DIGITAL COMMONS
@ UNIVERSITY OF SOUTH FLORIDA

University of South Florida
Digital Commons @ University of South Florida

USF Tampa Graduate Theses and Dissertations USF Graduate Theses and Dissertations

June 2018

Synthesis, Oxidation, and Distribution of Polyphenols in Strawberry Fruit During Cold Storage

Katrina E. Kelly
University of South Florida, ktakelly@gmail.com

Follow this and additional works at: https://digitalcommons.usf.edu/etd

Part of the Biology Commons

Scholar Commons Citation
Kelly, Katrina E., "Synthesis, Oxidation, and Distribution of Polyphenols in Strawberry Fruit During Cold Storage" (2018). *USF Tampa Graduate Theses and Dissertations.*
https://digitalcommons.usf.edu/etd/7314

This Thesis is brought to you for free and open access by the USF Graduate Theses and Dissertations at Digital Commons @ University of South Florida. It has been accepted for inclusion in USF Tampa Graduate Theses and Dissertations by an authorized administrator of Digital Commons @ University of South Florida. For more information, please contact digitalcommons@usf.edu.

Synthesis, Oxidation, and Distribution of Polyphenols in Strawberry Fruit During Cold Storage

by

Katrina E. Kelly

A thesis submitted in partial fulfillment
of the requirements for the degree
Master of Science
with a concentration in Cell and Molecular Biology
Department of Cell Biology, Microbiology, and Molecular Biology
College of Arts and Sciences
University of South Florida

Major Professor: Cecilia do Nascimento Nunes, Ph.D.
Stanley Stevens, Ph.D.
Vance Whitaker, Ph.D.

Date of Approval
May 31, 2018

Keywords: strawberry, polyphenols, anthocyanins, phenylalanine ammonia-lyase, polyphenol oxidase, abiotic stress

Copyright © 2018, Katrina E. Kelly

DEDICATION

This thesis is dedicated to my father, Michael Leo Kelly, and my grandfather, Daniel Leo Kelly. They were the major proponents of my education and I could have never made it this far without their support. I wish they were still here to share this momentous occasion with me. I also dedicate this thesis to my mother, Catherine Provenzano, who has always been there in my times of need. I'll always be grateful that you would answer my calls, even at 2 in the morning. This thesis is also dedicated to the rest of my family, Glenn Provenzano, Anna Provenzano, and Joseph Kelly for always believing in me and giving me the courage to see this through to the very end. Lastly, there are not the words to describe the magnitude of love and support I have received from Richard Witas throughout this journey in graduate school. I'm not sure how I would have managed without your never-ending encouragement. I hope that I can be the same pillar of strength for you as you complete your Ph.D.

ACKNOWLEDGMENTS

Firstly, I would like to acknowledge my major professor, Dr. Cecilia Nunes. I think it was more than just luck that brought me to your lab at USF. The time I have spent in your lab has taught me what it takes be a good scientist. I know I will carry the skills and values I have learned during my time with you for the rest of my career. I would also like to acknowledge my committee members, Dr. Stanley Stevens and Dr. Vance Whitaker. Dr. Stevens, I truly appreciate you remaining on my committee even all the way from Vermont. Dr. Whitaker, thank you for taking a chance on a young student without lab experience back in 2011. Working in your lab gave me a true appreciation for agriculture and the wealth of science behind it. I would also like to acknowledge my current and former lab partners Marvin Abountiolas, Robert Madden, Alyssa Smith, Rachel Kahler, Michelle Stein, and Justin Engel for helping me carry out my experiments. I would like to especially acknowledge Robert and Alyssa for assuming many of the lab responsibilities while I wrote my thesis. Finally, a special acknowledgment is given to Dr. Yavuz Yagiz at the University of Florida. You provided invaluable help and advice with the ever-temperamental HPLC for which I will always be grateful.

TABLE OF CONTENTS

List of Tables iv

List of Figures v

Abstract vii

Chapter One: Introduction 1

Chapter Two: Review of literature 3
- Strawberry fruit 3
  - Quality attributes 3
    - Appearance 3
    - Texture 4
    - Flavor 5
  - Health benefits 6
    - Nutritional composition 6
    - Antioxidants and disease prevention 6
    - Bioavailability of strawberry polyphenols 7
  - Handling requirements and shelf life 7
- Polyphenols 9
  - Functions and characteristics 9
    - Cell wall constituents 10
    - Pollinator and seed dispersal attractants 10
    - Signal transducers 11
    - Stress defense 11
    - Antioxidants 12
  - Classification and chemical structure 14
    - Phenolic acids 14
    - Tannins 15
    - Stilbenes 15
    - Lignin 16
    - Flavonoids 17
  - Biotic factors affecting polyphenolic content 19
    - Genetics 19
    - Plant and fruit maturity 19
    - Tissue type 21
  - Abiotic factors affecting polyphenolic content 22
    - Environmental conditions 22
    - Agricultural practices 24
    - Postharvest handling and storage conditions 25
  - Utilizing polyphenols to improve pre-harvest and postharvest quality 25
- Phenylpropanoid pathway and specific regulatory enzymes 26

Overview of the phenylpropanoid biosynthetic pathway 26
Phenylalanine ammonia-lyase (PAL) 27
Structure and function 27
Regulation 28
Factors affecting enzyme activity 29
Polyphenol oxidase (PPO) 31
Structure and function 32
Factors affecting enzyme activity 33

Chapter Three: Impact of cultivar and cold storage on the polyphenol profile of three strawberry cultivars 35
Introduction 35
Materials and methods 37
Plant Material 37
Storage conditions 37
Weight loss and dry weight 38
Total polyphenols and anthocyanin contents 38
Polyphenol profiles 39
Statistical analysis 40
Results and discussion 40
Total polyphenols content 40
Total anthocyanin content 42
Polyphenol profiles 43
Effect of cultivar on major, individual polyphenols 45
Effect of cold storage on major, individual polyphenols 47
Conclusions 49

Chapter Four: Synthesis and oxidation of polyphenols in three strawberry cultivars during cold storage 52
Introduction 52
Materials and methods 54
Plant material 54
Storage conditions 54
Sample collection 54
Phenylalanine ammonia-lyase (PAL) enzyme extraction and assay 55
Polyphenol oxidase (PPO) enzyme extraction and assay 55
Statistical analysis 56
Results and discussion 56
Phenylalanine (PAL) enzyme activity 56
Polyphenol oxidase (PPO) enzyme activity 59
Conclusions 60

Chapter Five: Morphological distribution of polyphenols in three strawberry cultivars 62
Introduction 62
Materials and methods 63
Plant material 63
Storage conditions 63
Sample collection 63
Total polyphenols, total anthocyanin, and polyphenol profiles 64
Statistical analysis 64
Results and discussion 65

Total polyphenol content and total anthocyanin content ... 65
Effect of cultivar on the distribution of major individual polyphenols ... 67
Distribution of major, individual polyphenols ... 70
Conclusions ... 73

Chapter Six: General Conclusions ... 78

References ... 80

LIST OF TABLES

Table 1: Retention times for major polyphenols in strawberries 43

LIST OF FIGURES

Figure 1: Plants respond to various biotic and abiotic stresses via the synthesis of a myriad of polyphenols 12

Figure 2: Mechanisms of free radical quenching by polyphenols 13

Figure 3: Phenolic acids are divided into benzoic acid derivatives and cinnamic acid derivatives........ 14

Figure 4: The flavonoid skeleton is comprised of two benzene rings connected by a heterocyclic ring 17

Figure 5: A cross-section of a strawberry 21

Figure 6: The phenylpropanoid pathway is connected to primary metabolism via the shikimate Pathway 27

Figure 7: Polyphenol oxidase (PPO) catalyzes the hydroxylation of monophenols to diphenols and the oxidation of diphenols to o-quinones........ 32

Figure 8: Total polyphenol content in Radiance, Beauty, and Sensation at day 0 and after 7 days of cold storage 40

Figure 9: Percent weight loss of Radiance, Beauty, and Sensation during 7 days of cold storage 40

Figure 10: Total anthocyanin content in Radiance, Beauty, and Sensation at day 0 and after 7 days of cold storage 42

Figure 11: A visual comparison of Radiance (left) and Sensation (right) 42

Figure 12: HPLC chromatograms of individual polyphenols (Sensation) at 250, 280, 360, and 520 nm 44

Figure 13: Differences in major, individual polyphenols of Radiance, Beauty, and Sensation at day 0 and after 7 days of cold storage and total area percentage using HPLC-DAD........ 50

Figure 14: Content major, individual polyphenols in Radiance, Beauty, and Sensation at day 0 and after 7 days of cold storage using HPLC-DAD 51

Figure 15: Enzyme activities of PAL in Radiance, Beauty, and Sensation during storage at 1 °C during Harvest 1 (A) and Harvest 2 (B) in 2016 and Harvest 3 (C) in 2017 57

Figure 16: Enzyme activities of PPO Radiance, Beauty, and Sensation during storage at 1 °C during Harvest 1 (A) and Harvest 2 (B) in 2016 and Harvest 3 (C) in 2017........ 59

Figure 17: Separation of the pith a cortex of a strawberry 64

Figure 18: Total polyphenol content of the pith and cortex of Radiance, Beauty and Sensation on the day of harvest (day 0) and after 7 days of cold storage 65

Figure 19: Total anthocyanin content of the pith and cortex of Radiance, Beauty and Sensation on the day of harvest (day 0) and after 7 days of cold storage 66

Figure 20: Cross section of Radiance, Beauty and Sensation at day 0 67

Figure 21: Differences in major, individual polyphenols of Radiance, Beauty, and Sensation on the day of harvest (day 0) in the pith and in the cortex using HPLC-DAD 74

Figure 22: Quercetin 3-glucoside, catechin, and cyanidin all derive from the same precursor, dihydroquercetin 69

Figure 23: Differences in major, individual polyphenols of Radiance, Beauty, and Sensation after 7 days of cold storage at 1 °C in the pith and in the cortex using HPLC-DAD 75

Figure 24: Differences in major, individual polyphenols of the pith and cortex of Radiance, Beauty and Sensation on the day of harvest (day 0) using HPLC-DAD 76

Figure 25: Differences in major, individual polyphenols of the pith and cortex of Radiance, Beauty and Sensation (C) after 7 days of cold storage at 1 °C using HPLC-DAD 77

ABSTRACT

Plants inherently produce polyphenols (i.e., antioxidants) as a response to reduce oxidative stress caused by abusive environmental pre- and postharvest conditions. These antioxidants, as well as vitamin C, are present in considerable levels in strawberries; however, excessive oxidative stress brought on by improper postharvest handling conditions can reduce the levels of antioxidants in the fruit and shorten the shelf-life of strawberries. Nevertheless, it may be possible to utilize strawberry's naturally occurring polyphenols to reduce postharvest stress and extend their shelf life. The polyphenolic profile has been previously investigated in several strawberry cultivars, however no studies have determined the unique polyphenolic profiles of three important Florida strawberry cultivars ('Florida Radiance', Sweet Sensation® 'Florida 127'and 'Florida Beauty') at harvest and during cold storage. Therefore, in order to better understand the distribution of individual polyphenols within these cultivars and their impact on postharvest shelf-life, this study examined the polyphenolic profiles throughout 7 days of cold storage (1 °C) using an HPLC-DAD. The activity of phenylalanine ammonia lyase (PAL), a key enzyme in the biosynthesis of polyphenols, and polyphenol oxidase (PPO), the enzyme responsible for polyphenol degradation, were also examined during cold storage to understand their possible influences on postharvest synthesis or degradation of polyphenols. This study revealed that the polyphenolic profile of strawberry fruit was genotype dependent; however, pelargonidin 3-glucoside was consistently the anthocyanin found in higher concentrations in the fruit regardless of the cultivar. Apart from the anthocyanins, the flavonols showed the most variation among the three cultivars. PAL was slightly induced during strawberry postharvest storage suggesting that a stress response occurred during cold storage while PPO showed variable induction patterns across all three cultivars most likely due to their different polyphenol profiles. Analysis of the distribution of polyphenols in the cortex and pith of strawberries showed that polyphenols

were mostly concentrated in the cortex of the fruit and that the concentration of individual polyphenol in each fruit tissue varied by cultivar. These results indicate that the oxidative stress response varies in each of the strawberry cultivars studied contributing to their unique polyphenolic profile. Results from this study can ultimately help to identify the polyphenols and enzymes related to superior postharvest quality in future studies.

CHAPTER ONE:

INTRODUCTION

The strawberry is a popular fruit worldwide which is evidenced by its upward trend in production and consumption over the last few decades. Since the 1980s, the consumption of fresh strawberries has nearly quadrupled due to a more health conscious consumer and to the increased availability of strawberries year-round. Strawberries are also available frozen at the retail level or they are processed into a variety of foods such as jams, jellies, yogurts, and beverages or used as ingredients in many food products.[3]

In the United States, California is the primary strawberry producer dominating the market with approximately 90% of the yearly production. The second largest producer is Florida which has carved out a niche in the strawberry market during the winter season. Mild winters have enabled Florida to become the principal supplier of strawberries to the United States and some part of Canada between the months of December and March. However, recent competition from Mexico has put a strain on the Florida strawberry industry. Imports of Mexican strawberries quadrupled from 2004 – 2014 and now constitute 95% of the imported strawberries.[4] Due to increased competition with other markets there is a need for improving the quality and postharvest shelf life of Florida strawberries to remain competitive in an increasing global market.

One of the challenges in delivering high-quality fruit to the consumer is the strawberry's relatively short postharvest shelf-life and their high susceptibility to mechanical injury and disease. Even with proper handling and storage conditions, such as keeping the fruit between 0 and 1 °C, the average self-life of a strawberry is approximately one week.[5] Extending the shelf-life and improving the postharvest quality of Florida strawberries might improve competition with imported fruit. However, even when handled under optimum conditions, strawberries undergo stress in their postharvest environment, as they have been

removed from their nutrient and water source, which can accelerate the onset of senescence. Nevertheless, plants possess natural stress-reducing mechanisms to resist aberrant changes in their environments, such as extreme temperatures, flooding, drought, excessive sunlight, pests and disease. These environmental strains can result in the production of excess free radical formation in the plant leading to oxidative stress. Oxidative stress can damage important cellular macromolecules (i.e., DNA, proteins, lipids) and ultimately result in cell death. Plants produce a wide array of antioxidants, or polyphenols, to reduce and prevent oxidative stress.[2, 6] Strawberries are rich in polyphenols, and it might be possible to utilize these natural stress-reducing compounds to extend their shelf-life. Further, the levels of polyphenols in different strawberry genotypes can be ultimately used as a selection indicator for genotypes with increased stress resistance and health benefits.

As a major supplier of winter strawberries to much of the United States, it is important that Florida strawberry cultivars maintain good postharvest quality in order to deliver superior fruit to distant markets. However, there have been no studies on the polyphenolic profiles of Florida strawberry cultivars during cold storage and the possible impact of polyphenolic variation on the postharvest quality of the fruit. Therefore, the objectives of the work presented in this thesis are 1) to identify the changes in the major, individual polyphenols of three important strawberry cultivars, 2) to examine the activity of two key enzymes involved in the synthesis and degradation of polyphenols, phenylalanine ammonia lyase and polyphenol, and 3) to observe the changes in the distribution of polyphenols within the strawberry during 7 days of storage at 1 °C. Results from this work will ultimately provide insight on possible mechanisms of postharvest stress resistance in strawberries.

Chapter Two:

Review of literature

Strawberry fruit

Quality attributes

The strawberry is a very attractive fruit with a bright and glossy, crimson color that contrasts against its leafy, green calyx. It is also an unusual fruit with its seeds displayed on the outside of the fruit. However, what the consumer considers 'seeds' are, botanically speaking, the true fruit of the strawberry plant while the flesh of the strawberry is a swollen receptacle.[7] Strawberry quality is measured by a complex balance of many sensory attributes such as appearance, texture, aroma, and taste which involves the interaction of many chemical compounds.[8] The taste of strawberries, and their perceived sweetness, is determined by the ratio of sugars, which are a mixture of sucrose, glucose, and fructose, to organic acids, a combination of mostly citric and malic acids.[8-9] The aroma of strawberry fruit arises from a combination of volatile compounds, mainly esters and furanones.[7] The texture, or firmness, is related to the disassembly of pectin during ripening.[10] Abnormality of any one of these attributes can cause the fruit to appear or taste disagreeable to the consumer.

Appearance

When selecting fresh fruits for consumption, consumer's choices are greatly driven by visual appearance. Visual color is considered one of the most important quality attributes in strawberries because the intensity of red coloration is usually related to the ripeness stage of the fruit [11-12]. That is, strawberries with a greener or whitish color are considered unripe and therefore too astringent to eat, while an overly dark red fruit can be indicative of over-ripeness and can be associated with a poor texture and flavor. In a consumer survey on quality, presentation, and marketing of strawberries, 'brilliant red berries' ranked only second to sweetness as top priority for consumers when purchasing strawberries. If one considers that

strawberries cannot usually be tasted before buying, then fruit color is a top priority for the decision to purchase.[13]

The red color of strawberries arises from the presence of anthocyanins within the ripe fruit. The two major anthocyanins in strawberries are pelargonidin 3-glucoside and cyanidin 3-glucoside. Pelargonidin 3-glucoside is the primary pigment and has been reported to contribute between 64% and 80% to the total anthocyanin content in strawberries.[14-15] In a comparative study of Californian strawberry cultivars 'Aromas', 'Diamante', and 'Selva', Pelayo-Zeldívar et al.[16] found the redder strawberry, 'Aromas', was also the fruit with the highest content of anthocyanins compared to the lighter red cultivars which had lower levels of anthocyanins. Thus, the amount of anthocyanins present in each cultivar also reflects their redness. 'Diamante', a pale red fruit, had the least amount of anthocyanins while 'Aromas', a dark red fruit, had the most. 'Selva', which was described as 'just red' and not pale or dark, had an anthocyanin content in between the two other cultivars.[16] This study also demonstrated the variation in analytical color between strawberry cultivars. Another study comparing three major Florida cultivars, 'Strawberry Festival', 'Florida Radiance', and Sweet Sensation® 'Florida127' also showed the variation in color and anthocyanin content between cultivars. Sweet Sensation® 'Florida127' is a much lighter red fruit than 'Florida Radiance' or 'Strawberry Festival' and consequently has the lowest anthocyanin content. The light color of Sweet Sensation® 'Florida127' also positively influences the subjective appearance ratings of this cultivar during cold storage, as 'Florida Radiance' and 'Strawberry Festival' fell into the 'unacceptable' category more quickly than Sweet Sensation® 'Florida127' due to excessive darkening of the fruit.[17]

Texture

The texture associated with a ripe strawberry is also a result of the natural ripening process. Not only do unripe strawberries lack the colorful pigments of the ripe fruit, they are also dissimilar in firmness, as they usually are very hard and not at all juicy. The unpleasant texture of green strawberries is one of the many ways plants deter herbivores until the young seeds are fully developed. As the seeds mature, ripening progresses and the flesh of the strawberry softens dramatically. This is largely due to disassembly of cell

wall structure, reduced cellular turgor pressure, and a breakdown of the pectin-rich middle lamellae, a layer of the cell wall that holds adjacent cells together, resulting in a decline in firmness (i.e., softening) of the fruit tissue.[10, 18]

Ripening is a ubiquitous process among strawberries; however, the degree of softening can differ from cultivar to cultivar.[18] Softening rate has a significant impact in the postharvest quality of strawberries as a consumer's overall liking of a strawberry is highly correlated to its texture.[7] The texture of strawberries indicates the degree of ripeness to the consumer, where a mushy fruit can be considered overripe while a crunchy fruit can be considered under ripe. Therefore, to be acceptable in terms of consumer taste perception, strawberries should have the correct balance of softness to firmness. The variation of firmness between strawberry cultivars and how this relates to the overall quality of a strawberry is evident from a study that compared the eating quality of 15 Florida strawberry genotypes using a trained sensory panel. Cultivars such as 'Dover', 'Winter Dawn', and 'Sweet Charlie' were described as soft while 'Florida Belle', 'Florida Radiance', and 'Strawberry Festival' were described as firm. Their analysis showed a negative correlation between the attributes of 'firmness' and 'overripe', therefore strawberries that were perceived as 'overripe' correlated to softer fruit.[19]

Flavor

The flavor of a strawberry arises from the combination of its aroma and taste. It is perceived by humans through a complex interaction between sugars, organic acids and volatiles.[7] The ratio of sugars to acids defines strawberry fruit taste where sweetness has one of the strongest correlations to consumer satisfaction.[20] The sugars fructose, glucose, and sucrose account for 99% of the sugars found in strawberries.[7] Glucose and fructose are generally found in equal ratios while sucrose is found in lesser amounts.[21-22] Sweetness perception is highly correlated to consumer satisfaction with strawberry flavor.[8]

Health benefits

Nutritional composition

Strawberries are considered a functional food due to their health promoting properties beyond just basic nutrition (i.e., nutrients such as fiber, vitamins and minerals) which may help reduce the risk of chronic disease.[23] They are a good natural source of dietary fiber, B vitamins, vitamin C and E, folate and potassium, and also an excellent source of non-nutrient compounds such as polyphenols.[24-26] In fact, strawberries are one of the richest natural sources of the essential folic acid.[27] While strawberry is not a dense source of energy (only 35 calories per 100 grams), if eaten as part of a balanced diet it will contribute to the overall human well-being, as they are one of the most consumed berries worldwide making them an important source of these key nutrients.[25-26]

Antioxidants and disease prevention

Along with their abundance of nutrients, including vitamin C, strawberries also contain an appreciable amount of non-nutrient antioxidants, namely polyphenols, which are efficient scavengers of reactive oxygen species (ROS).[27] The polyphenols with the highest antioxidant activities in strawberries are the anthocyanins and ellagitannins.[26, 28] Strawberries have a greater antioxidant capacity than other fruits such as apples, apricots, peaches, and kiwifruit as well as up to 10 times greater antioxidant capacity than vegetables and 40 times more than cereals.[27, 29] Consequently, strawberry consumption has been associated with an increase in total antioxidant capacity of blood plasma and resistance to oxidative destruction of red blood cells and lymphocytes.[30-31]

The cellular damage inflicted by ROS is associated with several chronic-degenerative diseases such as arthritis, cardiovascular disease, diabetes, obesity, and cancer. Many *in vitro* and *in vivo* studies have suggested that an increase in polyphenol consumption can possibly ameliorate and help prevent these diseases.[24] For example, on rats, oral administration of quercetin, a polyphenol found in strawberries, over a period of 28 days successfully reduced markers of inflammation and inhibited the onset of induced arthritis.[32] Strawberries also exert a cardioprotective effect on humans by blocking LDL uptake and by improving lipid profiles, antioxidant status, and the function of platelets.[33-34] In the case of diabetes,

administration of strawberry extracts to rats reversed the symptoms of induced diabetes.[35] Also, a powder produced from strawberries was effective at reducing pre-cancerous lesions in the esophagus of human subjects.[36]

Bioavailability of strawberry polyphenols

The antioxidant and anti-disease benefits of strawberry polyphenols are relevant only if the compounds are able to be absorbed by the gut and transported to the various parts of the body. After ingestion, strawberry polyphenols can be absorbed in the small intestine where the glycosidic forms undergo acid hydrolysis to release the aglycone.[37] This increases the lipophilicity of the polyphenols, and they passively transfuse across the epithelial cell membranes.[25] Within the cell, they are either metabolized into sulfate, glucuronide, or methylated forms before passing into the bloodstream or they are excreted back into the lumen of the small intestine. Once in the bloodstream they are further metabolized to make them more water soluble while they make their way to the liver where they undergo more metabolization.[37] It is possible for some of the polyphenols to end up in the liver bile where they are expelled back into the small intestine.[25] Flavonoids have another chance for absorption in the large intestine where the microflora of the colon metabolizes them into phenolic acids and hydroxycinnamates. Then they are absorbed and transported to the liver before being excreted in the urine.[25, 37] In human studies involving the consumption of strawberries, pelargonidin derivatives were detected in the blood serum and urine of patients.[24] However, the bioavailability of the main class of polyphenols in ripe strawberries, anthocyanins, has been reported to be very low with only 0.1% of the consumed amount being excreted in the urine and even lower amounts detected in blood.[25]

Handling requirements and shelf life

Strawberries are a non-climacteric fruit and, unlike climacteric fruit, they have no burst of respiration and ethylene production, a gaseous plant hormone, at the onset of ripening. The ripening of climacteric fruit can be controlled by the application of ethylene or inhibition of ethylene production; however, non-climacteric fruits do not respond to ethylene in such a way.[38] This means that strawberries

must be harvested at full maturity (i.e., three quarters to full red color) for maximum sensory and nutrient quality[39]. On the other hand, strawberries are also a soft fruit that, when ripe, is extremely vulnerable to mechanical damage and decay.[40] These two characteristics, combined with a high metabolic rate, translate to a fruit with a relatively short shelf life.[41] To maintain strawberry quality and extend shelf life, refrigeration is the best option because it slows their metabolic rate and thereby extends shelf life. However, even when kept at optimum temperature and humidity conditions (0-1°C and 90-95% humidity) strawberry shelf life is only approximately 5 to 8 days long, depending on the cultivar.[5]

The appearance of strawberries declines quickly during cold storage, and the biochemical composition of strawberries degrades even faster, before any deterioration of appearance is perceived. For example, after 6 days strawberries stored at 4 °C showed a 93% reduction in folate and a 89% reduction in pelargonidin 3-glucoside.[42] However, storage temperature higher than 4°C can be even more deleterious. Another study using 'Jewel' strawberries showed that the subjective quality of fruits kept at 10 °C decreased more quickly than fruits stored at 4 °C over a period of 12 days. The higher temperature also resulted in accelerated softening of the fruit during storage.[43]

