berry fruits for cancer prevention: Current status and future prospects. *Journal of Agricultural and Food Chemistry*, *56*(3), 630–635. https://doi.org/10.1021/jf072504n

Smirnoff, N. (2018). Ascorbic acid metabolism and functions: A comparison of plants and mammals. In *Free Radical Biology and Medicine* (Vol. 122, pp. 116–129). Elsevier Inc. https://doi.org/10.1016/j.freeradbiomed.2018.03.033

Snyder, C. (2019, December 2). *How much Vitamin C should you take?* https://www.healthline.com/nutrition/how-much-vitamin-c-should-i-take-daily

Stoner, G. D., & Gupta, A. (2001). Etiology and chemoprevention of esophageal squamous cell carcinoma. In *Carcinogenesis* (Vol. 22, Issue 11).

Strålsjö, L., Åhlin, H., Witthöft, C. M., & 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), 264–269. https://doi.org/10.1007/s00217-002-0656-1

Strålsjö, L. M., Witthöft, C. M., Sjöholm, I. M., & Jägerstad, M. I. (2003). Folate content in strawberries (Fragaria × ananassa): Effects of cultivar, ripeness, year of harvest, storage, and commercial processing. *Journal of Agricultural and Food Chemistry*, *51*(1), 128–133. https://doi.org/10.1021/jf020699n

Strawberry Plants.org. (2022, February 11). *Strawberry Plant: The Complete Guide*. http://strawberryplants.org/strawberry-plant/

Tulipani, S., Marzban, G., Herndl, A., Laimer, M., Mezzetti, B., & Battino, M. (2011). Influence of environmental and genetic factors on health-related compounds in strawberry. *Food Chemistry*, *124*(3), 906–913. https://doi.org/10.1016/j.foodchem.2010.07.018

Tulipani, S., Mezzetti, B., & Battino, M. (2009). Impact of strawberries on human health: Insight into marginally discussed bioactive compounds for the Mediterranean diet. *Public Health Nutrition*, *12*(9 SPEC. ISSUE 9A), 1656–1662. https://doi.org/10.1017/S1368980009990516

Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., de Vos, C. H. R., Capanoglu, E., Bovy, A., & Battino, M. (2008). Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. *Journal of Agricultural and Food Chemistry*, *56*(3), 696–704. https://doi.org/10.1021/jf0719959

Tulipani, S., Romandini, S., Suarez, J. M. A., Capocasa, F., Mezzetti, B., Busco, F., Bamonti, F., Novembrino, C., & Battino, M. (2008). Folate content in different strawberry genotypes and folate status in healthy subjects after strawberry consumption. *BioFactors*, *34*(1), 47–55. https://doi.org/10.1002/biof.5520340106

US National Institute of Health. (2022, November 30). *Folate- Health Professional Fact Sheet*. https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/#en16

US National Institutes of Health. (2021, March 26). *Vitamin C- Health Professional Facts Sheet*. https://ods.od.nih.gov/factsheets/vitaminC-HealthProfessional/#en4

Verwei, M., Arkbåge, K., Havenaar, R., van den Berg, H., Witthö, C., & Schaafsma, G. (2003). Nutritional Methodology Folic Acid and 5-Methyltetrahydrofolate in Fortified Milk Are Bioaccessible as Determined in a Dynamic In Vitro Gastrointestinal Model 1. In *J. Nutr* (Vol. 133). https://academic.oup.com/jn/article/133/7/2377/4688321

Wamucii Selina. (2022). *Strawberries production in Italy- Markets, Suppliers and Exporters Insights*. https://www.selinawamucci.com/insights/market/italy/strawberries/

Wang, S. Y., & Lewers, K. S. (2007). *Antioxidant Capacity and Flavonoid Content in Wild Strawberries*.

Wang, S. Y., & Lin, H. S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry*, *48*(2), 140–146. https://doi.org/10.1021/jf9908345

Wang, S. Y., & 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), 9651–9657. https://doi.org/10.1021/jf9020575

Watson, K. (2019, October 23). *Everything you need to know about flavonoids*. https://www.healthline.com/health/what-are-flavonoids-everything-you-need-to-know

Yamamoto, E., Kataoka, S., Shirasawa, K., Noguchi, Y., & Isobe, S. (2021). Genomic Selection for F1 Hybrid Breeding in Strawberry (Fragaria × ananassa). *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.645111

Yaman, M., Çatak, J., Uğur, H., Gürbüz, M., Belli, İ., Tanyıldız, S. N., Yıldırım, H., Cengiz, S., Yavuz, B. B., Kişmiroğlu, C., Özgür, B., & Yaldız, M. C. (2021). The bioaccessibility of water-soluble vitamins: A review. In *Trends in Food Science and Technology* (Vol. 109, pp. 552–563). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2021.01.056

Younis, A., Siddique, M. I., Kim, C. K., & Lim, K. B. (2014). RNA interference (RNAi) induced gene silencing: A promising approach of hi-tech plant breeding. *International Journal of Biological Sciences*, *10*(10), 1150–1158. https://doi.org/10.7150/ijbs.10452

Zafra-Stone, S., Yasmin, T., Bagchi, M., Chatterjee, A., Vinson, J. A., & Bagchi, D. (2007). Berry anthocyanins as novel antioxidants in human health and disease prevention. *Molecular Nutrition and Food Research*, *51*(6), 675–683. https://doi.org/10.1002/mnfr.200700002