The soft nature of strawberry fruit is a major concern for the strawberry industry and its ability to maintain postharvest quality.[44] The strawberry's soft flesh can be easily bruised or injured during harvesting and shipping, making strawberries highly susceptible to attack by fungi such as gray mold, *Botrytis cinerea*, which is a prominent postharvest disease in strawberries.[45-46] In an effort to control the incidence of postharvest diseases , studies involving the use of UV radiation[45], edible coatings,[47-48] essential oils[49-50], and heat[51] have been performed in an attempt to improve strawberry shelf life. For example, the application of UV radiation has been used as a germicide to kill surface pathogens on strawberries and to enhance their natural defense mechanisms. These postharvest treatments induce the synthesis of polyphenols and promote the activity of defense related enzymes such as phenylalanine ammonia-lyase (PAL), peroxidase, and polyphenol oxidase (PPO).[52] Chitosan has also been investigated as a potential edible coating that can be applied to the strawberry fruit to extend shelf life. Chitosan is a biopolymer derived from chitin, a natural component found in the cell walls of fungi, and it has been shown to stimulate plant defense as well as

prevent the incidence of disease. Postharvest dipping of strawberries in varying concentrations of chitosan (0.5, 1.0, or 1.5 mg/ 100 ml) has resulted in extended shelf life and better postharvest quality when compared to untreated strawberries.[53] However, small scale laboratory experiments of chitosan application are not representative of practical commercial utilization.[54] Feliziani et al.[47] investigated the rate of disease incidence on strawberries that had been sprayed with commercial grade chitosan prior to harvest. The incidence of disease on fruit sprayed with only a chitosan solution ranged between 62.6 – 77.6% compared to the 3.4 – 13.2% with conventional fungicide spray. Therefore, more research is needed to make the use of chitosan a viable option for improving the postharvest quality of strawberries. Along with edible coatings, the use of essential oils has also been examined in improving strawberry shelf-life. Essential oils are an appealing alternative to commercial fungicides due to their safety for the consumer and the environment. The bioactive vapors of essential oils are particularly applicable to strawberry fruit which can be sensitive to aqueous sanitation. Inhibition of *B. cinerea* on strawberry fruits has been achieved with the vapors derived from clove, mustard, and tea tree oil.[55-56] Heat treatment of strawberries has also been considered in the battle against postharvest diseases as the application of heat activates the plants natural defense system. Harvested strawberries that were incubated at 45 °C for 3.5 hours before being challenged with *B. cinerea* showed increased resistance to infection compared to fruit that had not been heated.[51]

Polyphenols

Functions and Characteristics

Plants have evolved a host of secondary metabolites to perform functions not directly related to cellular respiration. Polyphenols constitute an important group of these secondary metabolites. Once considered waste generated by primary metabolism, it is now accepted that polyphenols have a critical role in plants by serving as cell wall constituents, pollinator attractants, seed dispersal attractants, signal transducers, antioxidants as well as a defense system against a myriad of biotic and abiotic stresses (i.e., pathogens, UV radiation, drought, and temperature).[6] Indeed, even stresses on the strawberry plant will

affect the polyphenol content of the fruits. For example, strawberry plants grown under water deprivation produced fruit with a higher phenolic content than those plants that were grown with optimal water supply.[57] The same effect was observed in strawberries cultivated from plants that had been irrigated with slightly saline water. Fruit from the stressed plants contained higher anthocyanins and total phenolics.[58]

Cell wall constituents

Possession of a cell wall is one of the many features that distinguish plant cells from animal cells. As plants do not have skeletons, they rely on their cell walls to give the organism shape, turgidity, robustness and to stand upright.[59] Thus, plants rely heavily on a group of polyphenols called lignin to fortify their cell walls. Lignin is the second most abundant biopolymer after cellulose.[60] These large and complex polymers provide structural integrity to cell walls as well as impart strength and rigidity to stems.[60] Lignin also helps prevent cell desiccation by providing a barrier to water. This not only allowed plants to move from water to dry land millions of years ago, but it also enables the utilization of a vascular system for the movement of water and solutes throughout the plant.[60-61] As for strawberries, the inner vascular bundles and achenes contain most of the lignin whereas the fleshy receptacle contains very little amounts. The firmness and integrity of the fruit's receptacle is therefore related mostly to the concentration of pectin in the cell walls.[62-64]

Pollinator and seed dispersal attractants

The colorful pigments of many flowers, fruits, and seeds, which serve as a visual attractant for pollinators and seed dispersers, can be attributed to another large group of polyphenols classified as the flavonoids.[65] A subgroup of the flavonoids, the anthocyanins, give red, orange, purple, and blue hues to flowers and fruits.[66] In particular, ripe strawberries attract animals because of their high content of the anthocyanin pelargonidin 3-glucoside which gives the fruit an attractive bright, red color.[67] The strawberry's color combined with a high sugar content entices herbivorous animals to eat the fruit and seeds whereby the seeds are able to pass through the animal's digestive tract and be dispersed to new locations via feces. Flavonoids are also particularly well-suited to attract insect pollinators as they can absorb UV

light, and insects are able to visually detect into the UV spectrum. Thus, flowers pollinated by insects generally display a dark banded 'bullseye' when viewed under UV light.[61, 68]

Signal transducers

Polyphenols are also serve as signaling molecules between plant roots and nitrogen-fixing bacteria. They are excreted from the seeds and roots into the soil, and act as a chemoattractant to symbiotic fungi. This symbiotic relationship allows mycorrhizal plants to grow in nutrient-limited soil due to the fungus' ability to reduce inorganic nitrogen from the atmosphere. The reduced nitrogen is traded to the plant in return for carbohydrates. The most well-known symbiotic relationship between fungus and plant is the association between the fungus *Rhizobium* and the roots of legumes. Secretion of polyphenols, particularly flavonoids, attracts the fungus via chemotaxis. These polyphenols also regulate the expression of genes in the fungus which allows for the formation of specialized root nodules.[69-70]

Stress Defense

Stress defense is of particular importance to plants due to their sessile nature and their inability to move away from damaging or life-threatening agents. The threats, which induce stress, plants encounter in their environment can be classified as either biotic or abiotic. Biotic stresses include herbivores and pathogens, while abiotic stresses can arise from abusive changes in soil salinity, temperature, and water as well as from photooxidation and UV radiation (**Figure 1**).[71] Polyphenols provide a defense mechanism for all of these forms of stress.[6] For example, the polyphenols called tannins impart an astringent taste to the plant or fruit to discourage herbivores from eating them while phenolic acids, such as caffeic or chlorogenic acids, can be outright toxic to pathogens.[6] They also provide protection when a plant's living conditions are less than ideal. Yet, their most notable mechanism of defense is their ability to inhibit cellular oxidation which can occur as a consequence of stress, or from prolonged exposure to UV radiation. This creates a paradoxical situation for plants because while they need sunlight to perform photosynthesis, the Sun's UV radiation can lead to extensive, and sometimes lethal, oxidative damage of biomolecules. As they are unable to move away from the sunlight, plants must be able to control and prevent the harmful effects of UV-induced oxidation. Their protection comes from the combined utilization of antioxidant enzymes and

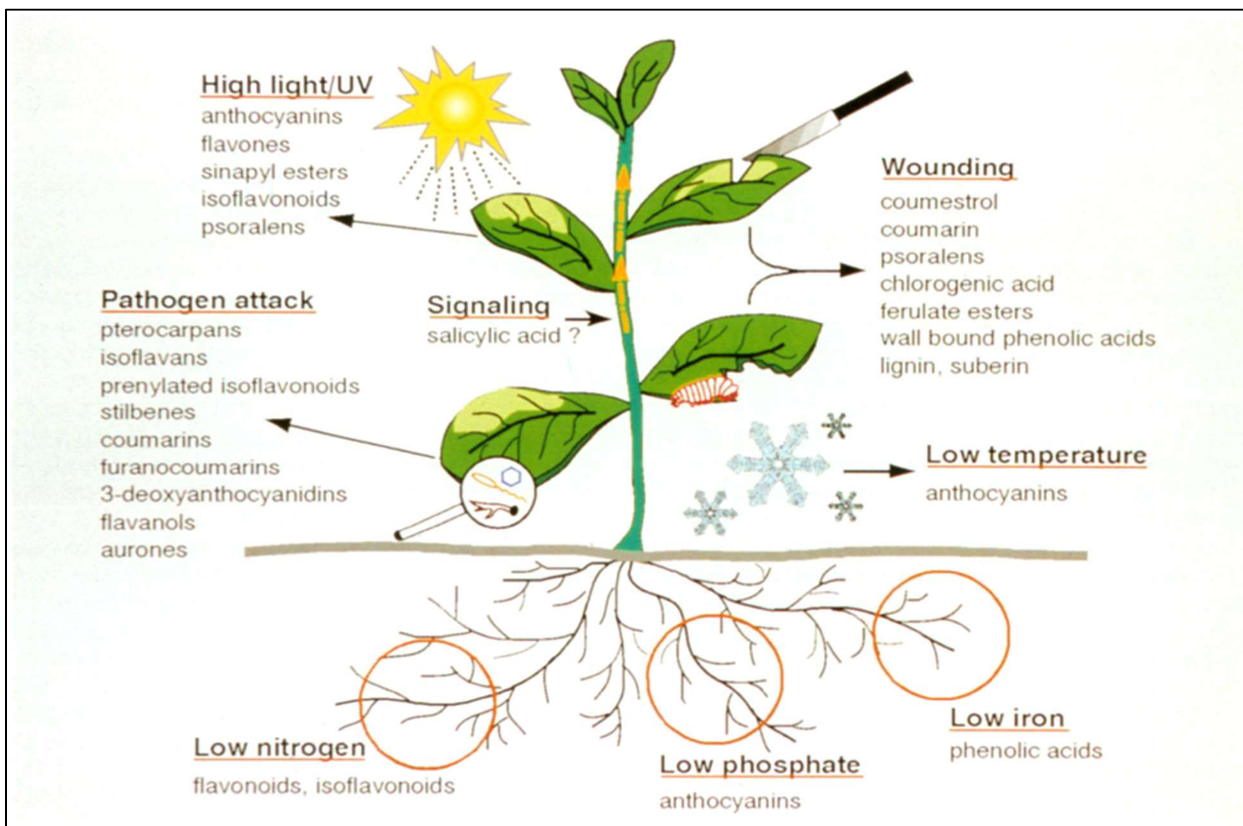

Figure 1. Plants respond to various biotic and abiotic stresses via the synthesis of a myriad of polyphenols. Photo source: Dixon and Paiva (1995).[1]

antioxidants. Antioxidant enzymes constitute the first line of defense against excessive radical formation. The major antioxidant enzymes in plants include superoxide dismutase, catalase, and ascorbate peroxidase.[72] The health of the cell depends on the balance of superoxide dismutase, catalase, and ascorbate peroxidase activities. Superoxide dismutase catalyzes the conversion of the O_2 radical to H_2O_2. Several genes for superoxide dismutase can be found in plants and they have been shown to be spatially regulated in plant tissue. H_2O_2 can then be catalyzed to two molecules of water by catalase or to one molecule of water by ascorbate peroxidase. Catalase is generally localized to peroxisomes while individual isoforms of ascorbate peroxidase can be found in the mitochondria, chloroplasts, peroxisomes, and in the cytosol.[71]

Antioxidants

Much attention has been brought to plant polyphenols over the years due to the growing evidence that they can be potent antioxidants. Their stress-reducing function in plants can be extended to humans that eat a diet rich in plant-based foods. Fruits and vegetables are an abundant source of polyphenols which help reduce oxidative stress, decreasing the imbalance of free radical formation and neutralization in the

body. Excessive oxidative stress has been implicated in several chronic and degenerative diseases such as Alzheimer's, cancer, arthritis, and cardiovascular disease. Common sources of oxidative stress include radiation, cigarette smoke, alcohol, poor diet, medications, and pollution.[73]

Polyphenols primarily function as antioxidants by scavenging ROS and oxidatively generated free radicals, RO▪ and ROO▪.[2] Chelating metal ions and inhibiting radical forming enzymes are, although to a lesser extent, two other antioxidant properties of polyphenols. ROS are naturally occurring within living organisms. Not only are they the natural by-products of cellular respiration and metabolism, they are also a vital component of the human immune system which utilizes free radicals to combat pathogens.[73] However, these highly reactive and unstable free radicals can severely damage the cell if left unchecked. They must be tightly controlled as they have the ability to oxidize macromolecules such as proteins, lipids, and DNA. This can lead to a loss of function, as with proteins, or mutagenesis in DNA. The oxidation of lipids can also generate a chain reaction of free radical formation, called lipid peroxidation, where a single oxidized lipid propagates the oxidation of the surrounding lipids. This can lead to extensive lipid and membrane damage which can have cytotoxic effects.[73]

Polyphenols neutralize free radicals via two mechanisms, hydrogen atom transfer (HAT) or single electron transfer (SET) (**Figure 2)**. The HAT mechanism relies on the donation of a hydrogen atom from the polyphenol to the free radical, while the SET mechanism relies on the donation of an electron.

Figure 2. Mechanisms of free radical quenching by polyphenols. Hydrogen atom transfer relies on the donation of a hydrogen atom while the single-electron transfer mechanism relies on the donation of an electron ion from the polyphenol. Adapted from Quideau et al.[2]

Both mechanisms neutralize the free radical and cause the polyphenol to become the oxidant; however, due to the inherent structural features of polyphenols the resulting unpaired electron on the polyphenol is more stable than the previously unpaired electron on the ROS. [2] The hydrophobic aromatic rings of a polyphenol coupled with hydrophilic hydroxyl groups create an amphiphilic molecule that is able

to act as a hydrogen-bond donor or acceptor.[2] This allows for the homolytic donation hydrogen and the subsequent resonance stabilization of the unpaired electron.[2]

Classification **and** ***chemical Structure***

The functional diversity of polyphenols and their wide array of bio-physiochemical properties arise from their chemical structure.[2] Polyphenols are characterized by an aromatic ring bearing one or more hydroxyl groups. The aromatic rings can be combined in nearly endless configurations to produce thousands of different polyphenols.[6] They can range from small, simple phenolic acids with a single ring, such as caffeic acid, to very large, complex polymers such as condensed tannins. Despite the wide variety of structures, all polyphenols arise from the deamination of the amino acids phenylalanine or tyrosine which yields the basic phenylpropanoid unit, C_6-C_3.[74] This unit is derived from either L-phenylalanine or L-tyrosine and serves as the building block for the synthesis of all polyphenols.[74] Due to their diversity, polyphenols have been divided based on their structure into a number of classes such as phenolic acids, tannins, stilbenes, lignin, and flavonoids.

<u>*Phenolic acids*</u>

Phenolic acids are the simplest phenolic metabolite containing a single aromatic ring. They are divided into two classes based on their carbon backbone namely the hydroxycinnamic acids (C_6-C_3) and hydroxybenzoic acids (C_6-C_1) (**Figure 3**). Hydroxycinnamic acids are derived from the deamination of L-phenylalanine to cinnamic acid. Variations of these acids arise from the hydroxylation and methylation of the aromatic ring. Fruits and vegetables contain high amounts of hydroxycinnamic acids with caffeic acid being the most predominant. Degradation of the sidechain on cinnamic acid yields the hydroxybenzoic acids. The various

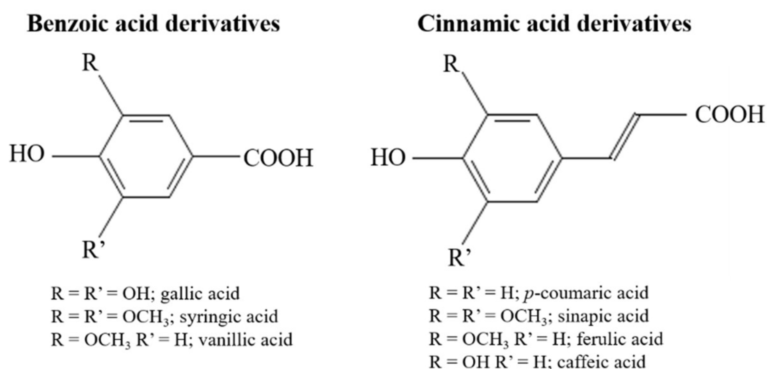

Figure 3. Phenolic acids are divided into benzoic acid derivatives and cinnamic acid derivatives.

hydroxybenzoic acids are also produced by the addition of hydroxyl or methyl groups to the aromatic ring and they are generally found in low levels in most foods except for some fruits in which their content can be very high. All phenolic acids are rarely present in their free form and are generally in a bound state. However, processing of foods can increase the occurrence of free phenolic acids.[75-76]

Unripe strawberries have high levels of phenolic acids with the abundance of individual phenolic acids varying during fruit maturation. Mandave et al.[77] showed that the hydroxybenzoic acid derivative accumulated in higher amounts during the green and white stages of strawberry development while ferulic and caffeic acids were more prevalent during the white and turning (pink) stages. Coumaric acids peaked at the fully ripe stage while the previous phenolic acids all decreased as ripening progressed.

Tannins

The tannins are high molecular weight polyphenols originally named for their ability to tan animal hide into leather. These water-soluble compounds typically have a molecular weight between 500 and 3000 g/mol. Tannins can be comprised of only gallic acid base units (gallotannins) or gallic acid and hexahydroxydiphenoyl subunits (ellagitannins).[78] Strawberries are one of the most important dietary sources of ellagitannins but these polyphenols are also found in blackberries and raspberries.[79] The content of ellagitannins varies by genotype but in general, unripe strawberries has significantly higher levels of ellagitannis compared to red ripe fruit.[79]

Tannins are divided into two groups, hydrolysable and condensed tannins. The difference between the two groups lies in the ability of weak acids or bases to break down the former while the latter are more resistant to hydrolyzation. Tannins are generally a feeding deterrent to herbivores due to their astringent taste. They also have anti-nutritional properties because they are able to precipitate proteins and other macromolecules in aqueous solutions. Although it was believed that tannins could disrupt digestion by interfering with essential digestive proteins, this notion is now under scrutiny.[78, 80]

Stilbenes

Stilbenes are a group of polyphenols with a 1,2 diphenylethylene backbone ($C_6 – C_2 – C_6$) generally found in pine, grapes, peanuts, and sorghum.[81] Stilbenes are considered phytoalexins because they

accumulate at the site of pathogen attack and serve as effective antimicrobial agents. Stilbenes are also involved in allelopathy, the inhibition of neighboring plant growth, and also in the oxidative stress response to UV radiation.[80-82] The most common stilbene is resveratrol which has been the center of extensive research over the years due to its presence in red wine and its proposed cardioprotective effects. Interestingly, resveratrol has recently been detected in strawberry fruits. Wang et al.[83] showed that several factors contribute to the amount of resveratrol found in strawberries such as temperature, tissue type, cultural system, soil composition, CO_2 concentrations, fruit maturity, and genotype. Elevated growing temperature, such as 30 °C during the day and 25 °C at night, significantly increased the levels of resveratrol in strawberries, with higher resveratrol levels detected in the achenes compared to the receptacle. Although resveratrol concentration significantly increases as the fruit ripens, genotype seemed to have the largest impact on resveratrol content. For example, out of 14 strawberry cultivars studied, 'Ovation' strawberries had five times greater amount of resveratrol compared to 'Allstar' strawberries.[83]

Lignin

As previously discussed, lignin is a vital component of plant cell walls. Their hydrophobic nature helps to prevent the intrusion of water and enables the use of a vascular system for the transportation of water throughout the plant organism. Lignin also provides mechanical support which allows plants to stand upright.[59] The dual roles of cellulose and lignin in plant cell walls have been likened to reinforced concrete where cellulose are the reinforcing steel rods that provide shape and lignin is the poured concrete that binds everything together.[84]

Lignin is a polymer of the monolignols *p*-coumaryl alcohol, conferyl alcohol, and sinapyl alcohol. These alcohols are derived from *p*-coumaric acid, ferulic acid, and sinapic acid respectively. The ratio of monolignols present in plant cell walls can vary from species to species.[84] Angiosperms contain lignin polymers mostly composed of conferyl alcohol and sinapyl alcohol while lignin polymers of *p*-coumaryl alcohol are much less frequent. In strawberries, this has been attributed to the low affinity of the enzyme and substrate (cinnamoyl-CoA reductase and *p*-coumaryl-CoA, respectively) needed to produce *p*-coumaryl alcohol.[85] The lignin polymers are strengthened by the random interlinking of monolignols from

different lignin polymers via the peroxidase enzyme. The randomness of peroxidase along with its broad substrate tolerance creates a structure that is highly resistant to enzymatic degradation and microbial attack.[84-85]

The firmness of strawberries is mostly attributed to the level of pectin degradation in the middle lamellae located between individual cells. However, recent studies have suggested that lignin content of strawberries also contributes to fruit firmness.[63, 85] A comparative microarray analysis by Salentijn et al.[63] revealed differences in gene expression of two strawberry cultivars with opposing firmness. Specifically, a large difference in gene expression of two enzymes from the lignin biosynthetic pathway, cinnamoyl-CoA reductase (CCR) and cinnamoyl alcohol dehydrogenase (CAD) was observed. Expression of CCR was higher in the soft cultivar while expression of CAD was higher in the firm cultivar. Yeh et al.[85] followed up on the previous study by overexpressing monolignol genes in strawberry fruit which resulted in greater fruit firmness.

Flavonoids

Flavonoids are the most pervasive polyphenol found in plants and the most prominent in strawberries.[27, 86] **Figure 4** shows the basic structure of a flavonoid which it is comprised of two benzene rings (A and B) connected by a heterocyclic pyrane ring (C). Flavonoids are divided into six subgroups: flavonols, flavones, flavandiols, chalcones, anthocyanins, and proanthocyanidins. The level of oxidation of the C ring determines the different classes of flavonoids while the individual compounds in each class are determined by the number and arrangement of hydroxyl and alkyl groups on the A and B rings.[86] The anthocyanins are the most distinguishing group of flavonoids due to their contribution to the color of many flowers and fruits. The bright, red color of strawberries can also be attributed to the anthocyanins, pelargonidin 3-glucoside and cyanidin 3-glucoside.[67] Various studies have shown these two anthocyanins to be the predominant polyphenols in ripe strawberries, with pelargonidin 3-glucoside being present in higher amounts than cyanidin 3-glucoside, and that the amount of each

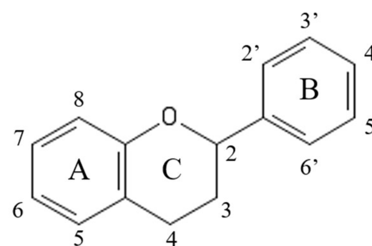

Figure 4. The flavonoid skeleton is comprised of two benzene rings (A and B) connected by a heterocyclic ring (C). Saturation of the C ring and placement of hydroxyl or alkyl groups on the A and B rings distinguish individual flavonoids.

anthocyanin present can vary depending on the cultivar. For example, a study on nine Italian strawberry cultivars showed that the concentration of pelargonidin 3-glucoside could range from 95.80 – 282.34 µg/g of strawberry tissue while the concentrations of cyanidin 3-glucoside were much lower and ranged from 1.06 – 11.15 µg/g of strawberry tissue.[15] In another study, Buendía et al.[87] reported the content of pelargonidin 3-glucoside and cyanidin 3-glucoside was also genotype dependent and ranged from 16.2 – 36.3 mg/100 g and from 0.5 – 4.2 mg/100g of fresh weight strawberry tissue, respectively.

Flavonoids are involved with many of the biological functions previously discussed, although pigmentation is their most distinctive role. However, flavonoids are also potent antioxidants and are considered second line in plant defense against oxidative stress after the antioxidant enzymes. Indeed, oxidative stress induces the production of flavonoids and flavonoid related enzymes in stress-sensitive plants.[88] For example, the primary flavonoid biosynthetic enzyme, chalcone synthase, was upregulated in a salt-sensitive rice genotype when exposed to salt stress whereas a salt-resistant genotype showed less upregulation of the same enzyme.[89] A similar trend was observed in two genotypes of beans, *Phaseolis vulgaris* L., with differing tolerance to ozone. The genotype more sensitive to ozone accumulated higher RNA transcripts of two enzymes related to flavonoid synthesis, chalcone synthase and chalcone isomerase when compared to the genotype that was more ozone tolerant.[90]

The antioxidant activity of flavonoids is dependent on their chemical structure with dihydroxyl substitutes on the B ring correlating to a higher antioxidant activity (**Figure 4**). Higher concentrations of flavonoids have been detected in the ROS generating centers of the cell such as the chloroplasts and the nucleus of mesophyll cells. Flavonoids also have the ability to absorb UV radiation which adds to their antioxidant capacity making them excellent protectants from the Sun's damaging rays.[86] For example, this photooxidative protection is most evident during autumn as the leaves of many trees transition from a green to a red/orange coloration. This is believed to provide extra protection against photooxidation to the plant cells during the nutrient retrieval process.[65, 91] In the same way, two subgroups of flavonoids, the anthocyanins and flavonols, protect the strawberry from UV radiation. Josuttis et. al.[92] studied the effect of UV radiation on the individual polyphenols of strawberries by comparing fruit that had been grown in open-

field to fruit grown under a tunnel covered by UV-blocking plastic films. While the presence or absence of UV radiation did not affect the overall anthocyanin content of the fruit, individual polyphenols such as the anthocyanin cyanidin 3-glucoside and the flavonol quercetin 3-glucuronide were found in significantly lower concentrations in the fruit grown under the UV-blocking tunnel. The primary polyphenol in strawberries, pelargonidin 3-glucoside, was unaffected by the amount of UV radiation. Flavonoids are also involved in pollen-tube germination and male fertility as well as the regulation of plant growth by inhibiting the transport of auxin.[93]

Biotic factors affecting polyphenolic content

Polyphenols in plants are either constitutively synthesized or induced by a stressor. Constitutive synthesis of polyphenols is a result of the coevolutionary process between plants, herbivores, and pathogens where plants that intrinsically produced protective polyphenols were better adapted to survival. This led to some plants being resistant to herbivory or infection by certain pathogens, like an innate immunity that gives the plant organism an advantage over other, less resistant plants. However, polyphenols can also be synthesized, or induced, upon pathogen or herbivore attack. These polyphenols can either be toxic to the attacking organism or give the plant an unpleasant taste to deter feeding.[6]

Plants synthesize polyphenols in response to specific biotic and abiotic factors which also dictate the types and levels of polyphenols synthesized. The biotic factor is heavily dependent on genetics, tissue type, and plant maturity.

Genetics

Polyphenol composition is genetically driven, and each species of plant has evolved to produce certain polyphenols to suit their environment and reproductive processes. For instance, anthocyanins are commonly expressed in flower petals to attract pollinator and tannins are a major component of tree bark to provide protection from pests and pathogens. Polyphenol composition can also vary among individuals within the same species. Over one-hundred species of strawberry are recognized (US National Plant Germplasm System) but the predominant cultivated strawberry is *Fragaria* x *ananassa* and within this

species there are numerous genotypes with major differences in polyphenol content. Immature, green strawberry fruit contain high concentrations of proanthocyanidins however this can vary significantly between genotypes and has been shown to be genetically dependent.[94] The major polyphenols present in the ripe fruit of *F. ananassa* are the anthocyanins pelargonidin 3-glucoside and cyanidin 3-glucoside which give the strawberry its distinctive, red color. Pelargonidin 3-glucoside is the most abundant anthocyanin and has been reported to constitute between 60-95% of the total anthocyanin content of strawberries.[11, 95-96] Cyanidin 3-glucoside is consistently detected in strawberries although in lower concentrations, only comprising 3-10% of the total anthocyanin content.[95]

In order to determine the effect of selective breeding on the polyphenol profile of the cultivated strawberry, Muñoz et al.[67] measured the concentration of various polyphenols in five genotypes within the *Fragaria* genus including two cultivars ('Chandler' and 'Parker') of the *F. ananassa* species. The levels of mRNA of four important enzymes in the phenylpropanoid pathway, namely phenylalanine ammonia-lyase, cinnamic acid 4-hydroxylase, chalcone synthase, and flavonoid 3'-hydroxylase were also measured. They found that the majority of the polyphenols, including phenolic acid derivatives, flavonols, and anthocyanins, were genotype dependent with the major polyphenols consistently being pelargonidin 3-glucoside and cyanidin 3-glucoside. The transcriptional analysis explained the differences in anthocyanins and phenolic acids between genotypes well. For instance, the cultivar 'Camarosa' had the highest levels of total anthocyanins and also the highest level of chalcone synthase transcripts, the first committed enzyme of flavonoid biosynthesis in the phenylpropanoid pathway that can lead into the production of anthocyanins. Another study compared the concentration of individual anthocyanins of five strawberry cultivars grown at two separate locations.[96] Three of the cultivars ('Frida', 'Polka', and 'Senga Sengana') showed similar anthocyanin levels between the two locations which pointed to a genetic basis of overall anthocyanin content independent of growing conditions. The other two cultivars ('Florence' and 'Korona') differed greatly between the locations however it was noted that despite the different level of total anthocyanins, the proportions between the two major anthocyanins, pelargonidin 3-glucoside and cyanidin 3-glucoside, remained constant.[96]

Plant and fruit maturity

Polyphenol profiles and concentrations are also dependent on the maturity of the plant and fruit. That is, polyphenols which are present in ripe strawberries differ from those present in the unripen fruit. Immature, green strawberries are more resistant to invasion by fungal pathogens such as *Colletotrichum acutatum* and *Botrytis cinerea* than red, ripe fruit because they contain higher levels of proanthocyanidins, namely catechin and epicatechin, which have antimicrobial properties and protect the immature embryos from pathogens.[97] These polyphenols also give the fruit a bitter taste to ward off herbivores allowing time for the seeds to develop. As the fruit matures, the concentration of these polyphenols decreases while there is a significant increase in the levels of anthocyanins, concomitantly with the development of a red coloration.[98] The rise in concentration of the brightly colored anthocyanins along with an increase in sugar content and softening of the fruit attracts seed dispersing herbivores. However, the decrease in the antimicrobial polyphenols results in the ripe fruit being more susceptible to fungal infection.[99]

Tissue type

The classes and levels of polyphenols also vary depending on plant organ and type of tissue. Each plant organ (i.e. roots, leaves, flowers, fruits) is designed to carry out a particular function and they each experience unique environmental pressures. For example, leaves must protect themselves from herbivory and the Sun's UV radiation as they contain the photosynthesizing cells and thereby provide the plant with energy, while roots must be able to endure abusive changes in soil chemistry and guard against pathogens present within the ground. Therefore, the tissues within plant organs also vary in their polyphenolic content. Moreover, the polyphenol distribution within a strawberry is not uniform. Lignin has been detected almost exclusively in the vascular bundles and achenes while the cortex has been found to contain more polyphenols than the pith (**Figure 5**).[52, 64, 92] Studies comparing the receptacle and achenes of

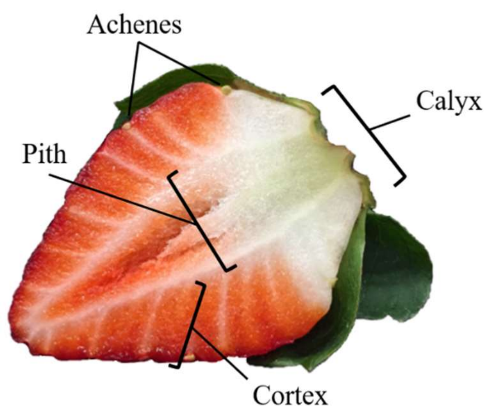

Figure 5. A cross-section of a strawberry. The achenes are the seeds on the outside of the fruit. The inner section is labeled as the pith while the outside is called the cortex. The calyx is the 'cap' of the strawberry and the white lines running from the pith through the cortex are the vascular bundles.

strawberry fruits have shown differences in their polyphenolic profiles and that the achenes contain a higher concentration of polyphenols.[83, 100] The higher content of polyphenols in the achenes probably serves to protect the seeds until conditions are right for germination, especially if the seeds pass through the digestive tract of a herbivore. Aaby et. al.[100] compared the polyphenolic content of the flesh and achenes of two strawberry cultivars, 'Totem' and 'Puget Reliance' and reported that, respectively, the total phenolic content of the achenes was about 11 to 14 times higher than that of the flesh. The ratios and abundance of individual polyphenols also differ between the achenes and the flesh of strawberry fruit. The predominant anthocyanin of the flesh was pelargonidin 3-glucoside with cyanidin 3-glucoside present in much smaller amounts. However, they were detected in nearly equal proportions in the achenes. Phenolic compounds differed greatly between the flesh and achenes as well. All phenolic compounds (ellagic acid, catechin, procyanidins, ellagitannins, flavonols) are in general more abundant in the achenes, especially the ellagitannins which concentration can be 833.0 mg/100 g fresh weight in the achenes and only 9.6 mg/100 g fresh weight in the flesh.

Abiotic factors affecting polyphenolic content

While plants have a preformed physical barrier (i.e., waxy layers, thorns, or resin ducts) to environmental pressures, they are also able to adapt their polyphenol profile to survive variations in their environment.[6] Abiotic factors such as environmental conditions, agricultural practices, and postharvest handling and storage conditions can all influence the amount and type of polyphenols present in plants as well as in fruits and vegetables.

Environmental conditions

Plants live in an ever-changing environment which can induce stress by a wide variety of factors such as high or low temperatures, nutrient deficiency, too little or too much sunlight, flooding or drought, and abusive changes in soil chemistry. Plants adapt and survive these changes by increasing polyphenol production.[84] Even the polyphenolic content of strawberries is affected by the environmental conditions of their cultivation site.

A significant part of the environmental conditions plants encounter is underground, in the soil. Soil composition has an impact on the health of the plant as it is where plants obtain many of their nutrients and water. However, soil composition can be controlled by the farmer and soils deficient in particular nutrients can be supplemented with fertilizers. Thus, there are relatively few studies regarding soil composition and polyphenols in strawberries. However, there has been recent interest in improving the health-promoting compounds in strawberries by manipulating the availability of nutrients in the soil. For example, strawberries grown in iron or phosphorous deficient soil produced more polyphenols. Most notably, the iron and phosphorous deficient fruit contained more anthocyanins and, in particular, the iron deficient fruit had 40% more pelargonidin 3-glucoside content, the predominant anthocyanin in strawberries, than the control.[101] Along with iron and phosphorous, increased concentrations of soil salinity has also been investigated as a way to enhance polyphenolic content in strawberries. Because strawberry plants are sensitive to saline environments, there is also a growing concern over the rising salinity of fresh water supplies and the possible impacts on strawberry production. However, a mild treatment with slightly saline irrigation has been shown to be beneficial with respect to the concentrations of polyphenols. Strawberries produced from plants treated with 40 mmol/l NaCl or 80 mmol/l NaCl had significantly higher anthocyanin and phenolic contents when compared to the control. More specifically, high phenolic contents correlated with an increase in the amount of (+)-catechin.[58, 102]

Many more studies have focused on the environmental conditions encountered above ground and the impact on polyphenols in strawberries. However, it is much more difficult to control the condition plants are exposed to above the soil. These include parameters such as temperature, rainfall, and sunlight which can positively or negatively affect the synthesis of polyphenol in strawberries. The effect of high temperature stress on anthocyanin accumulation was studied. For example, elevated day/night temperatures (30/15 °C) during fruit maturation resulted in a 60% reduction in anthocyanin content when the strawberries reached full ripeness.[103] Another study also reported that increasing the day/night temperature causes a decrease in the ellagic acid content of strawberry fruit.[104]

Temperature is one of the many factors influencing polyphenol content in strawberries. Palmieri et al.[105] examined the influence of growing site (altitude), sunlight duration, and UV-radiation on polyphenolic content of several strawberry cultivars in Italy. The strawberries were grown at three different altitudes, specifically at 59 m, 488 m, and 854 m above sea level. Interestingly, the content of anthocyanins was the least affected by growing site. By contrast, they discovered that flavonol content tended to increase at higher altitudes while the highest content of benzoic acid derivatives was at the lowest altitude. Longer duration of sunlight also correlated to a higher content of flavonols and ellagitannins while these compounds declined with increasing temperatures and UV-radiation.[105]

Agricultural practices

Agricultural practices can mimic and have the same effect as environmental conditions on the polyphenolic content of fruits and vegetables.[84] This is due to the farmer's effort to control the various abiotic stressors that can affect crop health and yield. For instance, temperature can be regulated using greenhouses or plastic tunnels. Soil composition can be manipulated by fertilizers and irrigation can help control water management of crops. These all help to alleviate the possible deleterious effects of climate variation which reduces stress on the plants and therefore affects the polyphenolic composition of the fruits or vegetables.

Different cultural systems utilized by strawberry growers have a marked effect on the polyphenol content of the fruit. The most common method of growing strawberries today is on a raised-bed that is covered by polyethylene (hill plasticulture). Hill plasticulture helps keeping the strawberries cleaner, makes harvesting easier, saves water, and reduces herbicide usage. Until the introduction of hill plasticulture, matted rows were the principal method of strawberry cultivation. Matted row involves planting the crop on the bare ground. The plants are spaced about two-feet apart from one another in row form. Then the runners from the mother plants are used to fill the spaces in between to create the "matte" of strawberry plants. A study comparing two agricultural systems, conventional matted row to hill plasticulture, showed that strawberries grown on the latter contained higher levels of polyphenols while also producing better quality fruits.[106] Strawberries can also be cultivated under plastic tunnels or in greenhouses. This offers a more

controlled environment to the grower and enables production to extend into winter months in Northern climates as these structures can be heated. Pincemail et al.[107] studied the effect of cultural system (open fields, tunnels, greenhouses) on 'Elsanta' strawberries. The fruit grown in open fields had a higher total phenolic content when compared fruit grown under a tunnel. Also, heated greenhouses produced fruit with lower total phenolic content than fruit grown under a tunnel.

Differences in polyphenol content have also been found between strawberries grown organically compared to conventional farming methods. Organically grown fruit in general have higher levels of polyphenols compared to conventionally grown fruit and thus tend to be redder due to a rise in anthocyanin synthesis. This has been attributed to increased stress on the plant and fruit due to lower pesticide applications.[108] A study spanning three years found the strawberries produced by organic farming had significantly more of the individual anthocyanins pelargonidin 3-glucoside and cyanidin 3-glucoside than fruit harvest under an integrated pest management system where less amount of pesticides were used.[109]

Postharvest handling and storage conditions

Fruits and vegetables continue to respire after they have been removed from the plant therefore they still have the ability to actively respond to their environment. However, senescence is inevitable since the fruit or vegetable is no longer attached to the mother plant and the supply of nutrition is cut off. The rate of senescence impacts the concentrations of polyphenols and the polyphenols, in turn, impact the quality of the produce.

Utilizing polyphenols to improve preharvest and postharvest quality

Many functions of polyphenols and the factors affecting their content in plants relates to the defense against stress. There is ample evidence showing that stress induces the production of polyphenols while a fruit or vegetable is still attached to the plant providing the organism with a level of protection.[101] Removal of the fruit or vegetable from the plant creates stress which can shorten shelf life. However, it has been suggested that a mild application of preharvest stress may improve the postharvest quality of crops due to an increase in polyphenol content.[6]

Galli et. al.[58] recently investigated the effect of applying mild salt stress (40 and 80 mmol/L NaCl) to strawberry plants and the subsequent effect on fruit quality. A significant increase in total anthocyanins was found in the fruit treated with 40 mmol/L NaCl while 80 mmol/L NaCl resulted in a significant increase in total polyphenols compared to the control treatment (distilled water). The increase in total polyphenols coincided with increased activity of the enzyme phenylalanine ammonia-lyase, the main regulatory enzyme of polyphenol synthesis. The content of individual polyphenols including gallic acid, ellagic acid, p-coumaric acid, (+)-catechin, p-hydroxybenzoic acid, and caffeic acid was also measured showing a significant increase in (+)-catechin with the salt stress.[58]

Phenylpropanoid pathway and specific regulatory enzymes

Overview of the phenylpropanoid biosynthetic pathway

All polyphenol compounds are derived from the general phenylpropanoid pathway and its numerous branches (**Figure 6**). The general phenylpropanoid pathway is defined as the first four enzymes that convert L-phenylalanine into *p*-coumaryl-CoA which can then be diverted into one of the various phenylpropanoid branches such as the monolignol biosynthetic pathway or the flavonoid biosynthetic pathway. The phenylpropanoid pathway is connected to primary metabolism via the shikimate pathway which generates the aromatic amino acids tyrosine, tryptophan, and phenylalanine. This plant-specific pathway is absent from animals who are unable to synthesize these amino acids *de novo* and for this reason the aforementioned aromatic amino acids are considered essential amino acids that must be obtained from diet. Phenylalanine ammonia-lyase (PAL) directs up to 30% of the fixed carbon source from the shikimate pathway to different phenolics.[110]

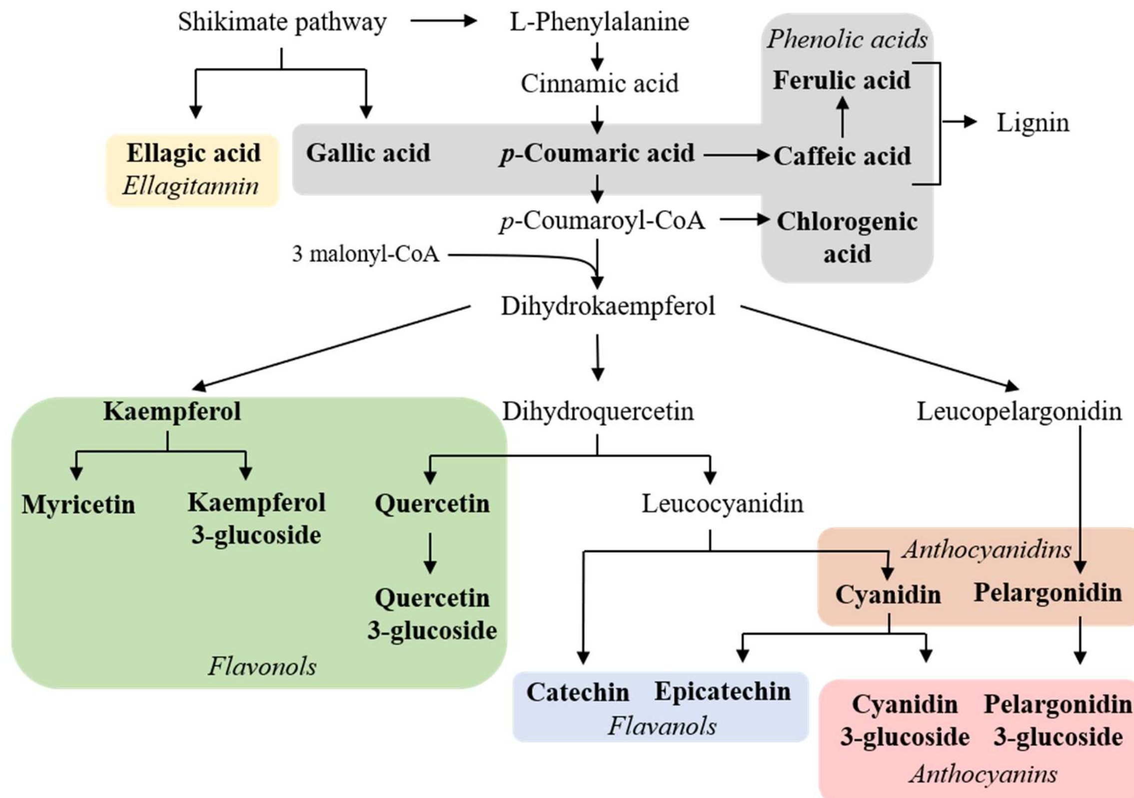

Figure 6. The phenylpropanoid pathway is connected to primary metabolism via the shikimate pathway. It begins with the conversion of l-phenylalanine to cinnamic acid and branches out into many pathways.

Phenylalanine ammonia-lyase (PAL) in plants

Structure and function

The first, and key regulatory, enzyme in the general phenylpropanoid pathway is phenylalanine ammonia-lyase (PAL). Carbon flux into this pathway, and consequently the biosynthesis of all secondary metabolites, is controlled by the activity of PAL, which is an enzyme related to the superfamily of histidine ammonia-lyases (HAL) found in bacteria from the histidine degradation pathway.[111] PAL differs from the HAL enzymes in that it contains an extra N-terminal domain which is believed to interact with other cellular components as well as function as a membrane anchor. PAL also contains a 'shielding' domain that can block the active site and thereby regulate its enzyme activity.[112-113] It is a tetrameric enzyme that catalyzes the non-oxidative removal of ammonia from L-phenylalanine to form *trans*-cinnamic acid without the aid of any external cofactor or coenzyme. This is a unique feature of PAL because it instead relies on an

electrophilic prosthetic group, 4-methylidene-imidazole-5-one (MIO), formed by the autocatalytic cyclization of three internal amino acids, namely Ala-Ser-Gly, situated in the active site, a motif conserved in the HAL superfamily of enzymes. Consequently, substitution of the serine for another amino acid inactivates the enzyme.

PAL exists as a multi-gene family with plants generally displaying several isoforms. Despite these isoforms showing redundant activity, there have been many studies indicating that specific isoforms are activated to produce distinct classes of polyphenols and the activity of the isoforms show spatial and temporal variation within the plant. For example, the four isoforms of PAL found in *Arabidopsis*, denoted PAL1-PAL4, are not uniformly expressed throughout the plant. PAL1 was found to be localized to vascular tissue while PAL2 and PAL4 expression was concentrated in the seeds.[114] Expression levels of PAL1, PAL2 and PAL4 were also high in stem tissue while PAL3 showed overall minimum expression in stem tissue but was highly expressed in leaf tissue. In the recently described PAL gene in strawberries, *FaPAL6* expression was only detected in the ripe fruit of the two cultivars studied ('Camarosa' and 'Toyonoka') and a higher gene expression was present in the cultivar with more anthocyanin content, 'Camarosa'. The lack of expression found in other plant tissues pointed to a PAL gene family in strawberries although it has not been elucidated.[52]

Regulation

PAL's position atop the phenylpropanoid pathway and the capability of its product, *trans*-cinnamic acid, to be converted into one of thousands of polyphenols means that plants have imposed many levels of regulation on this enzyme. The simplest form of regulation is feedback inhibition by *trans*-cinnamic acid and its derivatives.[115] PAL is also controlled at the transcriptional level by environmental and developmental cues. Evidence of MYB-mediated transcriptional control has been found in the promoter regions of PAL genes from many species as well as for many of the other biosynthetic genes in the phenylpropanoid pathway. This could indicate a coordinated expression of phenylpropanoids genes in response to different stimuli. MYB transcription factors have been reported to regulate PAL activity in carrots[116] and *Arabidopsis*.[117] While MYB transcription factors have not been reported to interact directly

with PAL genes in strawberries, they have been reported to interact with other genes in the phenylpropanoid pathway specifically in the synthesis of flavonoids, and anthocyanins. FaMYB1 isolated from strawberry and expressed in tobacco plants inhibited pigment production in tobacco flowers.[118]

De novo synthesis of PAL is rapidly induced when there is a need for phenylpropanoids.[119] This is followed by a spike in PAL activity and subsequently by a gradual reduction of activity indicating some level of post-translational control.[113, 120-121] This transient induction of PAL serves to prevent depletion of primary metabolites from the cell.[122] Post-translational regulation of PAL has been linked to the ubiquitin-proteasome degradation pathway opposed to inactivation of the enzyme.[110]

Factors affecting enzyme activity

PAL activity can be induced by a variety of endogenous and exogenous signals. First and foremost, PAL is stimulated by growth as some of the products of phenylpropanoid pathway are vital components of a healthy plant, such as lignin in the cell walls. A study on kenaf (*Hibiscus cannabinus*), a promising plant for use in biomass production, showed that PAL activity gradually increases in stem development reaching its peak in 4 week old seedlings.[123] Moreover, a quadruple knockout of the four PAL genes in *Arabidopsis thaliana* resulted in plants that were both stunted and sterile. The inflorescence stem of the quadruple knockout also contained significantly less lignin.[124] In developing strawberries, PAL activity expressed a biphasic pattern in the cultivar 'Tillikum' in which a spike in activity was observed at 7 and 27 days after anthesis. The first peak was associated with a rise in total phenol content of the fruit while the second peak in activity paralleled a rapid accumulation of anthocyanins.[125]

A variety of external stimuli can promote the activity of PAL as well. These are generally deviations in environmental conditions that could be detrimental to the survival of the organism which include abusive changes in temperature, light intensity, UV exposure, nutrient deprivation, and water (drought or flooding) which can result in oxidative stress. Temperature has a considerable effect on the health and growth of a plant as temperature is capable of influencing membrane fluidity, enzyme activity and photosynthesis.[126-127] Exposure to cold or hot temperatures induces metabolic and physiological changes in plants which includes higher PAL activity resulting in an accumulation of polyphenols. For example, the effect of

freezing temperatures on PAL activity in olive trees was examined and it was found that trees with 10 - 25% leaf death after exposure to freezing temperatures had higher PAL activity than trees that showed no signs of freezing stress. However, severely damaged trees with 50 - 75% leaf senescence after freezing exhibited significantly lower PAL activity than the non-stressed trees.[128] Excess heat is particularly dangerous to plant organisms as it can uncouple enzymes and metabolic pathways causing an accumulation of excess ROS. This is exacerbated by the sessile nature of plants and their inability to seek cooler environments during abusive weather changes. When heat stress (35 °C) was applied to tomato plants that have an optimal growing temperature of 15 – 22 °C, PAL activity doubled. This was accompanied by reduced shoot growth and high total phenolic content.[129]

Along with temperature, light waves ranging from the visible to the UV spectrum can also affect the activity of PAL. In particular, light affects flavonoid accumulation in some fruit where high light intensity increases flavonoid production while shading decreases flavonoid content. It is proposed that increased flavonoid content serves a photoprotective role during periods of elevated sunlight intensity.[130] Even the color of visible light was shown to have diverse effects on the activity of PAL and flavonoid accumulation in ripening strawberries. Ripe strawberries that were cultivated under red or yellow plastic films had higher PAL activity and higher flavonoid content than the control (white light) while fruit grown under green film exhibited reduced PAL activity and lower flavonoid content.[131] While most of the sunlight reaching the Earth's surface is in the visible spectrum, 7% is in the UV range (100 – 400 nm).[132] The UV spectrum is further divided into UV-A (315 – 400 nm), UV-B (280 -315 nm), and UV-C (100 – 280 nm) light. UV-C is completely absorbed by the Earth's ozone layer while UV-B is partially absorbed, and UV-A is not absorbed. UV radiation can cause extensive damage to DNA, membranes, and proteins. This is especially true of UV-B and UV-C radiation while UV-A is less directly harmful to cellular components, but it is still capable to inducing oxidative stress. However, all three categories of UV light have been shown to induce PAL expression. Guo et al.[132] examined the effect of UV-A exposure (365 nm) on PAL transcript expression (*SlPAL5*) in tomato cotyledons and hypocotyls. Transcript levels peaked in cotyledons after 6 hours of UV-A exposure and after 12 hours in hypocotyls before declining back to control levels at 24

hours. Excess UV-B is much more dangerous to living organisms however plants are able to effectively protect themselves from ambient UV-B exposure. A study on jack pines found that pine seedling grown with an inhibitor of PAL, 2-aminoindan-2-phosphonic acid (AIP), accumulated less polyphenols and were more susceptible to freezing when compared to the control.[133] Therefore, it was proposed that ambient UV-B makes plant organisms more stress tolerant via the production of polyphenols. Because of its germicidal nature, the use of UV-C in postharvest preservation of fruits and vegetables has been studied. UV-C treatment has been applied to postharvest strawberries in an effort to reduce the incidence of *Botrytis cinerea.* For example, Strawberries treated with 2.0 KJ m^{-2} UV-C radiation and subsequently inoculated with *B. cinerea* exhibited smaller lesions of mold growth and enhanced PAL activity when compared to the control.[45]

PAL activity is also promoted by the invasion of pathogens or herbivores, and it has been associated with disease resistance in several crops. Increased PAL activity can result in the deposition of more lignin in the cell walls to limit the spread of disease. It can also result in the formation of toxic polyphenols that have an antimicrobial effect on pathogens or deter feeding with their astringent taste. A study on 20 tomato cultivars with varying degrees of resistance to bacterial wilt caused by *Ralstonia solanacearum* found that resistant cultivars showed a more robust induction of PAL activity after inoculation with the pathogen.[134] Another study on aphid infestation in two cultivars of barley also showed that the more resistant cultivar had a higher PAL activity upon the introduction of the aphids.[135]

Polyphenol Oxidase (PPO) in plants

At the other end of the phenylpropanoid pathway is the enzyme polyphenol oxidase (PPO) which is responsible for the oxidation of polyphenols, and, unlike PAL, is found in animals as well as in plants, fungi, and bacteria. PPOs represent a diverse group of enzymes that are referred to by several different names such as tyrosinase, catechol oxidase, polyphenolase, catecholase, phenolase, or cresolase.[136-137] Polyphenols are generally sequestered in the vacuoles of plant cells and separated from oxidizing enzymes such as polyphenol oxidase (PPO).

Structure and function

PPO is a metalloenzyme with two copper atoms situated in its active site and each copper atom is associated with three histidine residues, a feature conserved across all PPO enzymes. Plants can possess several isozymes of this enzyme and the number of isozymes can vary widely between species. Studies on PPO from the strawberry cultivar ‘Selva’ uncovered only one isozyme; however, a previous study on the cultivar ‘Tioga’ revealed two isozymes which supports the notion that the number of isozymes can vary between organisms of the same species.[136, 138-139]

Two different reactions can be catalyzed by PPO in the presence of oxygen, namely the hydroxylation of monophenols and the oxidation of diphenols to *o*-quinones (**Figure 7**). While some PPOs found in plants can catalyze both reactions, most higher-plant PPOs only perform the latter.

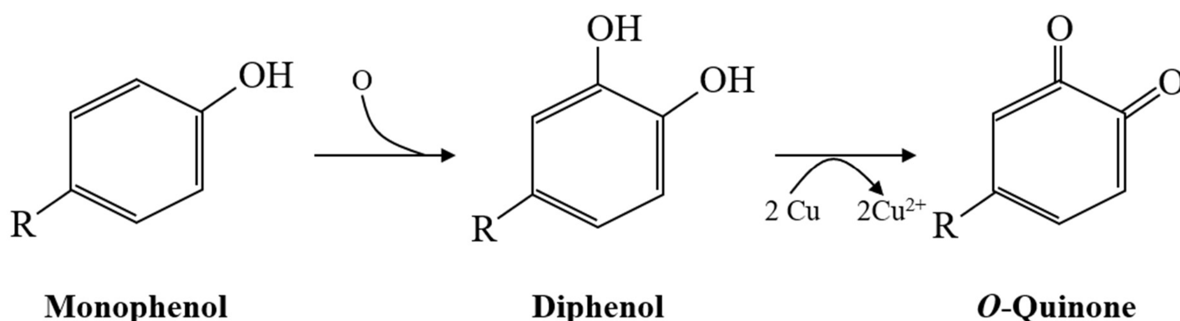

Figure 7. Polyphenol oxidase (PPO) catalyzes the hydroxylation of monophenols to diphenols and the oxidation of diphenols to *o*-quinones.

The *o*-quinones generated by PPO activity are capable of nonenzymatic polymerization which results in the formation of black or brown pigments called melanins. This discoloration can result in deterioration of postharvest plant products which reduces quality and consumer acceptance leading to loss of product at the market and increased generation of food waste. The reduction in quality is not only due to aesthetics as the browning also reduces the nutritional value as well as the organoleptic properties of the plant product. The nutritional loss is due to the destruction of essential amino acids, lower digestibility and inhibition of glycolytic and proteolytic enzymes.[140] Interestingly, PPO activity is a desired reaction in

the processing of coffee and leaf tea which gives these products a characteristic flavor and brown coloration.[141]

Undesired PPO activity is of critical concern in the postharvest quality of several horticultural crops such as wheat, mangoes, eggplants, potatoes, bananas, grapes, and apples. This is especially true if the food is to be cut, sliced, or otherwise processed in a way that results in damage to the product tissue. This negative consequence of PPO activity has generated extensive research on this enzyme and on the various ways to control its activity.[142] In the case of strawberries, PPO research has mainly centered on controlling its activity in purees as in the presence of oxygen the homogenized strawberries can quickly turn from red to brown. Inactivation of PPO in strawberry purees has been attempted by utilizing high temperature and high pressure during processing. However, this can lead to discoloration of the puree and destruction of the nutritive properties of the strawberry.[143]

PPO is generally bound to the thylakoid membranes of chloroplasts or associated with vesicles of other non-green plastids; however, some studies have reported that cytoplasmic PPOs not associated with any membranes.[144] The study on the strawberry cultivar ‘Selva’ indicated that PPO found in strawberries is likely membrane associated. This conclusion was drawn due to the higher enzyme activity associated with extracts using a detergent and salt.[139] The control of PPO activity in plants is usually due to separation and compartmentalization of PPO and its substrates where PPO is contained in the plastids while polyphenols are sequestered in the vacuoles. Stress or injury that causes the breakdown of these membranes results in association between enzyme and substrate. This results in the formation of highly reactive quinones that can react with amino acid, proteins, or phenols and the nonenzymatically polymerization of the quinones creates the brown pigmentation often associated with PPO activity.[144] The polymers are used as a barrier to pathogens and to prevent the spread of infection.[141]

Factors affecting enzyme activity

Many studies on strawberry PPO activity have focused on the processed fruit since strawberries are regularly incorporated into foods such as jams, jellies, and juices.[27] These studies have also focused on methods of inactivating enzymatic activity to preserve food color. These treatments can include high

pressure and heat; however, it has been noted that strawberry PPO is highly resistant to high pressure and thermal inactivation.[143, 145]

PPO activity in fresh harvested strawberries has been linked to water loss from the fruit during storage. In a study that compared strawberries stored in open plastic pint baskets (unwrapped) to fruit that been wrapped with PVC film, found that wrapped fruit lost less water and also had lower PPO activity. It was suggested that the higher PPO activity in unwrapped baskets was due to loss of turgor pressure on the cell membranes leading to increased membrane permeability.[11] Chisari et al.[146] also found that PPO activity in the strawberry cultivars 'Elsanta' and 'Madame Moutot' was highly correlated to browning of the fruit during cold storage at 4 °C. While generally viewed in a negative context in the food industry, PPO activity has been associated with increased disease resistance of plants in the field. For example, transgenic tomato overexpressing PPO that were challenged with the bacterial pathogen *Pseudomonas syringae* pv. *tomato.* The transgenic tomatoes exhibited better suppression of disease symptoms with 15-fold fewer lesions on the leaves than the controls at 7 days post-inoculation. Likewise, another study on tomatoes found that resistant cultivars to bacterial wilt had higher PPO activity than more susceptible cultivars.[134]

CHAPTER THREE:

IMPACT OF CULTIVAR AND COLD STORAGE ON THE POLYPHENOLIC PROFILE OF THREE STRAWBERRY CULTIVARS

Introduction

Strawberries are a popular fruit and the most consumed berry worldwide.[25] However, delivering high quality strawberries to distant markets is a challenge due to their delicate nature, perishability, and handling requirements. Ideally, strawberries should be kept between 0 and 1 °C and 90-95% relative humidity (RH) throughout the supply chain yet this is not always the case.[41, 147] Even when kept under optimum temperature and RH conditions, strawberry maximum shelf life can be as short as 7 to 10 days. This, combined with the fruit's high susceptibility to postharvest mechanical injury and decay, leads to loss of product, making it is necessary to find ways to maintain strawberry quality and extend their shelf-life.[47]

Rapid deterioration after harvest, is caused by an increased respiration rate due to the stress of strawberry fruit being removed from the mother plant and subsequent handling as the fruit are shipped to stores. Removal from its source of nutrients and heightened respiration results in serious water loss as well as a depletion of sugars and acids within the fruit.[148] Dehydration of the plant cells results in reduced cell wall turgor pressure which can lead to cell wall breakdown. Nutrient depletion, water loss, and high metabolic respiration results in production of excess reactive oxygen species (ROS) which can damage cellular components. Since senescence is a natural consequence of excessive ROS production[149], reduction of ROS can delay the onset of senescence. One way to reduce ROS production is to use refrigeration to slow down fruit metabolic respiration rate. This is key component of managing postharvest deterioration of strawberries as it not only slows respiration rate, but it also reduces water loss and nutrient depletion thus extending strawberry shelf-life strawberries.

Enhanced synthesis of polyphenols, part of the plant's natural defense system, might be another way to reduce excess ROS production and extend shelf-life[6] as polyphenols can scavenge and neutralize damaging ROS. Polyphenols function to reduce oxidative stress on plant cells as well as protect the organism from microbial invasion which both contribute to deterioration and spoilage of fresh produce. For example, a study on apples found that fruit with a higher content of polyphenols had better quality after 3 months of storage than the varieties with low polyphenol content.[150] Therefore, it might be possible to apply the same principle to strawberries to extend their shelf-life by identifying key polyphenols as markers of postharvest quality.

The major class of polyphenols in ripe strawberries are the anthocyanins and, in particular, pelargonidin 3-glucoside and cyanidin 3-glucoside which impart a red color to the fruit. The concentration of pelargonidin 3-glucoside, and all polyphenols present in strawberry, is genetically driven and can vary between genotypes.[67, 96] Aaby et al.[96] found a wide range of anthocyanin concentrations between 27 strawberry genotypes. Concentrations of pelargonidin 3-glucoside ranged from 8.5 mg/100 g in 'Marlate' to 66 mg/100 g in 'Rondo' strawberry cultivars. Indeed, pelargonidin 3-glucoside constitutes between 64 and 80% of the total anthocyanin content in strawberries whereas cyanidin 3-glucoside is about 5%.[14] Along with the anthocyanins, another important group of polyphenols in strawberry are the flavonols. These include quercetin, quercetin 3-glucoside, kaempferol, kaempferol 3-glucoside, and myricetin. The production of these colorless polyphenols in strawberries has been linked to changes in environmental conditions, especially to UV radiation.[92, 105] For example, Josuttis et al.[92] reported that strawberries grown in the absence of UV radiation contained only 15 to 25% the amount of quercetin derivatives as strawberries that were grown in an open field.

Although there are several studies that compared the levels of polyphenols in different strawberry cultivars, to our knowledge there are no studies reporting on the major, individual polyphenols of important Florida strawberry cultivars and the effect of cold storage on their polyphenol profiles. Therefore, the objectives of this study were 1) to identify variations in total phenolics and total anthocyanin contents and on major, individual polyphenols in three important Florida strawberry cultivars on the day of harvest and

2) to determine the effect of cold storage on total phenolics and total anthocyanin contents and on concentrations of major, individual polyphenols for each cultivar. The long-term aim is to determine if the enhancement of polyphenol content in strawberries correlates to a longer shelf life. Moreover, the aim is also to identify key polyphenols that could possibly relate to strawberry quality as affected by genetic variability, agricultural practices, and postharvest environmental stress. Such key polyphenols could ultimately be used as biomarkers to determine abiotic stress on the fruit.

Materials and Methods

Plant Material

'Florida Radiance', Sweet Sensation® 'Florida127', and 'Florida Beauty' strawberry cultivars (hereafter referred to as Radiance, Sensation, and Beauty, respectively) were harvested from fields at the University of Florida Gulf Coast Research and Education Center in Wimauma, Florida, two times during the 2016 production year and three times during the 2017 production year. The fruit were transported to the USF- Food Quality Laboratory in Tampa, with minimal delay after harvest. Immediately upon arrival at the laboratory, three replicated samples of 15 fruit from each cultivar were selected for uniformity of color and freedom from defects, carefully packed into three polyethylene terephthalate (PET) vented clamshells (capacity ≈453 g). Initial evaluations were performed after harvest (day 0) using 15 fruits per cultivar.

Storage Conditions

After selection, strawberries were stored for seven days inside a temperature- and humidity-controlled chamber (Forma Environmental Chambers Model 3940 Series, Thermo Electron Corporation, OH, USA) set at 1 °C and 85% RH, which simulates the lowest temperature and highest RH measured during real strawberry field-to-store trials.[17] Temperature and RH were monitored throughout the experiments using battery-powered data loggers (Hobo® U10 Temp/RH data logger, Onset Computer Corporation, Pocasset, MA, USA).

Weight loss and dry weight

Weight loss of three replicated samples of 15 strawberries each was calculated from the initial weight of the fruit and every day during a seven-day storage period**.** Concentrations of chemical constituents were expressed in terms of dry weight in order to show the differences between cultivars and treatments that might be obscured by differences in water content. The following formula was used for water loss corrections: [chemical components (fresh weight) × 100 g / strawberry dry weight + weight loss during storage (g)]. Strawberry dry weight was determined by drying three weighed aliquots of homogenized strawberry tissue at 80 °C, and until weight stabilized.

Total polyphenols and anthocyanin contents

Total soluble phenolic compounds were determined using the Folin-Ciocalteau Assay as described by Nunes et al.[11] This assay uses an oxidizing reagent (Folin-Ciocalteau's Phenol Reagent) to reduce tyrosine and tryptophan residues which results in a blue color with a maximum absorbance at 765 nm.[151] The level of absorbance is proportional to the concentration of phenolic compounds within the sample.[151] Pureed fruit were centrifuged for 20 minutes at 4700 rpm at a refrigerated temperature (4ºC). The clear supernatant was filtered through cheese cloth. Then 0.5 g of supernatant was diluted in 9.5 ml of deionized water. Each sample was evaluated in triplicate to account for error. Five ml of diluted Folin-Ciocalteau reagent (1:9 reagent to water dilution) was added to the diluted sample and vortexed. Between 30 seconds and 8 minutes afterwards, 4 ml of a sodium carbonate (0.71 M) solution was added and vortexed. The sample was incubated at 30ºC for 1 hour then chilled for 30 minutes on ice. The absorbance was read at 765 nm and plotted on a standard curve to determine the concentration of total soluble phenolic compounds. Gallic acid was used to create a standard curve for this assay. The standard concentrations were 0.04 mg/ml, 0.08 mg/ml, 0.12 mg/ml and 0.16 mg/ml.

The concentration anthocyanin contents were determined using the method described in Nunes et al.[11] Two grams of pureed fruit were mixed with 18 ml 0.5% (v/v) HCL in methanol. The pigments were extracted for 1 hour at 4ºC in the dark then filtered through a single layer tissue. Total anthocyanin contents

of the clear liquid were measured at 520 nm absorbance. Anthocyanin pigments are sensitive to degradation from light, therefore the entire extraction procedure took place under low light conditions. Total polyphenols and anthocyanin contents were reported in a dry weight basis to compensate for water loss during storage.

Polyphenol profiles

For polyphenol extraction, triplicates of 5 mL of homogenate were mixed with 15 mL of acetone, sonicated for 10 minutes and filtered through Whatman paper No.4. The filtrate was concentrated to 5 mL in a SPD121P SpeedVac® Concentrator (Thermo Fisher Scientific Inc., Asheville, NC, USA) and passed through a classic C_{18} Sep-Pack cartridge (Waters Technologies Corp., USA) previously activated with methanol, followed by water and 3% formic acid. Anthocyanins and other phenolics were absorbed onto the column whereas sugars, acids, and other water-soluble compounds were eluted with acidified water. The polyphenols were then recovered by passing 2.0 mL of methanol containing 3% formic acid through the cartridge. The extract was filtered through a 0.20 µm syringe filter into 2 mL autosampler vials and stored at -30°C until the time of analysis.

Individual polyphenol compounds were identified and quantified using the extracts prepared as described above. Analysis of phenolic compounds was conducted using a Hitachi LaChroma Ultra HPLC system coupled with a photodiode array detector (Hitachi, Japan). Samples were injected at 40°C onto a reverse-phase Hypersil Gold C_{18} column (100 × 2.1 mm; particle size, 1.9 µm) (Thermo Fisher Scientific Inc., USA). The mobile phase was acidified water containing 0.5% formic acid (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) in an isocratic mixture. The flow rate was 0.3 µL/min, and the wavelength detection was set at 250, 280, 360 and 520 nm. Sample injection volume was 10 µL. Retention times and spectra were compared with pure standards of 16 compounds from different polyphenol classes: flavonoids (anthocyanidins: cyanidin and pelargonidin; anthocyanins: cyanidin 3-glucoside and pelargonidin 3-glucoside; flavonols: quercetin, kaempferol, quercetin 3-glucoside, kaempferol 3-glucoside and myricetin; flavanols: catechin and epicatechin), phenolic acids (p-coumaric acid, ferulic acid, caffeic

acid and chlorogenic acid) and hydrolysable tannins (ellagic acid). Quantification of individual polyphenols was based on surface area (%) of each peak.

Statistical analysis

The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used for the analysis of the data from these experiments. The data were analyzed via two-way analysis of variance (ANOVA) with cultivar and storage time as main effects. Significant differences between harvests were observed, but a similar trend in polyphenolic content was observed. Therefore, for ease of interpretation, the data is represented as an average of two years totaling five harvests. Significant differences between cultivars and storage times were detected using the least significant difference (LSD) at the 5% level of significance.

Results and discussion

Total polyphenols content

The total polyphenols content (TPC) of Radiance, Beauty, and Sensation are shown in **Figure 8**. At harvest (day 0), Sensation had a significantly lower TPC than Radiance and Beauty which were

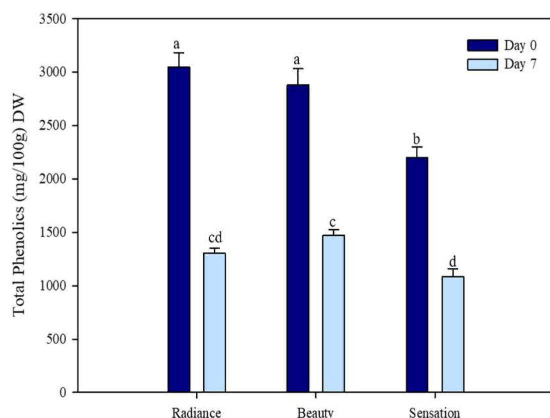

Figure 8. Total polyphenol content in Radiance, Beauty, and Sensation at day 0 and after 7 days of cold storage. Each bar represents an average of 5 harvests. Bars headed by the same letter are not statistically different from one another.

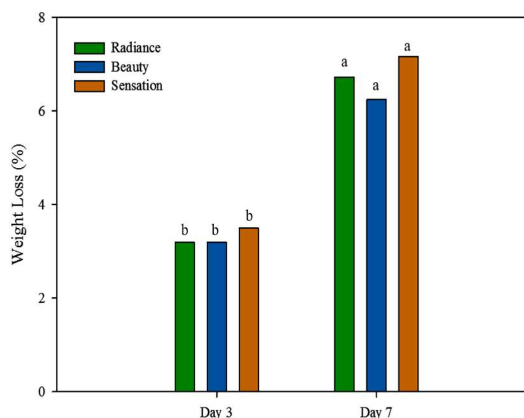

Figure 9. Percent weight loss of Radiance, Beauty, and Sensation during 7 days of cold storage (1 °C). Each data point represents an average of 5 harvests. Bars headed by the same letter are not statistically different from one another.

statistically similar to one another. All three cultivars showed a decline in TPC during storage which agrees with previous studies also showing a decrease in strawberry TPC during cold storage.[17, 152] Beauty had the highest TPC after 7 days of cold storage and was significantly higher than Sensation. After 7 days of cold storage, TPC of Radiance was not significantly different from either Beauty or Sensation. Despite Radiance having the highest TPC at day 0, it had the largest reduction (57%) after 7 days while TPC of Beauty and Sensation decreased by 49% and 51% during storage, respectively. The decrease in TPC during storage is likely related to water loss that results in reduced turgor pressure of the cells leading to membrane breakdown. Although not statistically significant, after 7 days of cold storage Beauty had lower weight loss compared to Radiance and Sensation (**Figure 9**) and thus higher TPC after 7 days (**Figure 8**). On the other hand, Sensation had the highest weight loss after storage resulting in lower TPC compared to the other cultivars. Collapse of cellular membranes allows for the interaction of polyphenol degrading enzymes, such as polyphenol oxidase (PPO), to come into contact with polyphenols.[153] The difference in TPC between Radiance and Sensation is in agreement with previous studies showing that Radiance has a much higher TPC than Sensation.[17] The variation in TPC between cultivars used here is in agreement with previous published studies [15, 107] that also showed that there is a significant difference in TPC between strawberry genotypes. For instance, Pincemail et al.[107] reported that the TPC of 'Clery' strawberries was 160 mg/100 g fruit while 'Isaura' strawberries contained nearly five time as much TPC with 594 mg/100 g fruit. Therefore, it is evident that the genetic factor also plays heavily into the total amount of polyphenols present in Radiance, Beauty, and Sensation.

Overall, cold storage had a negative impact on the TPC of all cultivars; however, the decrease in TPC was not uniform amongst strawberry cultivars. After 7 days of cold storage, TPC of Radiance decreased significantly and it was not statistically different from TPC of Sensation. However, TPC of Beauty which was at harvest (day 0) significantly different from Sensation remained so after storage (day 7). The differences in the percent reduction of TPC between each cultivar indicates that genotype impacts the TPC of strawberries as the fruit were grown at the same location, picked on the same day, and stored in

the same temperature-controlled chambers. Based on TPC alone, Beauty performed the best during cold storage as it had higher TPC at harvest and TPC decreased the least after 7 days.

Total anthocyanin content

At harvest (day 0), each of the three cultivars studied had significantly different total anthocyanin content (ANC) from one another **(Figure 10)**. Radiance had the highest amount of ANC while Sensation had the least. ANC of Beauty was between Radiance and Sensation which mirrors TPC for each strawberry cultivar (**Figure 8).** Similarly, to that observed for TPC, there was a decline in ANC during storage. Again, Beauty had the lowest ANC percent decrease, (38%) while Radiance and Sensation had similar ANC percent decreases (46% and 44%, respectively). Anthocyanins are the predominant polyphenol and the major pigment in ripe strawberries, in particular the anthocyanin pelargonidin 3-glucoside.[96] Yoshida et al.[154] reported that pelargonidin 3-glucoside comprised between 66.2 and 93.6% of the total anthocyanin content in several strawberry cultivars. Not only do anthocyanins impart color to the strawberry, they have also been claimed to have many health benefits due to their antioxidant nature.[66] The greater amount of anthocyanins in

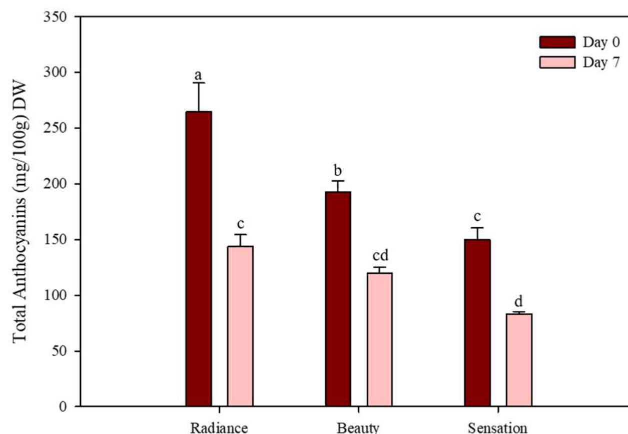

Figure 10. Total anthocyanin content in Radiance, Beauty, and Sensation at day 0 and after 7 days of cold storage. Each bar represents an average of 5 harvests.

Figure 11. A visual comparison of Radiance (left) and Sensation (right). Sensation is a light red cultivar whereas Radiance is dark red.

Radiance when compared to Sensation correlates to Radiance being a redder fruit (**Figure 11**). This observation agrees with a previous study involving Radiance and Sensation.[17] As the dominant class of polyphenols, higher ANC also correlates to higher TPC (**Figure 8**). This has been shown in previous studies on strawberry anthocyanin content. For instance, Nour et al.[155] also noted that the redder 'Magic' strawberry cultivar had a greater amount of ANC and TPC when compared to the lighter 'Premial' strawberry. It is important to note that, while the Folin-Ciocalteau method is a good indicator of polyphenol content in strawberries, it also reacts with ascorbic acid and reducing sugars within the sample.[156] The greater similarity between the TPC of Radiance and Beauty when compared to their ANC might be due to the presence of higher levels of non-anthocyanin polyphenols, as well as ascorbic acid, or reducing sugars in Beauty than in Radiance.

Polyphenol Profiles

Polyphenols mainly absorb light in the UV spectrum while the anthocyanins are the only polyphenols to absorb light in the visible spectrum. Previous studies have detected strawberry polyphenols using wavelengths between 250-520 nm and reported that flavonols have a maximum absorption at 280 nm, phenolic acids between 300-320, and anthocyanins between 500-520 nm.[28] Therefore, four wavelengths were chosen for this study namely, 250, 280, 360, and 520 nm. To obtain the retention time

Table 1

Retention times for major polyphenols in strawberries.

Compound No.	Class	Group	Polyphenol	Abbrv.	t_R[a]	UV bands (nm)[b]
1	**Flavonoids**	Anthocyanidins	Cyanidin	CYA	5.00	250, 280, 360, **520**
2			Pelargonidin	PGN	5.64	250, 280, **520**
3		Anthocyanins	Cyanidin 3-glucoside	C3G	3.81	250, 280, 360, **520**
4			Pelargondin 3-glucoside	P3G	4.15	250, 280, **520**
5		Flavonols	Quercetin	QRN	7.84	**250**, 280, 360
6			Quercetin 3-glucoside	Q3G	5.60	**250**, 280, 360
7			Kaempferol	KPF	8.87	250, 280, **360**
8			Kaempferol 3-glucoside	K3G	6.10	**250**, 280, 360
9			Myricetin	MYR	6.56	250, 280, **360**
10		Flavanols	Catechin	CTN	3.62	250, **280**
11			Epicatechin	ECN	4.29	250, **280**
12	**Phenolic Acids**	Cinnamic Acids	*p*-coumaric acid	PCA	5.07	250, **280**
13			Ferulic acid	FRA	5.58	250, **280**, 360
14			Caffeic acid	CFA	4.09	250, 280, **360**
15		Benzoic Acids	Gallic acid	GLA	1.41	**250**, 280
16		Hydroxycinnamic esters	Chlorogenic acid	CGA	3.81	250, **280**, 360
17	**Hydrolysable tannins**	Ellagitannins	Ellagic acid	EGA	4.95	**250**, 280, 360

[a]t_R = Retention time.

[b]Bold indicates optimum absorbency.

for each polyphenol standard at 250 nm, 280 nm, 360 nm, and 520 nm wavelengths, the retention times of individual polyphenol standards were analyzed by HPLC-DAD. Afterwards a mixture containing all polyphenol standards (cyanidin, pelargonidin, cyanidin 3-glucoside, pelargonidin 3-glucoside, quercetin, quercetin 3-glucoside, kaempferol, kaempferol 3-glucoside, myricetin, catechin, epicatechin, p-coumaric

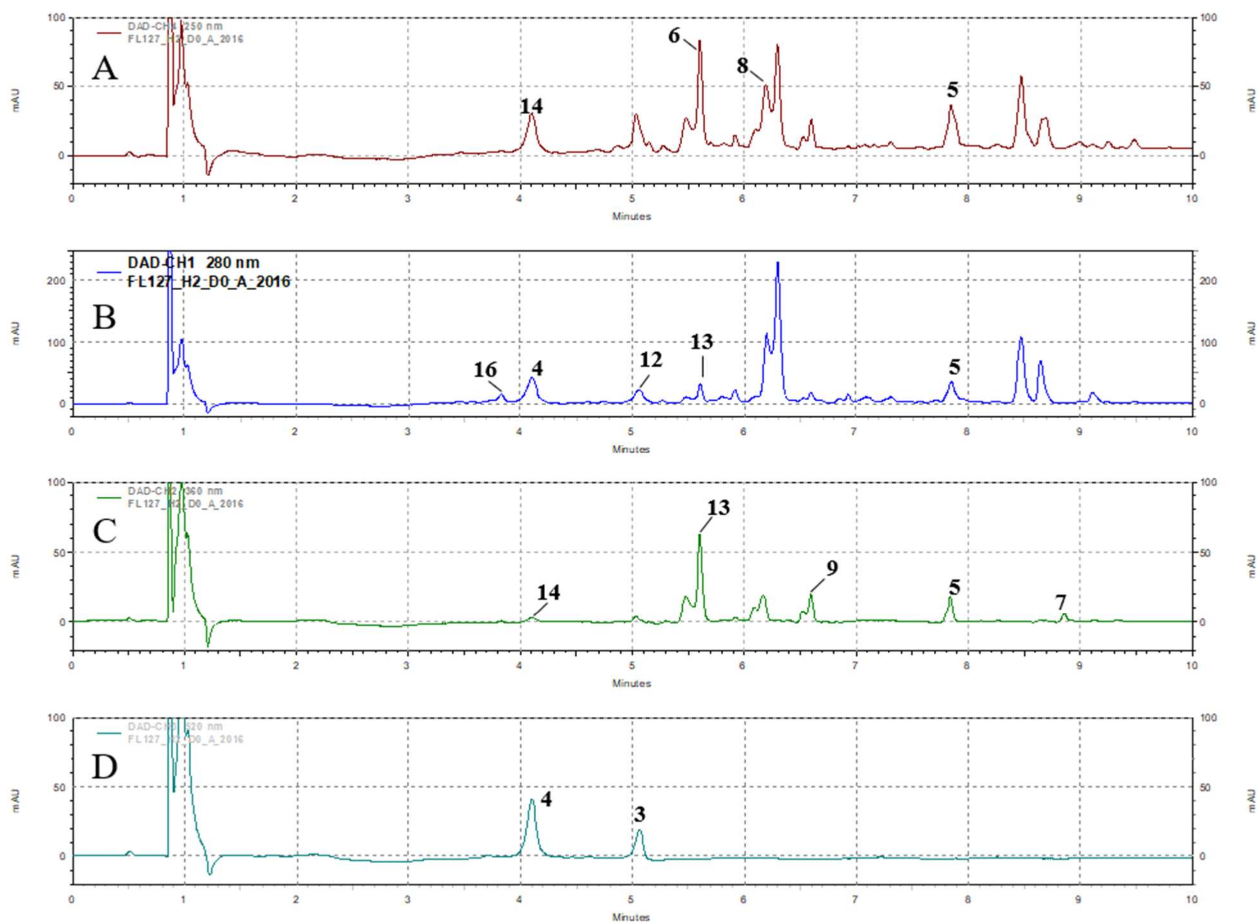

Figure 12. HPLC chromatograms of individual polyphenols (Sensation) at 250 (**A**), 280 (**B**), 360 (**C**), and 520 (**D**) nm. Peak numbers refer to Table 1

acid, ferulic acid, caffeic acid, gallic acid, chlorogenic acid, and ellagic acid) was analyzed to obtain the retention times (**Table 1**).

The retention times from the mixture were used to analyze strawberry samples instead of the individual standards to account for shifts in retention by polyphenolic interaction. The optimum wavelength absorbency was determined by comparing the chromatograms of each sample and using the strongest peak wavelength (**Figure 12**). In some cases, the optimum wavelength could not be used due to interference

from other polyphenols that eluted at similar times. For example, caffeic acid had the strongest absorbency at 280 nm however frequently eluted at the same time as pelargonidin 3-glucoside. Therefore, to analyze caffeic acid content, the 360 nm wavelength was used instead where pelargonidin 3-glucoside's absorbency is weaker.

Flavonoids, namely the anthocyanins pelargonidin 3-glucoside and cyanidin 3-gucoside and their non-glycosylated counterparts pelargonidin and cyanidin, represented the major class of polyphenols detected in strawberry, regardless of the cultivar. The flavonols quercetin, quercetin 3-glucoside, kaempferol, kaempferol 3-glucoside, and myricetin; and the flavanol catechin were also identified in all cultivars. Polyphenols from the class of phenolic acids were also detected including p-coumaric acid, ferulic acid, caffeic acid, gallic acid and chlorogenic acid. The hydrolysable tannin, ellagic acid, was also analyzed in the strawberry samples. Epicatechin was not detected in our strawberry samples and therefore omitted when representing the data. Määttä et al.[157] did not detect epicatechin in their strawberries as well whereas Gasperotti et al.[158] found that amount of epicatechin to be about 10-times lower than catechin in strawberry. Therefore, it is possible that our method was not sensitive enough to detect such low epicatechin amounts. There were also several peaks that were unidentified, the largest eluting at 6.3 minutes. These unidentified peaks could possibly be polymerized polyphenols which occur as a natural consequence of senescence.

Effect of cultivar on major, individual polyphenols

Figure 13 shows a comparison of individual polyphenols between strawberry cultivars Radiance, Beauty, and Sensation at harvest (day 0; **Figure 13A**) and after 7 days of cold storage at 1 °C (**Figure 13B**). At harvest, there was not a significant difference between the three cultivars studied for most polyphenols detected. Only the concentrations of pelargonidin 3-glucoside, quercetin, kaempferol 3-glucoside, myricetin, catechin, and ellagic acid were significantly different among strawberry cultivars. Of these six polyphenols, Radiance had the highest percent area for all of these polyphenols except catechin for which Sensation had the highest levels. Nour et al.[155] also detected variability in quercetin, myricetin, and ellagic acid in the cultivars 'Magic' and 'Premial'. However, another study done by Hernanz et al.[159] on five

strawberry cultivars did not detect any differences in the amount of quercetin and sometimes quercetin was not detected at all. The results from **Figure 13A** correlated with TPC where Radiance also had the highest total polyphenol content (**Figure 8**). Among the four anthocyanins detected, pelargonidin 3-glucoside was the most abundant in all cultivars. This agrees with previous studies that have shown pelargonidin 3-glucoside to be the major polyphenol in ripe strawberries.[15, 159]

After 7 days of cold storage, pelargonidin 3-glucoside remained the major polyphenol present in all cultivars and Radiance continued to have the highest concentration compared to the other cultivars (**Figure 13B**). Again, only six polyphenols showed a significant difference between cultivars; however, they were different from day 0. These six polyphenols included pelargonidin 3-glucoside, quercetin, quercetin 3-glucoside, kaempferol 3-glucoside, myricetin, and caffeic acid. Four of these polyphenols were flavonols while three of the six polyphenols from day 0 were flavonols as well. It might be possible that flavonols show more genetic variability among the cultivars as opposed to the phenolic acids. Phenolic acids are synthesized early in the phenylpropanoid pathway and are the precursors to the more complex polyphenols. Therefore, it is likely that the phenolic acids are regularly being shuttled to the various branches of the pathway causing variable levels of phenolic acids to occur. The constant flux of phenolic acids might explain why there is less variation in the cultivars with the exception of caffeic acid. Palmieri et al.[105] reported that the concentration of caffeic acid in strawberries was influenced by environment. Environmental conditions during storage might have contributed to the difference in caffeic acid content seen between the three cultivars after cold storage. The production of flavonols has also been linked to growing conditions, and particularly to the exposure of UV radiation. The production of quercetin and kaempferol and their glycosides has been shown to increase in strawberries upon exposure to UV radiation.[92] Radiance might interact more strongly with its growing conditions which could account for its higher prevalence of flavonols when compared to Beauty or Sensation.

Figure 13C shows the total area percentage of each cultivar at day 0 and after 7 days of cold storage. There was an increase in the total area percentage of polyphenols in Radiance and Beauty while the total area percentage of polyphenols in Sensation decreased slightly from 29.1% on day 0 to 27.4% on

day 7. The increase in polyphenols seen with Radiance and Beauty contrasts with the TPC and ANC data which showed a decrease in polyphenol content (**Figures 8 & 10**). The increase in polyphenol content (**Figure 13**) was most likely due to a concentration effect where the fruit lose water during storage due to respiration and transpiration and thereby caused the polyphenolic content to become more concentrated. For the TPC and ANC data, this water loss was compensated for by calculating the contents in dry weight. However, because area percentage was used to estimate the levels of individual polyphenols detected in strawberries, it did not seem accurate to calculate these values in dry weight. Therefore, the increase in polyphenols seen on day 7 was most likely an increase in *concentration* rather than on the overall *amount.* Data shown on **Figure 13C** also differs from that shown on **Figure 8** in that no significant difference between the cultivars at day 0 was observed. The Folin-Ciocalteau method is used only as an estimation of polyphenols present in a solution as the oxidizing agent of this method also reacts with ascorbic acid and reducing sugars which are present in significant amounts in strawberries[156]. This can skew the results obtained from the Folin-Ciocalteau method. The HPLC determination of total polyphenols is a more accurate estimation of the amount of polyphenols present in each cultivar. Chaves et al.[14] also showed a discrepancy between polyphenols measured with the Folin-Ciocalteau method and with HPLC. The Folin-Ciocalteau method indicated that the strawberry cultivar 'Monterey' had higher polyphenol content than 'Camarosa' however data from HPLC showed the opposite with 'Camarosa' having the most polyphenol content.

Effect of cold storage on major, individual polyphenols

Data shown on **Figure 14** is a comparison between each cultivar regarding the change in the concentration of polyphenols during storage. For Radiance, after 7 days of cold storage, there was an increase in polyphenol concentration for 12 out of the 16 polyphenols when compared to at harvest (day 0) (**Figure 14A**). Pelargonidin 3-glucoside showed the most dramatic increase from day 0 to day 7. Cyanidin levels also increased significantly from initial values at day 7 and paralleling with a decrease in cyanidin 3-glucoside. Strawberries turn redder during storage and this can be related to the increase in concentration

of pelargonidin 3-glucoside and cyanidin 3-glucoside as they contribute to the color of strawberries.[17, 160] The only other polyphenol to show a significant increase between day 0 and day 7 was myricetin.

Beauty showed a similar increase in pelargonidin 3-glucoside and cyanidin during storage (**Figure 14B**). There was also a decrease in pelargonidin and cyanidin 3-glucoside however not significant. More polyphenols were significantly different at day 7 in Beauty than in Radiance. Besides cyanidin and pelargonidin, quercetin, quercetin 3-glucoside, and ellagic acid were all significantly higher at day 7. Gasperotti et al.[79] reported that genotype was the major factor influencing ellagic acid content. Ellagic acid is a subunit of ellagitannins and belong to the class of hydrolysable tannins. As their name implies, hydrolysable tannins are easily broken down.[80] The statistically significant increase of ellagic acid at day 7 might be due to the hydrolyzation of ellagitannins into their constitutive components, ellagic acid.

In contrast to Radiance and Beauty, pelargonidin 3-glucoside, pelargonidin, cyanidin 3-glucoside and cyanidin were not significantly different between day 0 and day 7 in Sensation despite their increase (**Figure 14C**). Sensation also showed an increase in cyanidin 3-glucoside which departs from the trend of the other two cultivars while pelargonidin still showed a decrease over time.

Without running standard curves for each polyphenol, it is difficult to ascertain whether any increase in polyphenol content is a true increase due to *de novo* synthesis or merely a concentration effect due to water loss from the fruit during storage (**Figure 9**). However, it is possible to examine the amount of each polyphenol present in relation to each other. For example, the ratio between pelargonidin 3-glucoside and pelargonidin increased from day 0 to day 7. For each cultivar, the amount of pelargonidin 3-glucoside increased during storage concomitant with a decrease in the pelargonidin. It could be assumed that Sensation, the cultivar that lost the most amount of weight during storage, would be the one to have the largest increase in pelargonidin 3-glucoside if the increase is due to a concentration effect. However, that is not the case in this study. Sensation had the smallest increase of pelargonidin 3-glucoside at day 7 and it was not a significantly different amount than day 0. Degradation of pelargonidin 3-glucoside might account for this discrepancy. Also, Beauty was the only cultivar to show significant differences in the amount of quercetin and quercetin 3-glucoside between day 0 and day 7 (**Figure 14B**). This could be a

concentration effect; however, Beauty lost the least amount of weight during storage and the other two cultivars did not show the same trend despite a higher weight loss. As the production of quercetin and quercetin 3-glucoside has been shown to be stimulated by environmental conditions[92, 105], the increase seen here could be synthesis of polyphenols in response to postharvest stress.

Conclusions

Strawberry cultivars used in this study showed varying trends with respect to each individual polyphenol during cold storage and between the cultivars themselves. However, a common trend between all three cultivars during storage was an increased concentration of pelargonidin 3-glucoside. This study shows that the levels of individual polyphenols varies by cultivar and is dependent on genotype. The increase in some of the individual polyphenols and in the overall polyphenol content was more likely due to a concentration effect rather than the *de novo* synthesis of polyphenols as the TPC and ANC, expressed in dry weight, indicates a decrease. However, it is possible that synthesis of individual polyphenols, especially the flavonols, may be induced during postharvest storage due to a time-temperature stress which can have an effect on the overall postharvest quality of a strawberry cultivar.

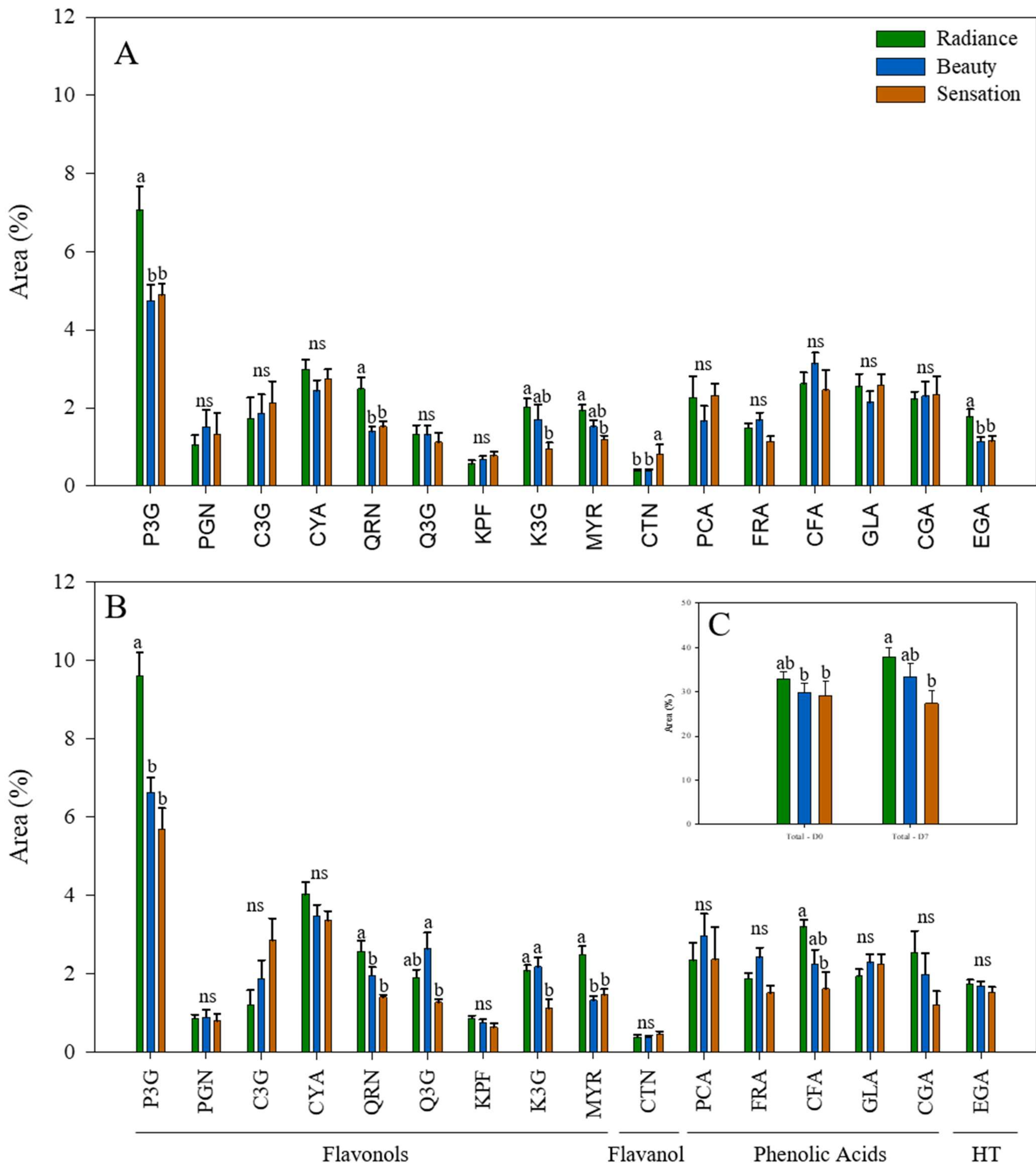

Figure 13. Differences in major, individual polyphenols of Radiance, Beauty, and Sensation at day 0 (**A**) and after 7 days of cold storage (**B**) and total area percentage (**C**) using HPLC-DAD. (P3G – pelargonidin 3-gllucoside; PGN – pelargonidin; C3G – cyanidin 3-glucoside; CYA – cyanidin; QRN – quercetin; Q3G – quercetin 3-glucoside; KPF – kaempferol; K3G – kaempferol 3-glucoside; MYR – myricetin; CTN – catechin; PCA – p-coumaric acid; FRA – ferulic acid; CFA – caffeic acid; GLA – gallic acid; CGA – chlorogenic acid; EGA – ellagic acid) HT – hydrolysable tannin

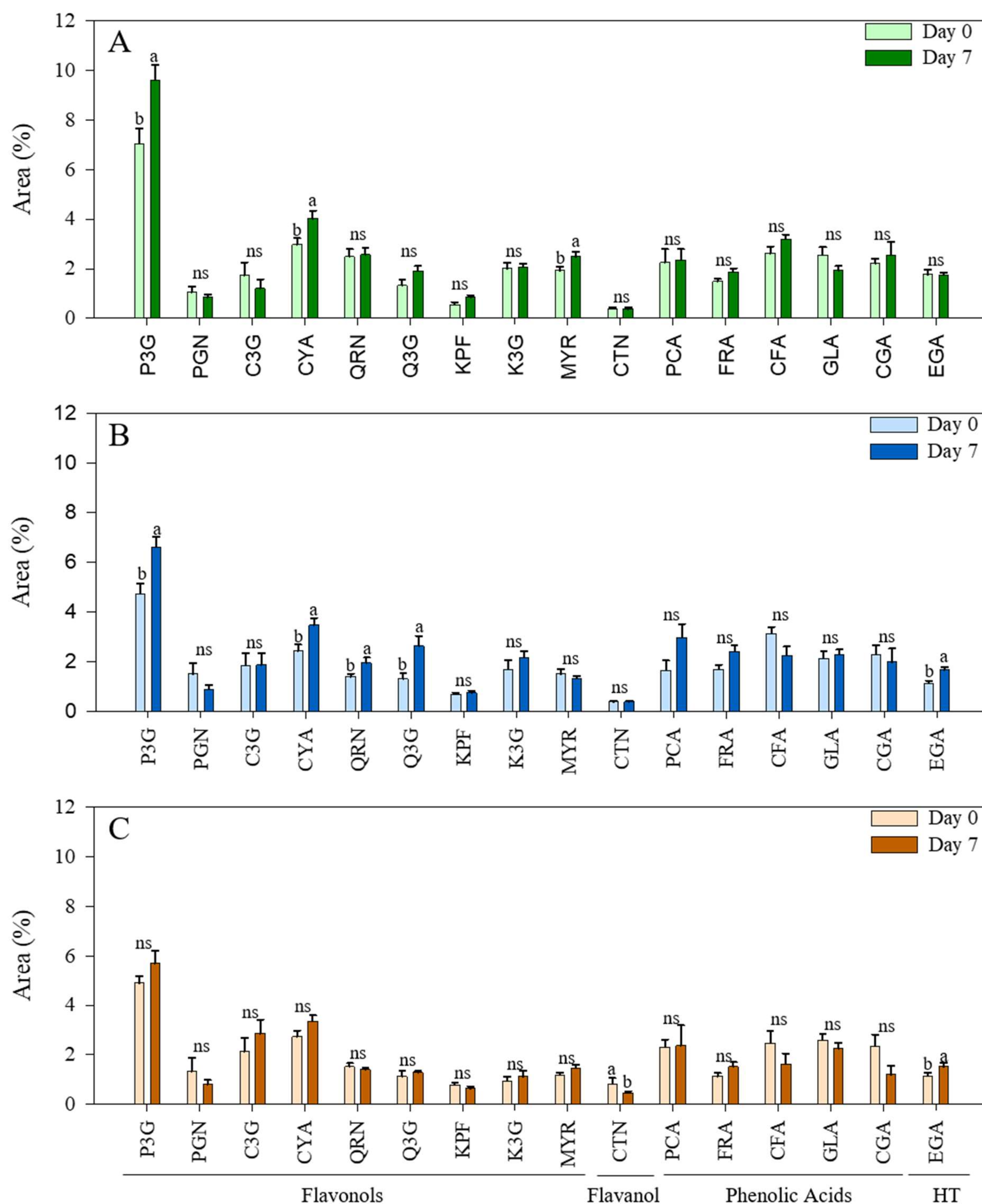

Figure 14. Content major, individual polyphenols in Radiance (**A**), Beauty (**B**), and Sensation (**C**) at day 0 and after 7 days of cold storage using HPLC-DAD. (P3G – pelargonidin 3-gllucoside; PGN – pelargonidin; C3G – cyanidin 3-glucoside; CYA – cyanidin; QRN – quercetin; Q3G – quercetin 3-glucoside; KPF – kaempferol; K3G – kaempferol 3-glucoside; MYR – myricetin; CTN – catechin; PCA – p-coumaric acid; FRA – ferulic acid; CFA – caffeic acid; GLA – gallic acid; CGA – chlorogenic acid; EGA – ellagic acid) HT – hydrolysable tannin

CHAPTER FOUR:

SYNTHESIS AND OXIDATION OF POLYPHENOLS IN THREE STRAWBERRY CULTIVARS DURING COLD STORAGE

Introduction

As a soft fruit, ripe strawberries are sensitive to postharvest stress which can result from physical injury, changes in temperature or humidity, or pathogen attack.[146] Such adverse conditions can also result in undesirable changes in texture, color, and flavor thereby reducing the quality of strawberries and accelerating the onset of senescence.[161] This problem is exacerbated as strawberries already have a short postharvest life due to a high metabolic rate.[146] Therefore, adding stress will cause increased metabolic rate, nutrient depletion and premature senescence of the fruit.

When exposed to biotic (i.e., pathogen or insect attack) or abiotic stresses (i.e., damage, UV radiation, or extreme temperatures) plants can synthesize thousands of secondary metabolites such as polyphenols via the phenylpropanoid pathway. Production of such secondary metabolites constitutes the plant defense mechanisms. Therefore, thousands of different polyphenols can be produced; however, they all arise from the initial deamination of L-phenylalanine to *trans*-cinnamic acid catalyzed by the enzyme phenylalanine ammonia-lyase (PAL). This enzyme regulates the entry of primary metabolites into secondary metabolism. PAL is induced when a plant is exposed to abusive environmental changes which can arise from variations in temperature, drought, water intrusion, nutrient deficiency or pathogen attack. For example, Jin et al.[51] saw a significant increase in the PAL activity of strawberries that were exposed to 45 °C for 3.5 hours or inoculated with *Botrytis cinerea* spores when compared to non-inoculated fruit stored under optimum conditions. The same induction of PAL activity was also observed by Xu et al.[121] when strawberries were stored under blue light irradiation. Increased PAL activity has been associated with

increased stress resistance.[128, 162-163] For example, upon pathogen exposure, tomato cultivars with a high resistance to bacterial wilt showed a stronger induction of PAL activity when compared to susceptible cultivars.[134] Likewise, transgenic tobacco constitutively expressing PAL had a higher resistance to the tobacco pathogens *Phytophthora parasitica* and *Cercospora nicotianae*.[164] Detachment of a strawberry from the mother plant may also create a stress within the fruit because it deprives the fruit from water and nutrient supply. While PAL activity has been studied during fruit ripening, and after induced stress, there are currently no studies that report the effect of genotype variability and cold storage on the PAL activity between different strawberry cultivars.[51-52, 121, 125, 165]

While PAL is a key enzyme in the synthesis of polyphenols, the enzyme polyphenol oxidase (PPO) is responsible for their oxidation and degradation. PPO performs two reactions namely the *o*-hydroxylation of monophenols and the oxidation of diphenols to *o*-quinones.[11] These quinones then undergo non-enzymatic polymerization resulting in the formation of black or brown pigments, producing undesirable discoloration of fresh fruits and vegetables and reducing their overall quality.[146] PPO is generally sequestered to the thylakoid membranes of the chloroplasts while polyphenols reside in the vacuoles. Break down of cellular membranes allows enzyme and substrate to come into contact, as a result of fruit senescence, or physical injury, due to decreased membrane integrity.[146] Browning in strawberries has been attributed to the activity of PPO, especially in processed strawberries found in jams or jellies.[166] This is concurrent with the loss of color and the degradation of anthocyanins, the main pigment in strawberry.[11] In fresh strawberries, PPO activity is correlated to water loss which leads to a reduction of cellular turgor pressure which results in the collapse of cellular membranes and disintegration of organelles. Water loss was positively correlated with increased PPO activity and browning of the strawberry cultivar 'Oso Grande'.[11] PPO activity was also attributed to the browning of the strawberry cultivars 'Elsanta' and 'Madame Moutot' when stored at 4 °C.[146]

The effect of stressors (i.e., UV, pathogens) and ripening have been studied on the activities of PAL and PPO; however, there are no studies on the effect of genotype variability and the impact of optimal cold storage on activities of PAL and PPO in strawberry cultivars. Therefore, the objectives of this study were

1) to determine the effect of genotype on the activities of PAL and PPO in three important Florida strawberry cultivars and 2) to determine the effect of storage at 1 °C on the activities of PAL and PPO.

Materials and methods

Plant material

'Florida Radiance', Sweet Sensation® 'Florida127', and 'Florida Beauty' (hereafter referred to as Radiance, Sensation, and Beauty, respectively) strawberry cultivars were harvested two times during the 2016 production year and three times during the 2017 production year from the University of Florida Gulf Coast Research and Education Center, Wimauma, Florida. The fruit were transported to the USF- Food Quality Laboratory in Tampa, with minimal delay after harvest. Immediately upon arrival at the laboratory, 10 to 15 fruits from each cultivar were selected for uniformity of color and freedom from defects, carefully packed into two polyethylene terephthalate (PET) vented clamshells (capacity ≈453 g). Initial evaluations were performed right after harvest (day 0) using 10 to 15 fruits.

Storage conditions

After selection, strawberries were stored for seven days inside a temperature- and humidity-controlled chamber (Forma Environmental Chambers Model 3940 Series, Thermo Electron Corporation, OH, USA) set at 1 °C and 85% RH, which simulates the lowest temperature and highest RH measured during real strawberry field-to-store trials.[17] Temperature and RH were monitored throughout the experiments using battery-powered data loggers (Hobo® U10 Temp/RH data logger, Onset Computer Corporation, Pocasset, MA, USA).

Sample collection

For the PAL assay 10 whole strawberries were sliced and either placed directly into a -80 ºC freezer or frozen in liquid nitrogen and placed into a -30 ºC freezer until the time of analysis. For the PPO assay, 15 fruits were blended to a smooth puree and kept in a -30 °C freezer until the time of analysis.

Phenylalanine ammonia-lyase (PAL) enzyme extraction and assay

PAL activity was determined using the method described in Pombo et. al. (2011)[52] with some modifications. Five grams of whole, frozen strawberry tissue was ground into a powder using a mortar and pestle prechilled with ice. Hereafter, the samples were kept on ice at all times. The ground tissue was homogenized in 40 ml of extraction buffer (0.1 M sodium borate, 5 mM 2-mercaptoethanol, 2 mM EDTA and 3% w/v PVPP) with Protease Inhibitor Cocktail VI for plant cells (Alfa Aesar, Ward Hill, MA, USA). The enzyme was extracted by stirring the solution for 1 hour at 4 ^{0}C. After extraction, the solution was centrifuged for 30 minutes at 4,700 rpm. The supernatant was filtered through cheesecloth, the pellet discarded and the supernatant analyzed for enzyme activity and protein content. Protein concentration was determined using the Pierce® BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA).

After extraction, 1500 µl of the supernatant was added to a preincubated (37 ^{0}C) test tube containing 2550 µl 0.3 M sodium borate buffer and 450 µl l-phenylalanine in 0.03 M sodium borate buffer. The test tube was vortexed and 300 µl of the solution was placed in three wells of a 96-well quartz microplate. The microplate was incubated at 37 ^{0}C and read every 30 minutes for three hours at 290 nm using a PowerWave HT multiplate reader (BioTek Instruments, Winooski, VT, USA). PAL activity was calculated as the change in optical density (OD) per second and per gram of total protein.

Polyphenol oxidase (PPO) enzyme extraction and assay

PPO activity was determined using the method described in Flurkey and Jen[167] with some modifications. Twenty grams of previously blended strawberry tissue was stirred with 100 ml of cold acetone (-20 ^{0}C) for 20 minutes then filtered under vacuum through a Whatman #2 filter. The tissue was then mixed with an additional 100 ml of cold acetone and filtered again to obtain a pinkish white tissue. The tissue was then ground to a fine powder using a mortar and pestle. This powder was allowed to dry overnight after which it was stored in a desiccator until use.

Enzyme activity was determined by mixing 0.1 g of the powder with a 0.1 M sodium citric-acid phosphate buffer, pH 6.5, and allowed to rehydrate for 10 minutes while stirring then filtered under vacuum with a Whatman #42 filter. One milliliter of a 0.1 M catechol solution will be mixed with 1 ml of the filtrate. The absorbance of this solution was read at 420 nm every 5 minutes for 40 minutes using a standard GENESYS 10 vis spectrophotometer (Thermo Electron Scientific Instruments Corporation, Madison WI, USA). One unit of PPO activity was defined as an increase of 0.1-unit absorbency per minute.

Statistical analysis

The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used for the analysis of the data from these experiments. The data was treated by two-way analysis of variance (ANOVA) with cultivar and time as main effects. Each harvest was analyzed separately to detect any trends between different harvest dates on enzyme activity. Significant differences between cultivars and storage times were detected using the least significant difference (LSD) at the 5% level of significance.

Results and discussion

Phenylalanine ammonia-lyase (PAL) enzyme activity

To determine the effect of optimal storage conditions on the activity of PAL in strawberries, enzyme activity assays were performed on the day of harvest (day 0) and after 3 and 7 days of storage at 1 °C (**Figure 15A-C**). The data was collected from two harvests during 2016 and from one harvest during 2017. No data was collected for Sensation during on the first harvest of 2016 due to lack of sufficient amount fruit to perform the experiments (**Figure 15A**).

Overall, PAL activity tended to increase from day 0 to day 3 followed by a decrease in activity at day 7 (**Figure 15A-C**). In the first harvest of 2016 and 2017, Radiance deviated from this trend where it showed an increase in activity by day 7 (**Figure 15A & C**). It is possible that a subsequent decrease would have been observed beyond 7 days of storage. The overall induction of PAL activity at day 3 might be a response to the stress on the fruit, from first being removed from the plant and then from handling. However,

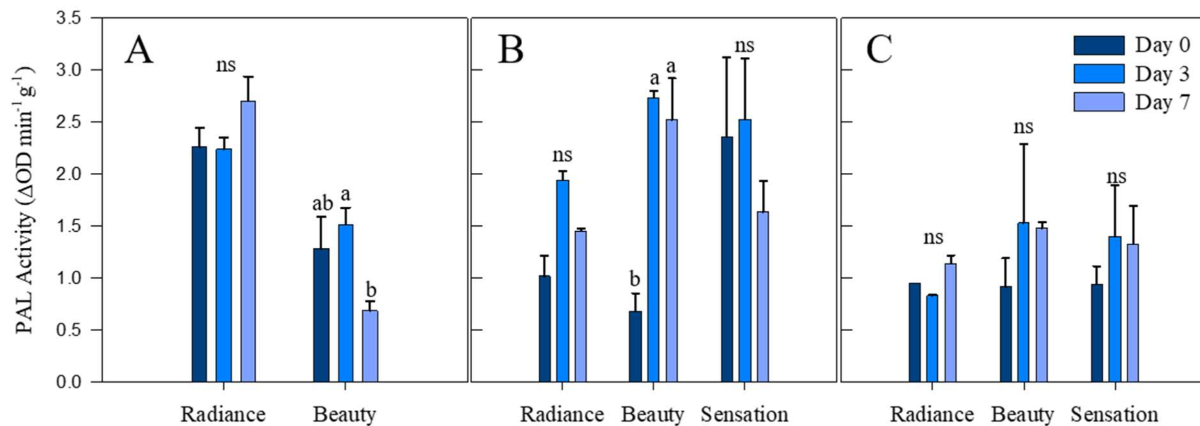

Figure 15. Enzyme activities of PAL in Radiance, Beauty, and Sensation during storage at 1 °C during Harvest 1 (A) and Harvest 2 (B) in 2016 and Harvest 3 (C) in 2017. Grouped bars headed by the same letter are not significantly different from one another. ns – Not significant.

this was only a brief increase in activity as it decreased again several days later. These results are in agreement with earlier studies on PAL activity which also showed transient induction after exposure to stress, attributed to post translational regulation of the enzyme.[113] For example, Jin et al.[51] and Nigro et al.[168] subjected fresh harvested strawberries to UV stress (UV-C and UV-A light, respectively) and found a transient induction of PAL as well. Similar PAL activity has been observed in mildly stressed peaches. Spadoni et al.[169] exposed peaches to heat stress following a couple days storage at 0 °C. PAL activity was induced by dipping the fruit in hot water (60 °C for 20s). The heat-treated fruit showed an immediate PAL induction 15 minutes after the heat treatment, followed by a reduction in activity after 6 hours. Interestingly, the non-heated peaches (control) also showed an induction of PAL activity 3 hours after being treated with water at room temperature. This was concurrent with a second induction of PAL in the heat-treated fruit, albeit lower than the activity at 15 minutes. This could be an effect of holding the fruit in cold storage (0 °C) prior to the experiment and then moving the fruit to room temperature (20 °C). The change from low to high temperature might have induced a mild stress response or resulted from exposure of the fruit to cold temperature (0 °C).

Despite the general transient induction of PAL activity, the level of activity was not consistent between cultivars or between the same cultivar throughout all three harvests. As previously mentioned, PAL activity in Radiance did not increase after 3 days of storage in two harvests. In fact, PAL activity

dropped slightly at day 3 before increasing on day 7 (**Figure 15A & C**). On harvest 2 of 2016, PAL activity in Radiance fell more in line with the other cultivars showing a transient induction of enzyme activity at day 3 (**Figure 15B**). Although in Beauty PAL showed transient induction during all three harvests, the level of induction was not consistent. On the second harvest of 2016, Beauty showed initially a very low PAL activity followed by a very dramatic increase at day 3 and a mild decrease at day 7 (**Figure 15B**). In contrast, during the first harvest of 2016 PAL activity in Beauty did not show a dramatic increase at day 3 but rather a significant decrease at day 7 (**Figure 15A**). In Sensation, PAL activity also showed inconsistency between harvest 2 of 2016 and harvest 1 of 2017 (**Figure 15B & C**). During harvest 2 of 2016, in Sensation PAL only had a slight increase in activity on day 3 followed by a larger decrease on day 7 whereas the opposite is true for PAL activity in Sensation during harvest 1 of 2017. Overall, in most cases the increase in PAL activity was not significant except for Beauty from harvest 2 of 2016 in which there was a significant induction of PAL activity from day 0 to day 3. The lack of significant changes in PAL activity could have been a result of a low-stress environment used in this study. The strawberries were kept at optimal storage conditions (1 °C) which are known to slow down metabolic activity and deterioration of strawberry fruit.[41] Despite being removed from the plant, the low temperature probably reduced the stress enough to lower the activation of PAL and consequently lowered PAL activity. This study also does not take into account the growing conditions of fruit prior to harvest. Despite the fruit originating from the same field over the three harvests, the weather could have had a substantial impact on the PAL activities observed during cold storage. This could also account for the variability observed between the three harvests.

Regarding cultivar variability, a previous study reported higher PAL activity in 'Camarosa' strawberry, a high anthocyanin content cultivar, when compared to 'Toyonoka' strawberry, a low anthocyanin content cultivar.[52] This was not observed in the current study as Radiance, an anthocyanin-rich strawberry, had lower PAL activity than Sensation, an anthocyanin-poor cultivar (**Figure 10; Figure 15B & C**).[17] These varying results can be attributed the different cultivars used in each of the studies. The postharvest performance of strawberries is dependent on cultivar (i.e., genetics) as well as storage conditions. Individual organisms have a predisposed stress response based on their genetic makeup and this

extends to the activation of stress response enzymes, such as PAL. Based on the findings from this study and the study with ‘Camarosa’ and ‘Toyonoka’, it seems that PAL activity and the anthocyanin content of a strawberry are independent of one another. Indeed, there several enzymes between the product of PAL, *trans*-cinnamic acid, and the flavonoid branch of the phenylpropanoid pathway. All of the intermediate enzymes can have an effect on the production of anthocyanins. For example, chalcone synthase (CHS) is the first and key regulatory enzyme of the flavonoid branch. Anthocyanin production is dependent on the activity of CHS. Therefore, the activity of PAL means little in the formation of anthocyanins without the subsequent activity of CHS, which is most likely more active in the anthocyanin-rich cultivars of ‘Camarosa’ and Radiance.

Polyphenol oxidase (PPO) enzyme activity

The PPO activities of Radiance, Beauty, and Sensation during cold storage are shown in **Figure 16A-C**. The first harvest from 2016 shows that the PPO activity of Radiance increased at the third day of storage followed by a decrease at the seventh day while Beauty showed a decrease on day 3 and maintained the same activity at day 7 (**Figure 16A**). In contrast to the first harvest, Radiance had a significant drop in PPO activity by the third day of storage during harvest 2 of 2016 while both Beauty and Sensation showed an increasing trend of PPO activity throughout cold storage (**Figure 16B**). Radiance once again, during the first harvest of 2017, had a spike in activity at the third day of storage followed by a decline at day 7 (**Figure**

16C). Beauty showed the opposite trend where the activity of PPO declined at day 3 and then increased again at day 7 and Sensation had an increase in activity at day 3 which plateaued at day 7.

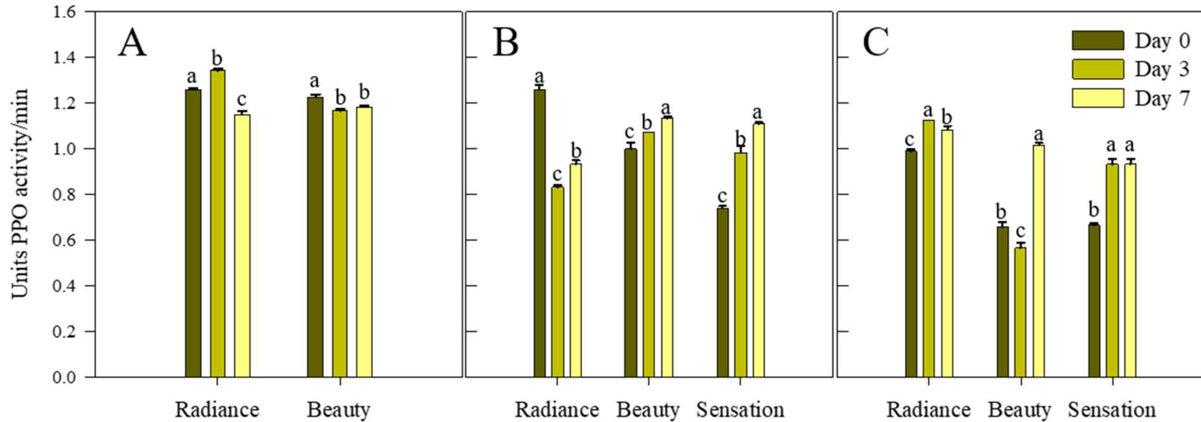

Figure 16. Enzyme activities of PPO in Radiance, Beauty, and Sensation during storage at 1 °C during Harvest 1 (A) and Harvest 2 (B) in 2016 and Harvest 3 (C) in 2017. Grouped bars headed by the same letter are not significantly different from one another.

As with PAL activity, the PPO data does not account for the conditions of the field prior to harvest. The environmental conditions could have an effect on postharvest PPO activity. This might explain why in harvest 1 of 2016 (Figure 16A), there is no difference between cultivars at harvest and there was an overall decline in activity during storage while in the subsequent two harvests (Figure 16B&C) PPO activity tended to increase during storage. Indeed, there was an overall trend toward an increasing PPO activity during storage however this was not always the case, especially with Radiance. At day 0, during harvest 2 of 2016, Radiance had the highest PPO activity followed by Beauty while Sensation had the lowest activity. (**Figure 16B**). A similar trend followed during harvest 1 of 2017 where Radiance had the highest activity but this time Beauty and Sensation had similar activities at day 0 (**Figure 16C**). This correlates with Radiance having the highest anthocyanin content followed by Beauty while Sensation the least anthocyanin content (**Figure 10**). It might be possible that strawberries with a higher content of anthocyanins tend to have a higher PPO activity at the time of harvest (day 0). In fact, in another study comparing 'Elsanta' and 'Madame Moutot' strawberry cultivars with different anthocyanin contents showed the same trend.[146] The anthocyanin-rich cultivar, 'Elsanta', also had a higher PPO activity at harvest compared to 'Madame Moutot' which had about three times less anthocyanin content.[146] 'Elsanta', like Radiance, also had a

transient increase in PPO activity during 10 days of storage at 4 °C while 'Madame Moutot', like Sensation, had a gradual increase in activity during storage.[146] Also, Radiance is a cultivar that darkens considerably during cold storage and this might be reflected in its high PPO activity at the time of harvest (day 0).[17] Chisari et al.[146] also found an inhibitory effect of fructose and glucose on the activity of PPO.[146] Sensation has a higher concentration of sugars than Radiance, particularly glucose and fructose.[17] It is also possible that the lower PPO activity in Sensation at harvest is also related to its higher sugar content compared to Radiance. The low anthocyanin content and high sugar content and low PPO activity could also account for the postharvest color stability of Sensation reported in a previous study.[17] This might indicate the role of genotype on PPO activity as genotype dictates the amount of anthocyanins and sugars present in strawberries.

Conclusions

While strawberry PAL and PPO activities did not show a strong, definite trend during cold storage, there was a tendency for a transient induction of PAL and for PPO activity to increase. The lack of strong induction for either enzyme might be due to the optimal storage conditions (1 °C at 85% RH) used in this study combined with the fact that the cultivars chosen were high quality, commercial-grade strawberries. Different results might have been seen if cultivars not well suited to the Florida climate were used instead. Despite the optimum storage conditions, the fruit experienced a certain amount of stress that is evident from PAL and PPO induction. It is possible that other enzymes in the phenylpropanoid pathway are induced during postharvest storage, such as chalcone synthase which is the first enzyme in the flavonoid branch of this pathway. Further experiments using more stressful conditions (i.e., higher storage temperatures) might be needed to gain a better insight of the enzymatic response to postharvest stress in strawberries.

CHAPTER FIVE:

MORPHOLOGICAL DISTRIBUTION OF POLYPHENOLS IN THREE STRAWBERRY CULTIVARS

Introduction

While all plants can synthesize polyphenols, the amount and types of polyphenols produced differ from plant to plant. Indeed, variations can even occur between individuals of the same species and between tissues within a singular plant. The expression of different polyphenols within distinct plant tissues reflects the specific function of that organ in the overall survival of the plant organism. For example, plant stem are rich in lignin to allow the plant to stand upright.[59] Cells performing photosynthesis, such as in leaves, are generally rich in flavonoids to protect the cell from oxidative damage from the sun's radiation. Anthocyanins can be a large components of flower petals to impart color to attract pollinating insects and birds.[68]

Fruits also synthesize specific polyphenols for the purpose of attracting herbivores to consume the fruit and thereby spread the plant's seed through feces. These are generally colorful anthocyanin such as pelargonidin 3-glucoside, in the case of strawberries, which give the fruit its distinct red color. It has been shown with other fruits that the expression of individual polyphenols is not homogenous throughout the fruit. A study on eight different apple cultivars showed that polyphenols were more concentrated in the peel than in the flesh of the fruit. It was also found that the peel contained high amounts quercetin glycosides whereas they were barely detectable in the flesh.[170] However, very few studies have examined the distribution of polyphenols in strawberry fruit and even less have studied the effect of storage on polyphenol distribution. Therefore, the objectives of this study were to 1) measure total polyphenol and anthocyanin contents in the outer cortex and inner pith of three strawberry cultivars and 2) identify major, individual

polyphenols in the cortex and pith using an HPLC-DAD and finally 3) examine the effect of cold storage on total polyphenols and anthocyanins as well as on the concentrations of individual polyphenols.

Materials and methods

Plant Material

'Florida Radiance', Sweet Sensation® 'Florida127', and 'Florida Beauty' strawberry cultivars (hereafter referred to as Radiance, Sensation, and Beauty, respectively) were harvested from fields at the University of Florida Gulf Coast Research and Education Center in Wimauma, Florida, three times during the 2017 production year. The fruit were transported to the USF- Food Quality Laboratory in Tampa, with minimal delay after harvest. Immediately upon arrival at the laboratory, three replicated samples of 10 fruit from each cultivar were selected for uniformity of color and freedom from defects, and carefully packed into three polyethylene terephthalate (PET) vented clamshells (capacity ≈453 g). Initial evaluations were performed after harvest (day 0) using 15 fruits per cultivar.

Storage Conditions

After selection, strawberries were stored for seven days inside a temperature- and humidity-controlled chamber (Forma Environmental Chambers Model 3940 Series, Thermo Electron Corporation, OH, USA) set at 1 °C and 85% RH, which simulates the lowest temperature and highest RH measured during real strawberry field-to-store trials.[17] Temperature and RH were monitored throughout the experiments using battery-powered data loggers (Hobo® U10 Temp/RH data logger, Onset Computer Corporation, Pocasset, MA, USA).

Sample Collection

Ten fruits were used each for the initial evaluation (day 0), and after 3 and 7 days of cold storage. Fruits of similar size were chosen for this experiment to preserve the pith to cortex ratio. Each fruit was cut transversely across the top at the shoulder, to remove the calyx and any white portion of the fruit, and then

cut across the bottom tip (**Figure 17**). The calyx and tip were discarded. An apple corer (Oxo Good Grips®) was used to separate the pith from cortex. The piths and cortexes were blended separately and held at -30 °C until analysis.

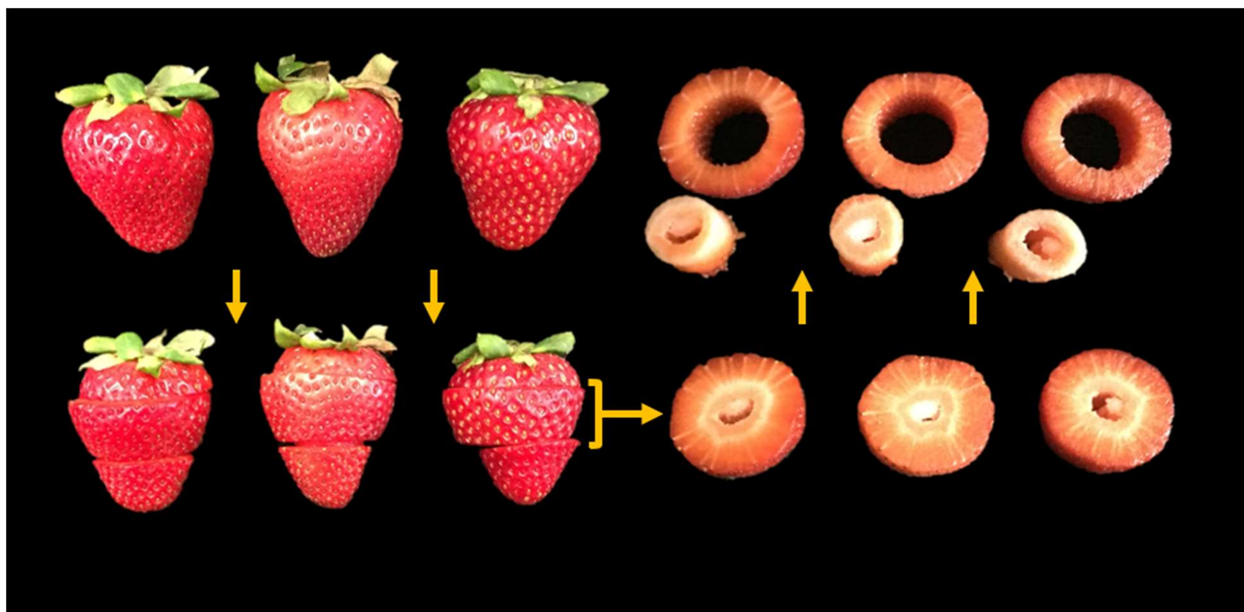

Figure 17. Separation of the pith a cortex of a strawberry. The fruit were first cut transversely through the shoulder and tip. The middle section of the fruit was used for analysis. An apple corer was used to separate the pith from the cortex.

Total polyphenols, total anthocyanin, and polyphenol profiles

Total polyphenol and anthocyanin contents, and polyphenol profiles were determined using the methods described in Chapter Three.

Statistical analysis

The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used for the analysis of the data from these experiments. The data was treated by two-way analysis of variance (ANOVA) with cultivar and storage time as main effects. Significant differences between harvests were observed but a similar trend in polyphenolic content was observed. Therefore, for ease of interpretation, the

data is represented as an average of three harvests. Significant differences between cultivars and storage times were detected using the least significant difference (LSD) at the 5% level of significance.

Results and discussion

To determine the distribution of polyphenols in three strawberry cultivars at harvest (day 0) and after 7 days of cold storage, the pith and cortex of the strawberry were separated and analyzed separately for total polyphenol content, total anthocyanin content, and for major, individual polyphenols using HPLC-DAD.

Total polyphenol and total anthocyanin contents

The total polyphenol content (TPC) of the pith and cortex of Radiance, Beauty, and Sensation are shown in **Figure 18**. For all three cultivars, the cortex contained higher TPC than the pith, regardless of the day where samples were collected. Josuttis et al.,[92] also detected higher TPC in the peel (cortex) than in the flesh (pith) of 'Everest' strawberries. Radiance and Beauty had similar TPC in their cortex at day 0 while Sensation had significantly less TPC. This correlates with the TPC of the whole fruit shown on Chapter 3 (**Figure 8)**. After 7 days cold storage, the TPC of the cortexes showed no significant difference between the three cultivars (**Figure 18**) and the pith of Radiance, Beauty, and Sensation were not statistically different from one another at day 0 or at day 7. Once again, Radiance lost the most TPC during storage with a 48% reduction overall (pith and cortex combined) while Beauty and Sensation lost

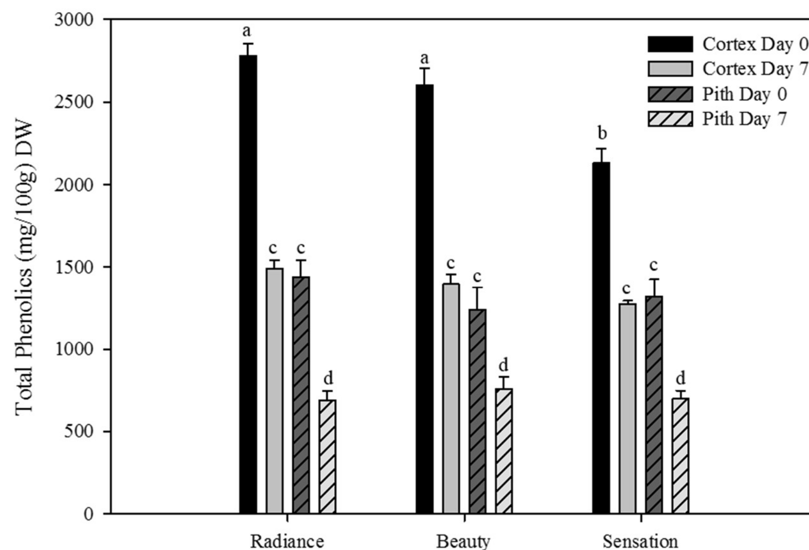

Figure 18. Total polyphenol content of the pith and cortex of Radiance, Beauty and Sensation on the day of harvest (day 0) and after 7 days of cold storage. Bars headed by the same letter are not significantly different from one another.

similar amounts at 44% and 43%, respectively. In Radiance, there was a greater reduction of TPC in the pith than in the cortex during storage (52% and 46% respectively). Sensation showed the same trend losing 46% of its TPC from the pith and only 40% from the cortex. However, Beauty lost more TPC from its cortex (46%) than from its pith (40%). These results suggest that the differences in overall TPC are most likely related to the concentration of polyphenols in the cortex rather than the pith, due to the higher TPC of the cortex.

Anthocyanins are the primary pigments in strawberries that give them their red color. The most prominent anthocyanin in strawberries is pelargonidin 3-glucoside which can constitute up to 90% of their total anthocyanin content. Depending on the cultivar and pre-harvest conditions, the intensity of the red coloration of strawberries can be localized to the external tissues of the fruit while the internal tissues remain white or they can be red throughout. ANC analysis was performed on the cortex and pith of Radiance, Beauty, and Sensation to determine the effect of genotype on anthocyanin accumulation and distribution within the fruit (**Figure 19**). On the day of harvest (day 0) Radiance had the highest levels of ANC in the cortex followed by Beauty and then Sensation with the lowest cortex ANC. This trend between the cortex of the cultivars also correlates to the ANC of the whole fruit (**Figure 10**). The pith of Radiance also contained significantly higher ANC than the piths of both Beauty and Sensation which were not statistically different from one another at day 0 (**Figure 19**). In accordance with previous research, Radiance is the redder strawberry with a higher anthocyanin content while Sensation is the least red.[17] In all cultivars, anthocyanins were concentrated to the cortex of the fruit which agrees with previous studies.[52, 171] However, the proportion of

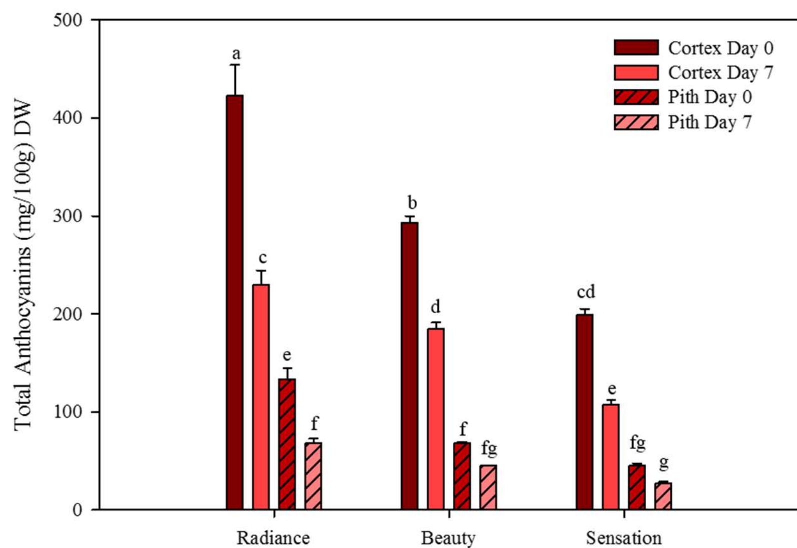

Figure 19. Total anthocyanin content of the pith and cortex of Radiance, Beauty and Sensation on the day of harvest (day 0) and after 7 days of cold storage. Bars headed by the same letter are not significantly different from one another.

anthocyanins between the cortex and pith varied between cultivars. Radiance had the smallest variation between cortex and pith, with the pith containing 32% the amount of anthocyanins found in the cortex while the piths of Beauty and Sensation each contained 23% ANC of the cortex. This would make Radiance appear redder throughout the inside of the fruit compared to Beauty or Sensation (**Figure 20**).

Figure 20. Cross section of Radiance, Beauty and Sensation at day 0.

After 7 days, the ANC of all cultivars decreased in both the pith and the cortex. The ANC of the cortex in Radiance, Beauty, and Sensation was reduced by 46%, 37%, and 46%, respectively, while the ANC of their piths decreased by 50%, 34%, and 40%, respectively. Radiance had an overall (pith and cortex combined) reduction of 47% while Beauty had a 37% reduction and Sensation decreased by 45%. By the end of storage, all cultivars were still significantly different from one another with Radiance containing the most ANC, followed by Beauty and with Sensation containing the least.

Effect of cultivar on the distribution of major individual polyphenols

Figure 21 compares the differences in major individual polyphenols between Radiance, Beauty, and Sensation on the day of harvest (day 0). Individual polyphenols in the piths (**Figure 21A**), showed much less variation between the three cultivars than the cortex (**Figure 21B**), with most polyphenols in the pith showing no significant difference between strawberry cultivars. Regarding the colored anthocyanins, only pelargonidin 3-glucoside and pelargonidin varied among the cultivars (**Figure 21A**) while there were no significant differences in the levels of cyanidin 3-glucoside and cyanidin between cultivars. Amongst all cultivars Radiance had the highest concentration of pelargonidin 3-glucoside in the pith while the levels

of these polyphenols in Beauty were in the middle and not significantly different from either Radiance or Sensation. This correlates to the results obtained from total anthocyanin content which also showed Radiance having the highest concentration of anthocyanins in the pith and Sensation having the lowest (**Figure 19**). As pelargonidin 3-glucoside is the predominant pigment in strawberries, the data from the pith of the strawberries (**Figure 21A**) also correlates to **Figure 20** which shows their internal visual color. Radiance, which had the highest pelargonidin 3-glucoside content, is very red throughout the center of the fruit while Sensation is whiter inside. Other than pelargonidin and pelargonidin 3-glucoside, the only polyphenols to show differences among the cultivars in the pith were kaempferol, kaempferol 3-glucoside, *p*-coumaric acid, and ellagic acid. For example, Beauty had higher concentrations of kaempferol in the pith compared to the other two cultivars whereas p-coumaric acid was higher in the pith of Radiance compared to Sensation. Sensation had the lowest content of ellagic acid in the pith compared to the other strawberry cultivars.

At harvest (day 0), over half of the polyphenols significantly varied in concentration between Radiance, Beauty, and Sensation in the cortex of the fruit (**Figure 21B**). Radiance and Beauty had significantly higher concentrations of pelargonidin 3-glucoside than Sensation. This agrees with previous studies showing that Radiance is a redder fruit.[17] Beauty had a much higher concentration of cyanidin in the cortex which differs from the pith in which it had more pelargonidin than Radiance or Sensation. Along with cyanidin, Beauty also had higher concentrations of quercetin 3-glucoside and ferulic acid. Quercetin 3-glucoside and cyanidin are derived from the same precursor, dihydroquercetin (**Figure 22**).[172] Thus, it is possible that the enzymes involved in the conversion of dihydroquercetin to cyanidin and quercetin 3-glucoside, such as flavonol 3-O-glucotransferase (F3GT) and anthocyanidin synthase (ANS), are more active in Beauty than in Radiance or Sensation. Catechin is also derived from dihydroquercetin and it was detected in significantly lower amounts in cortex of Beauty (**Figure 21B**). Therefore, it seems like the production of catechin is suppressed in Beauty while formation of cyanidin, and quercetin 3-glucoside is enhanced. A predominant polyphenol in the cortex of the fruit was *p*-coumaric acid. Oszmiajski and Wojdylo[173] also reported high concentrations of *p*-coumaric acid in relation to the other polyphenols, apart

from pelargonidin 3-glucoside and the proanthocyanidins, in the strawberry cultivars 'Senga Sengana' and 'Elkat'. The synthesis of *p*-coumaric acid occurs very early in the phenylpropanoid pathway (**Figure 6**) and results by the hydroxylation of cinnamic acid via 4-cinnamic acid hydroxylase. Afterwards, *p*-coumaric acid has the ability to be converted into any of the polyphenols, except into gallic and ellagic acids. Previous studies have reported that the content of *p*-coumaric acid increases as the strawberry ripens.[174-176] The high content of *p*-coumaric acid in the cortex of strawberry fruit might be due to ripening of the fruit and active synthesis of polyphenols.

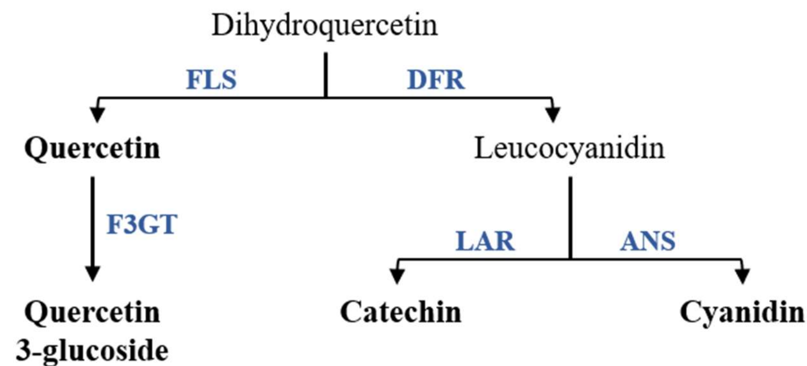

Figure 22. Quercetin 3-glucoside, catechin, and cyanidin all derive from the same precursor, dihydroquercetin. ANS – anthocyanindin synthase; DFR – dihydroflavonol 4-reductase; FLS – flavonol synthase; F3GT – flavonol 3-O-glucotransferase; LAR – leucoanthocyanidin reductase

The distribution of polyphenols was analyzed again after 7 days of cold storage at 1 °C (**Figure 23A & B**). Overall, there was much less variation between Radiance, Beauty and Sensation after 7 days. Only two polyphenols showed significant differences in the pith, namely pelargonidin 3-glucoside and myricetin (**Figure 23A**). Beauty had a significantly higher content of pelargonidin 3-glucoside in the pith at day 7 when compared to Sensation. Radiance was not statistically different from either which departs from trend at day 0 where pelargonidin 3-glucoside was significantly higher in Radiance than Sensation (**Figure 21A**). Radiance most likely had a greater degradation of pelargonidin 3-glucoside in the pith paralleling its decline in ANC (**Figure 19**). The ANC of the pith in Beauty and Sensation decreased from day 0 to day 7 but the effect of storage was not statistically significant; however, the pith of Radiance had a significant decrease in ANC from day 0 to day 7. The substantial degradation of ANC in Radiance is likely reflected in the HPLC analysis of pelargonidin 3-glucoside content of the pith at day 7 and could be the reason why the pith of Radiance is no longer significantly different from Sensation (**Figure 23A**).

Once again, individual polyphenols in the cortex showed more variation among Radiance, Beauty and Sensation than the pith at day 7 (**Figure 23B**). Radiance had significantly more pelargonidin 3-glucoside in the cortex than Beauty and Sensation who were not statistically different from one another. This departs again from the trend seen at day 0 (**Figure 21B**) where the pelargonidin 3-glucoside content was similar between Radiance and Beauty. Beauty had a higher reduction of TPC in the cortex of the fruit than in the pith whereas both Radiance and Sensation had a higher reduction in the pith (**Figure 18**). It is possible that Beauty experienced a greater degradation of pelargonidin 3-glucoside in the cortex which would reduce its levels to that of Sensation. This could explain why the levels of pelargonidin 3-glucoside in Beauty are more like Sensation at day 7 (**Figure 21B**).

At day 7, flavonols present in the cortex (quercetin, quercetin 3-glucoside, kaempferol, kaempferol 3-glucoside, and myricetin) showed variation among Radiance, Beauty and Sensation (**Figure 21B**). Radiance consistently had a higher content of ellagic acid in the cortex of the fruit compared to the other two strawberry cultivars (**Figures 21B & 23B**). The biological role of ellagic acid in plants is still unclear. The proposed roles include antioxidant activities, regeneration of cellular antioxidants, and antimicrobial activities. The present study and others have shown that ellagic acid concentrations are cultivar dependent.[96, 155, 177] Da Silva Pinto et al. (2008) measured the quantity of ellagic acid in seven strawberry cultivars and the concentration of free ellagic acid ranged from 0.61 mg/100 g fruit in ‘Piedade’ to 2.60 mg/100 g fruit in ‘Dover’. Another study detected ellagic acid in greater amounts in the strawberry cultivars ‘Magic’ and ‘Premial’ with concentrations of 9.03 and 6.79 mg/100 g fruit, respectively. (Nour 2017)

Distribution of major, individual polyphenols

Figure 24A-C shows the comparison between the distribution of individual polyphenols in Radiance, Beauty, and Sensation at harvest (day 0). Radiance and Beauty had a significantly higher amount of pelargonidin 3-glucoside in the cortex of the fruit (**Figure 24A & B**) compared to Sensation. This is agreement with previous studies that also showed pelargonidin 3-glucoside to be more concentrated in the cortex.[92] As pelargonidin 3-glucoside is the main pigment in ripe strawberries, this data shows that Radiance

and Beauty have a larger difference in color between the pith and cortex than does Sensation. This is shown by their relative concentrations of pelargonidin 3-glucoside in the pith compared to the cortex. Sensation has a much smaller difference in concentration which means its internal color is more similar to its external color. Beauty differed from the other two cultivars in that it had a significantly higher concentration of pelargonidin in the pith compared to the cortex. It also had significantly more cyanidin in the cortex than in the pith. The higher concentration of cyanidin in the cortex at harvest (day 0) might explain why Beauty is redder that Radiance or Sensation which is indicated by its lower hue (unpublished data). Beauty also contained significantly higher quercetin 3-glucoside and kaempferol 3-glucoside in the cortex than in the pith compared to Radiance and Sensation. Catechin was detected in higher amounts in the cortex of Radiance and Sensation whereas the levels were nearly equal in Beauty. The lower amount of catechin in the cortex of Beauty might be related its higher content of cyanidin within the same tissue. Leucocyanidin is a precursor to both catechin and cyanidin (**Figure 22**).[172] It is possible that the higher production of cyanidin diminishes the concentration of catechin as leucocyanidin is preferentially converted into the former. Higher amounts of *p*-coumaric acid were detected in the cortex for all three cultivars (**Figure 24A-C**). As mentioned previously, *p*-coumaric acid content increases during ripening and the cortex contains overall more polyphenols than the pith (**Figure 18**). As *p*-coumaric acid is a precursor to nearly all polyphenols, it seems reasonable that a greater amount of *p*-coumaric acid would be found in the same tissue that also contains the most polyphenols. Ferulic acid was also more abundant in the cortex of all three cultivars than in the pith (**Figure 24A-C**). Ferulic acid is a precursor to lignin synthesis (**Figure 18**). The high ferulic acid content of the cortex could likely be due to the presence of the achenes on the outside of the fruit which contain a large amount of lignin.[64] Ellagic acid content was also significantly higher in the cortex of Radiance, Beauty and Sensation (**Figure 24A-C**).

There were many more differences between the cortex and pith of Radiance, Beauty and Sensation after 7 days of cold storage (**Figure 25A-C**). Radiance had the most variation between the pith and cortex after cold storage with ten out of the sixteen major polyphenols being significantly more concentrated in the cortex (**Figure 25A**). Nine polyphenols exhibited this same trend in Beauty while Sensation only had

six (**Figure 25B & C**). This indicates that the polyphenol distribution in Sensation is more homogenous than Radiance or Beauty.

Significantly more pelargonidin 3-glucoside was detected in the cortex than in the pith for all cultivar (**Figure 25A-C**). Interestingly, the cortex to pith ratio of pelargonidin 3-glucoside increased during storage for Radiance and Sensation whereas the ratio decreased for Beauty. It was previously mentioned that Beauty lost more TPC from the cortex than from the pith (**Figure 17**) while Radiance and Sensation both lost more TPC from the pith. The greater reduction of polyphenolic content, which is predominantly pelargonidin 3-glucoside, in the cortex than in the pith would account for the lower cortex to pith ratio at day 7.

Along with pelargonidin 3-glucoside, cyanidin also showed greater concentration in the cortex in all three cultivars after 7 days of cold storage (**Figure 25A-C**). Likewise, cyanidin 3-glucoside concentrations were higher in the cortex of Radiance and Beauty when compared to their piths whereas Sensation showed no significant difference between pith and cortex. As for the rest of the flavonols, quercetin 3-glucoside was in higher concentrations in the cortex for all three cultivars. At day 0, only Beauty had differing concentrations of quercetin 3-glucoside between the cortex and pith (**Figure 24B**). The greater concentration differences seen at day 7 (**Figure 25A-C**) might be a result of water loss from the cortex which would make the cortex seem to have a higher concentration or it could be active synthesis of quercetin 3-glucoside in the cortex of the fruit. Beauty had a much larger concentration of kaempferol 3-glucoside in its cortex than in its pith. Radiance had a smaller difference in concentration however it was still significant whereas there was no significant difference in Sensation. This could be indicative of a genotype-dependent response to cold storage where the production of kaempferol 3-glucoside is more robust in Beauty while not induced in Sensation. As from day 0 (**Figure 24A-C**), *p*-coumaric acid, ferulic acid, and ellagic acid showed significant differences between the pith and cortex in all three cultivars after 7 days (**Figure 25A-C**). The high ferulic acid content in the cortex is still probably due to the presence of the achenes on the outside of the fruit. Removal of the achenes before polyphenol extraction might produce different results and more equal concentrations.

Conclusions

Results from this study showed that the distribution of polyphenols within strawberries is genotype dependent; however, the cortex of each cultivar consistently had a higher concentration of polyphenols than the pith. The degradation of total polyphenols and total anthocyanins also varied among the cultivars as well, with greater deterioration of polyphenols seen in the cultivar Radiance. The differences in the percent reduction of total polyphenols and total anthocyanins contents in the pith and cortex of the three cultivars indicates that each cultivar responds to cold storage in a different manner. Radiance also showed the most variation between the pith and cortex while the cultivar Sensation was had a more homogenous distribution of polyphenols. The variation, or lack thereof, between the cortex and pith could have an effect on the overall postharvest quality of a particular strawberry cultivar.

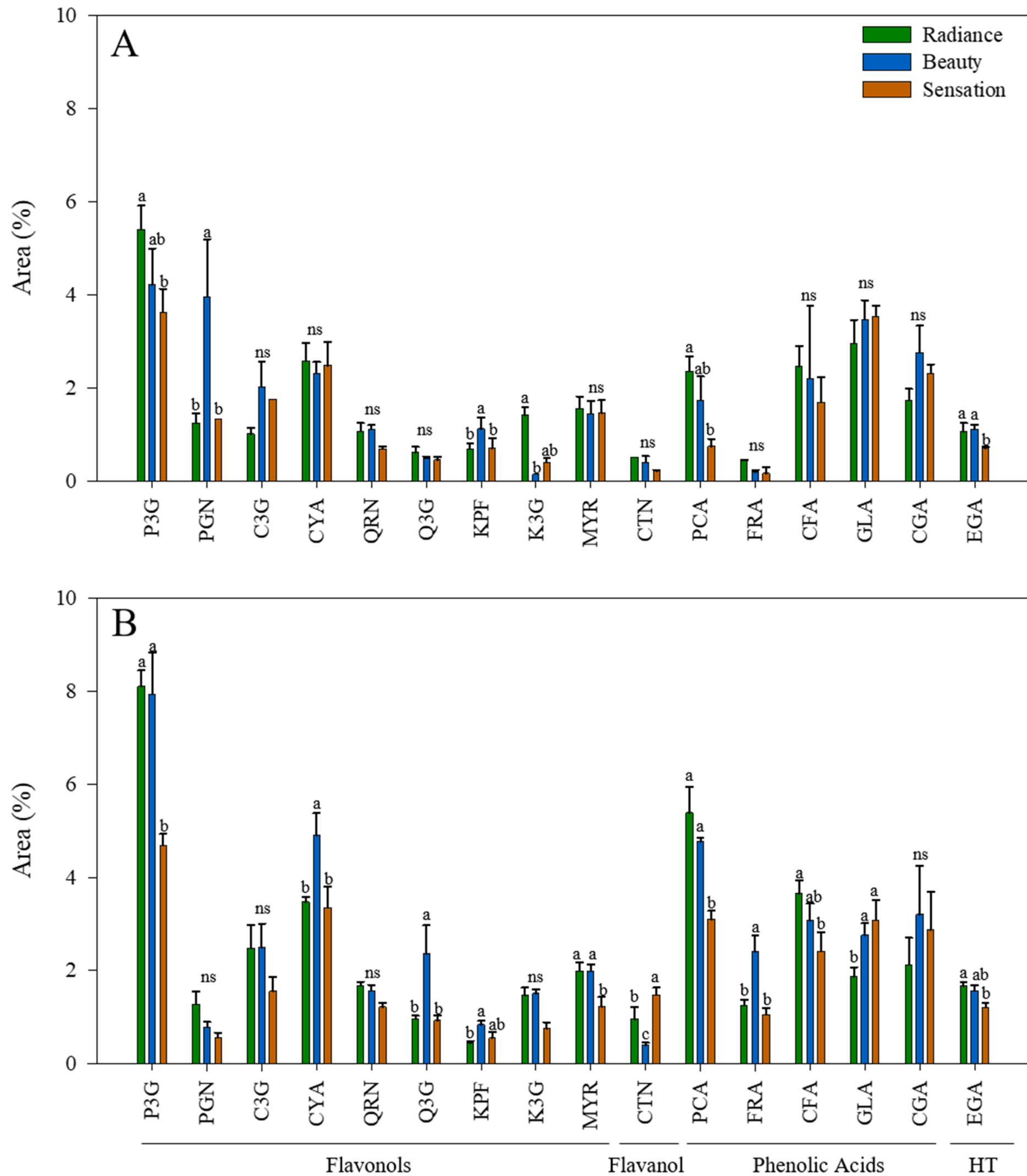

Figure 21. Differences in major, individual polyphenols of Radiance, Beauty, and Sensation on the day of harvest (day 0) in the pith (**A**) and in the cortex (**B**) using HPLC-DAD. (P3G – pelargonidin 3-gllucoside; PGN – pelargonidin; C3G – cyanidin 3-glucoside; CYA – cyanidin; QRN – quercetin; Q3G – quercetin 3-glucoside; KPF – kaempferol; K3G – kaempferol 3-glucoside; MYR – myricetin; CTN – catechin; PCA – p-coumaric acid; FRA – ferulic acid; CFA – caffeic acid; GLA – gallic acid; CGA – chlorogenic acid; EGA – ellagic acid) HT – hydrolysable tannin. Grouped bars headed by the same letter not significantly different from one another. ns – Not significant

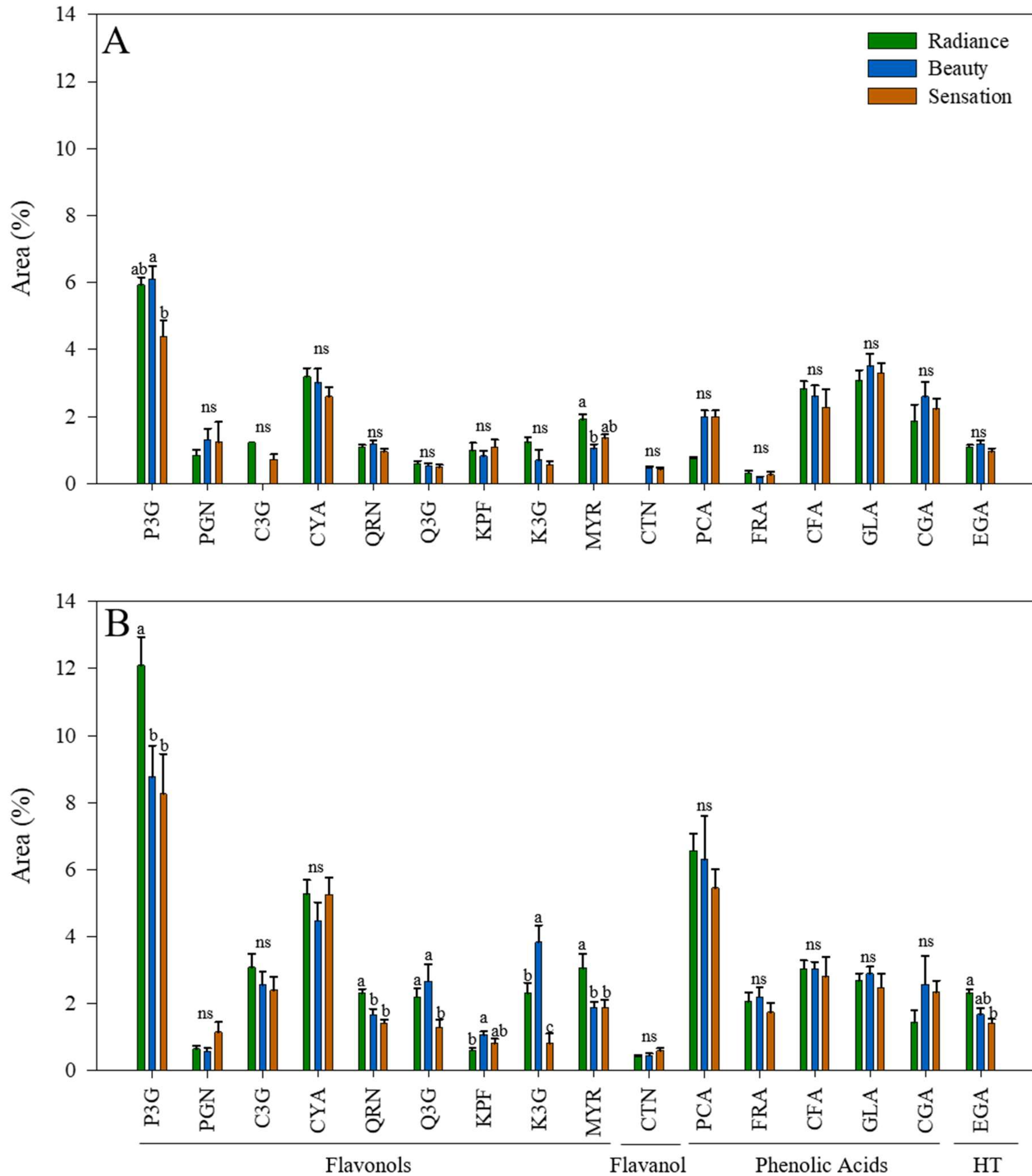

Figure 23. Differences in major, individual polyphenols of Radiance, Beauty, and Sensation after 7 days of cold storage at 1 °C in the pith (**A**) and in the cortex (**B**) using HPLC-DAD. (P3G – pelargonidin 3-gllucoside; PGN – pelargonidin; C3G – cyanidin 3-glucoside; CYA – cyanidin; QRN – quercetin; Q3G – quercetin 3-glucoside; KPF – kaempferol; K3G – kaempferol 3-glucoside; MYR – myricetin; CTN – catechin; PCA – p-coumaric acid; FRA – ferulic acid; CFA – caffeic acid; GLA – gallic acid; CGA – chlorogenic acid; EGA – ellagic acid) HT – hydrolysable tannin. Grouped bars headed by the same letter not significantly different from one another. ns – Not significant

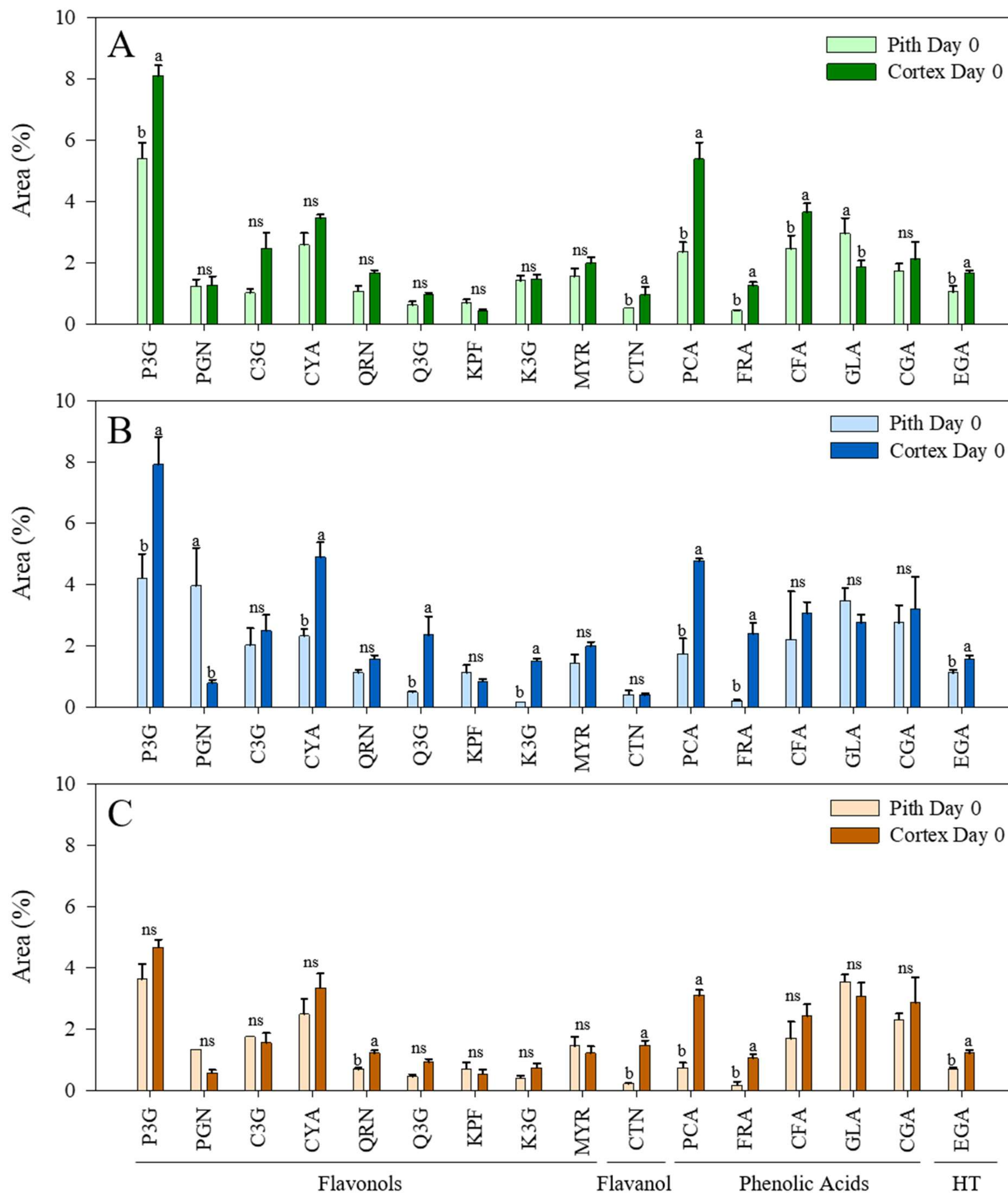

Figure 24. Differences in major, individual polyphenols of the pith and cortex of Radiance (**A**), Beauty (**B**) and Sensation (**C**) on the day of harvest (day 0) using HPLC-DAD. (P3G – pelargonidin 3-gllucoside; PGN – pelargonidin; C3G – cyanidin 3-glucoside; CYA – cyanidin; QRN – quercetin; Q3G – quercetin 3-glucoside; KPF – kaempferol; K3G – kaempferol 3-glucoside; MYR – myricetin; CTN – catechin; PCA – p-coumaric acid; FRA – ferulic acid; CFA – caffeic acid; GLA – gallic acid; CGA – chlorogenic acid; EGA – ellagic acid) HT – hydrolysable tannin. Grouped bars headed by the same letter not significantly different from one another. ns – Not significant

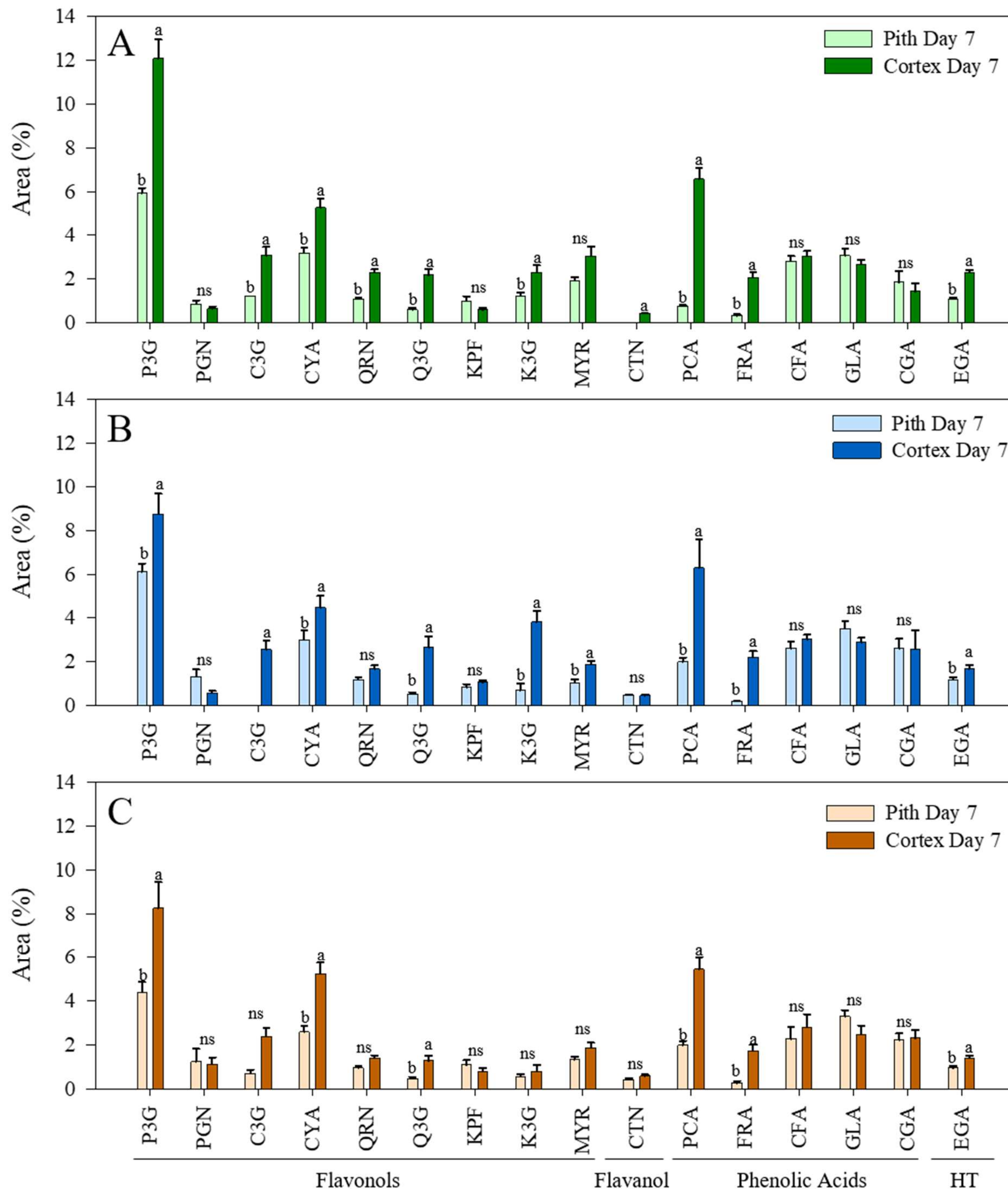

Figure 25. Differences in major, individual polyphenols of the pith and cortex of Radiance (**A**), Beauty (**B**) and Sensation (**C**) after 7 days of cold storage at 1 °C using HPLC-DAD. (P3G – pelargonidin 3-gllucoside; PGN – pelargonidin; C3G – cyanidin 3-glucoside; CYA – cyanidin; QRN – quercetin; Q3G – quercetin 3-glucoside; KPF – kaempferol; K3G – kaempferol 3-glucoside; MYR – myricetin; CTN – catechin; PCA – p-coumaric acid; FRA – ferulic acid; CFA – caffeic acid; GLA – gallic acid; CGA – chlorogenic acid; EGA – ellagic acid) HT – hydrolysable tannin. Grouped bars headed by the same letter not significantly different from one another. ns – Not significant

Chapter 6:

General Conclusions

The results from the work presented in this thesis show that polyphenol profiles and concentrations in strawberry cultivars Radiance, Beauty and Sensation respond differently during 7-days of storage at 1 °C. During cold storage, the concentrations of polyphenols did not decrease uniformly across cultivars or within the individual fruit however it seems that the degradation of polyphenols is due to the loss of polyphenols from the cortex of the fruit rather than the pith. Pelargonidin 3-glucoside was consistently the predominant polyphenol in the three strawberry cultivars studied, showing a wide variation between cultivars with the cultivar Sensation containing the least amount of pelargonidin 3-glucoside compared to the other two cultivars. Apart from the anthocyanins, the flavonols showed the most variation among the cultivars and between the pith and cortex of the fruit. The key biosynthetic enzyme, phenylalanine ammonia lyase (PAL), did not show a strong induction during cold most likely due to the optimal postharvest conditions used in this study. However, polyphenol oxidase (PPO) showed a general induction during cold storage with the cultivar with the highest concentration of polyphenols exhibiting the highest enzyme activity at the time of harvest.

Future studies should focus on quality parameters alongside the content of individual polyphenols and explore a possible correlation between individual polyphenols and positive postharvest quality traits and strawberry shelf life. Furthermore, the induction of PAL and PPO as well as the content of individual polyphenols should be investigated under abuse conditions to further reveal polyphenols and enzyme activity linked with superior postharvest quality. Because the phenylpropanoid pathway is a very large and branched pathway, other important regulatory biosynthetic enzymes such as chalcone synthase, the key enzyme of the flavonoid branch need to be studied. Based on this study, future studies should focus on the

content of flavonols, and their respective biosynthetic enzymes, as these have shown the most variability between cultivars and during storage.

References

1. Dixon, R. A.; Paiva, N. L., Stress-induced phenylpropanoid metabolism. *The plant cell* **1995,** *7* (7), 1085.
2. Quideau, S.; Deffieux, D.; Douat-Casassus, C.; Pouységu, L., Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. *Angewandte Chemie International Edition* **2011,** *50* (3), 586-621.
3. Giampieri, F.; Forbes-Hernandez, T. Y.; Gasparrini, M.; Alvarez-Suarez, J. M.; Afrin, S.; Bompadre, S.; Quiles, J. L.; Mezzetti, B.; Battino, M., Strawberry as a health promoter: an evidence based review. *Food & Function* **2015,** *6* (5), 1386-1398.
4. Guan, Z.; Hee Suy, D.; Khachatryan, H.; Wu, F. Import Growth and the Impact on the Florida Strawberry Industry **2017**. http://edis.ifas.ufl.edu/fe1022 (accessed May 15, 2018).
5. Nunes, M. C., Soft Fruits and Berries. In *Color Atlas of Postharvest Quality of Fruits and Vegetables*, **2009**.
6. Sarkar, D.; Shetty, K., Metabolic Stimulation of Plant Phenolics for Food Preservation and Health. *Annual Review of Food Science and Technology* **2014,** *5* (1), 395-413.
7. Schwieterman, M. L.; Colquhoun, T. A.; Jaworski, E. A.; Bartoshuk, L. M.; Gilbert, J. L.; Tieman, D. M.; Odabasi, A. Z.; Moskowitz, H. R.; Folta, K. M.; Klee, H. J.; Sims, C. A.; Whitaker, V. M.; Clark, D. G., Strawberry Flavor: Diverse Chemical Compositions, a Seasonal Influence, and Effects on Sensory Perception. *PLoS ONE* **2014,** (2).
8. Jouquand, C.; Chandler, C.; Plotto, A.; Goodner, K., A Sensory and Chemical Analysis of Fresh Strawberries Over Harvest Dates and Seasons Reveals Factors That Affect Eating Quality. **2008**, p 859.
9. Montero, T. M.; Mollá, E. M.; Esteban, R. M.; López-Andréu, F. J., Quality attributes of strawberry during ripening. *Scientia Horticulturae* **1996,** *65* (4), 239-250.
10. Brummell, D. A., Review: Cell wall disassembly in ripening fruit. **2006**, p 103.
11. Nunes, M. C. N.; Brecht, J. K.; Morais, A. M. B.; Sargent, S. A., Possible Influences of Water Loss and Polyphenol Oxidase Activity on Anthocyanin Content and Discoloration in Fresh Ripe Strawberry (cv. Oso Grande) During Storage at 1 °C. *Journal of Food Science* **2005,** *70* (1), S79-S84.
12. Nunes, M. C. d. N., Correlations between subjective quality and physicochemical attributes of fresh fruits and vegetables. *Postharvest Biology and Technology* **2015,** *107*, 43-54.
13. Colquhoun, T. A.; Levin, L. A.; Moskowitz, H. R.; Whitaker, V. M.; Clark, D. G.; Folta, K. M., Framing the perfect strawberry: An exercise in consumer-assisted selection of fruit crops. *Journal of Berry Research* **2012,** *2* (1), 45-61.
14. Chaves, V. C.; Calvete, E.; Reginatto, F. v., Quality properties and antioxidant activity of seven strawberry (Fragaria x ananassa duch) cultivars. *Scientia Horticulturae* **2017**.

15. Tulipani, S.; Mezzetti, B.; Capocasa, F.; Bompadre, S.; Beekwilder, J.; de Vos, C. H. R.; Capanoglu, E.; Bovy, A.; Battino, M., Antioxidants, Phenolic Compounds, and Nutritional Quality of Different Strawberry Genotypes. *Journal of Agricultural and Food Chemistry* **2008,** *56* (3), 696-704.

16. Pelayo-ZaldÍVar, C.; Ebeler, S. E.; Kader, A. A., CULTIVAR AND HARVEST DATE EFFECTS ON FLAVOR AND OTHER QUALITY ATTRIBUTES OF CALIFORNIA STRAWBERRIES. *Journal of Food Quality* **2005,** *28* (1), 78-97.

17. Kelly, K.; Whitaker, V. M.; Nunes, M. C. d. N., Physicochemical characterization and postharvest performance of the new Sensation® 'Florida127' strawberry compared to commercial standards. *Scientia Horticulturae* **2016,** *211*, 283-294.

18. Goulao, L. F.; Oliveira, C. M., Review: Cell wall modifications during fruit ripening: when a fruit is not the fruit. *Trends in Food Science & Technology* **2008,** *19*, 4-25.

19. Plotto, A.; Whitaker, V.; Chandler, C., Eating Quality of Old and New University of Florida Strawberry Cultivars. **2010**, p 290.

20. Mikulic-Petkovsek, M.; Schmitzer, V.; Slatnar, A.; Weber, N.; Veberic, R.; Stampar, F.; Munda, A.; Koron, D., Alteration of the Content of Primary and Secondary Metabolites in Strawberry Fruit by Colletotrichum nymphaeae Infection. *Journal of Agricultural and Food Chemistry* **2013,** *61* (25), 5987-5995.

21. Manning, K., Soft fruit. In *Biochemistry of Fruit Ripening*, Seymour, G. B.; Taylor, J. E.; Tucker, G. A., Eds. Springer Netherlands: Dordrecht, **1993**; pp 347-377.

22. Gündüz, K.; Özdemir, E., The effects of genotype and growing conditions on antioxidant capacity, phenolic compounds, organic acid and individual sugars of strawberry. *Food Chemistry* **2014,** *155*, 298-303.

23. Hasler, C. M., Functional Foods: Benefits, Concerns and Challenges—A Position Paper from the American Council on Science and Health. *The Journal of Nutrition* **2002,** *132* (12), 3772-3781.

24. Afrin, S.; Gasparrini, M.; Forbes-Hernandez, T. Y.; Reboredo-Rodriguez, P.; Mezzetti, B.; Varela-Lopez, A.; Giampieri, F.; Battino, M., Promising Health Benefits of the Strawberry: A Focus on Clinical Studies. *J Agric Food Chem* **2016,** *64* (22), 4435-49.

25. Giampieri, F.; Alvarez-Suarez, J. M.; Battino, M., Strawberry and Human Health: Effects beyond Antioxidant Activity. *Journal of Agricultural and Food Chemistry* **2014,** *62* (18), 3867-3876.

26. Basu, A.; Nguyen, A.; Betts, N. M.; Lyons, T. J., Strawberry As a Functional Food: An Evidence-Based Review. *Critical Reviews in Food Science and Nutrition* **2014,** *54* (6), 790-806.

27. Giampieri, F.; Tulipani, S.; Alvarez-Suarez, J. M.; Quiles, J. L.; Mezzetti, B.; Battino, M., The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition* **2012,** *28* (1), 9-19.

28. Aaby, K.; Ekeberg, D.; Skrede, G., Characterization of Phenolic Compounds in Strawberry (Fragaria × ananassa) Fruits by Different HPLC Detectors and Contribution of Individual Compounds to Total Antioxidant Capacity. *Journal of Agricultural and Food Chemistry* **2007,** *55* (11), 4395-4406.

29. Scalzo, J.; Politi, A.; Pellegrini, N.; Mezzetti, B.; Battino, M., Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition* **2005,** *21* (2), 207-13.

30. Tulipani, S.; Alvarez-Suarez, J. M.; Busco, F.; Bompadre, S.; Quiles, J. L.; Mezzetti, B.; Battino, M., Strawberry consumption improves plasma antioxidant status and erythrocyte resistance to oxidative haemolysis in humans. *Food Chemistry* **2011,** *128* (1), 180-186.

31. Tulipani, S.; Armeni, T.; Giampieri, F.; Alvarez-Suarez, J. M.; Gonzalez-Paramás, A. M.; Santos-Buelga, C.; Busco, F.; Principato, G.; Bompadre, S.; Quiles, J. L.; Mezzetti, B.; Battino, M., Strawberry intake increases blood fluid, erythrocyte and mononuclear cell defenses against oxidative challenge. *Food Chemistry* **2014,** *156*, 87-93.

32. Gardi, C.; Bauerova, K.; Stringa, B.; Kuncirova, V.; Slovak, L.; Ponist, S.; Drafi, F.; Bezakova, L.; Tedesco, I.; Acquaviva, A.; Bilotto, S.; Russo, G. L., Quercetin reduced inflammation and increased antioxidant defense in rat adjuvant arthritis. *Archives of biochemistry and biophysics* **2015,** *583*, 150-157.

33. Burton-Freeman, B.; Linares, A.; Hyson, D.; Kappagoda, T., Strawberry Modulates LDL Oxidation and Postprandial Lipemia in Response to High-Fat Meal in Overweight Hyperlipidemic Men and Women. *Journal of the American College of Nutrition* **2010,** *29* (1), 46-54.

34. Alvarez-Suarez, J. M.; Giampieri, F.; Tulipani, S.; Casoli, T.; Di Stefano, G.; González-Paramás, A. M.; Santos-Buelga, C.; Busco, F.; Quiles, J. L.; Cordero, M. D.; Bompadre, S.; Mezzetti, B.; Battino, M., One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. *The Journal of Nutritional Biochemistry* **2014,** *25* (3), 289-294.

35. Abdulazeez, S. S.; Ponnusamy, P., Antioxidant and hypoglycemic activity of strawberry fruit extracts against alloxan induced diabetes in rats. *Pakistan Journal of Pharmaceutical Sciences* **2016,** *29*, 255+.

36. Chen, T.; Yan, F.; Qian, J.; Guo, M.; Zhang, H.; Tang, X.; Chen, F.; Stoner, G. D.; Wang, X., Randomized Phase II Trial of Lyophilized Strawberries in Patients with Dysplastic Precancerous Lesions of the Esophagus. *Cancer prevention research (Philadelphia, Pa.)* **2012,** *5* (1), 41-50.

37. Del Rio, D.; Borges, G.; Crozier, A., Berry flavonoids and phenolics: bioavailability and evidence of protective effects. *British Journal of Nutrition* **2010,** *104* (S3), S67-S90.

38. Symons, G. M.; Chua, Y. J.; Ross, J. J.; Quittenden, L. J.; Davies, N. W.; Reid, J. B., Hormonal changes during non-climacteric ripening in strawberry. *Journal of Experimental Botany* **2012,** *63* (13), 4741-4750.

39. Cordenunsi, B. R.; Nascimento, J. R. O.; Lajolo, F. M., Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage. *Food Chemistry* **2003,** *83* (2), 167-173.

40. Cordenunsi, B. R.; Genovese, M. I.; Oliveira do Nascimento, J. R.; Aymoto Hassimotto, N. M.; José dos Santos, R.; Lajolo, F. M., Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. *Food Chemistry* **2005,** *91* (1), 113-121.

41. Pelletier, W.; Brecht, J. K.; Cecilia do Nascimento Nunes, M.; Émond, J.-P., Quality of Strawberries Shipped by Truck from California to Florida as Influenced by Postharvest Temperature Management Practices. *HortTechnology* **2011,** *21* (4), 482-493.

42. Octavia, L.; Choo, W. S., Folate, ascorbic acid, anthocyanin and colour changes in strawberry (Fragaria × annanasa) during refrigerated storage. *LWT - Food Science and Technology* **2017,** *86*, 652-659.

43. Shin, Y.; Ryu, J.-A.; Liu, R. H.; Nock, J. F.; Watkins, C. B., Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit. *Postharvest Biology and Technology* **2008,** *49* (2), 201-209.

44. Paniagua, C.; Santiago-Doménech, N.; Kirby, A. R.; Gunning, A. P.; Morris, V. J.; Quesada, M. A.; Matas, A. J.; Mercado, J. A., Structural changes in cell wall pectins during strawberry fruit development. *Plant Physiology and Biochemistry* **2017,** *118*, 55-63.

45. Jin, P.; Wang, H.; Zhang, Y.; Huang, Y.; Wang, L.; Zheng, Y., UV-C enhances resistance against gray mold decay caused by Botrytis cinerea in strawberry fruit. *Scientia Horticulturae* **2017,** *225*, 106-111.

46. Romanazzi, G.; Smilanick, J. L.; Feliziani, E.; Droby, S., Integrated management of postharvest gray mold on fruit crops. *Postharvest Biology and Technology* **2016,** *113*, 69-76.

47. Feliziani, E.; Landi, L.; Romanazzi, G., Preharvest treatments with chitosan and other alternatives to conventional fungicides to control postharvest decay of strawberry. *Carbohydrate Polymers* **2015,** *132*, 111-117.

48. Li, L.; Sun, J.; Gao, H.; Shen, Y.; Li, C.; Yi, P.; He, X.; Ling, D.; Sheng, J.; Li, J.; Liu, G.; Zheng, F.; Xin, M.; Li, Z.; Tang, Y., Effects of Polysaccharide-Based Edible Coatings on Quality and Antioxidant Enzyme System of Strawberry during Cold Storage. *International Journal of Polymer Science* **2017**, 1-8.

49. Aguilar-González, A. E.; Palou, E.; López-Malo, A., Antifungal activity of essential oils of clove (Syzygium aromaticum) and/or mustard (Brassica nigra) in vapor phase against gray mold (Botrytis cinerea) in strawberries. *Innovative Food Science and Emerging Technologies* **2015,** *32*, 181-185.

50. Liu, S.; Shao, X.; Wei, Y.; Li, Y.; Xu, F.; Wang, H., Solidago canadensis L. Essential Oil Vapor Effectively Inhibits Botrytis cinerea Growth and Preserves Postharvest Quality of Strawberry as a Food Model System. *Frontiers in Microbiology* **2016,** *7* (1179).

51. Jin, P.; Zheng, C.; Huang, Y.-p.; Wang, X.-l.; Luo, Z.-s.; Zheng, Y.-h., Hot air treatment activates defense responses and induces resistance against Botrytis cinerea in strawberry fruit. *Journal of Integrative Agriculture* **2016,** *15* (11), 2658-2665.

52. Pombo, M. A.; Martinez, G. A.; Civello, P. M., Cloning of FaPAL6 gene from strawberry fruit and characterization of its expression and enzymatic activity in two cultivars with different anthocyanin accumulation. *Plant Sci* **2011,** *181* (2), 111-8.

53. Wang, S. Y.; Gao, H., Effect of chitosan-based edible coating on antioxidants, antioxidant enzyme system, and postharvest fruit quality of strawberries (Fragaria x aranassa Duch.). *LWT - Food Science and Technology* **2013,** *52* (2), 71-79.

54. No, H. K.; Meyers, S. P.; Prinyawiwatkul, W.; Xu, Z., Applications of Chitosan for Improvement of Quality and Shelf Life of Foods: A Review. *Journal of Food Science* **2007,** *72* (5), R87-R100.

55. Aguilar-González, A. E.; Palou, E.; López-Malo, A., Antifungal activity of essential oils of clove (Syzygium aromaticum) and/or mustard (Brassica nigra) in vapor phase against gray mold (Botrytis cinerea) in strawberries. *Innovative Food Science & Emerging Technologies* **2015,** *32*, 181-185.

56. Shao, X.; Wang, H.; Xu, F.; Cheng, S., Effects and possible mechanisms of tea tree oil vapor treatment on the main disease in postharvest strawberry fruit. *Postharvest Biology and Technology* **2013,** *77*, 94-101.

57. Weber, N.; Zupanc, V.; Jakopic, J.; Veberic, R.; Mikulic-Petkovsek, M.; Stampar, F., Influence of deficit irrigation on strawberry (Fragaria × ananassa Duch.) fruit quality. *Journal of the Science of Food and Agriculture* **2017,** *97* (3), 849-857.

58. Galli, V.; da Silva Messias, R.; Perin, E. C.; Borowski, J. M.; Bamberg, A. L.; Rombaldi, C. V., Mild salt stress improves strawberry fruit quality. *LWT - Food Science and Technology* **2016,** *73*, 693-699.

59. Volkmann, D.; Baluška, F., Gravity: one of the driving forces for evolution. *Protoplasma* **2006,** *229* (2), 143-148.

60. Boerjan, W.; Ralph, J.; Baucher, M., Lignin Biosynthesis. *Annual Review of Plant Biology* **2003,** *54* (1), 519-546.

61. Parr, A. J.; Bolwell, G. P., Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture* **2000,** *80* (7), 985-1012.

62. Bartley, I. M.; Knee, M., The chemistry of textural changes in fruit during storage. *Food Chemistry* **1982,** *9* (1), 47-58.

63. Salentijn, E. M. J.; Aharoni, A.; Schaart, J. G.; Boone, M. J.; Krens, F. A., Differential gene expression analysis of strawberry cultivars that differ in fruit-firmness. *Physiologia Plantarum* **2003,** *118* (4), 571-578.

64. Suutarinen, J.; Änäkäinen, L.; Autio, K., Comparison of Light Microscopy and Spatially Resolved Fourier Transform Infrared (FT-IR) Microscopy in the Examination of Cell Wall Components of Strawberries. *LWT - Food Science and Technology* **1998,** *31* (7), 595-601.

65. Falcone Ferreyra, M. L.; Rius, S. P.; Casati, P., Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science* **2012,** *3*, 222.

66. de Pascual-Teresa, S.; Sanchez-Ballesta, M. T., Anthocyanins: from plant to health. *Phytochemistry Reviews* **2008,** *7* (2), 281-299.

67. Muñoz, C.; Sánchez-Sevilla, J. F.; Botella, M. A.; Hoffmann, T.; Schwab, W.; Valpuesta, V., Polyphenol Composition in the Ripe Fruits of Fragaria Species and Transcriptional Analyses of Key Genes in the Pathway. *Journal of Agricultural and Food Chemistry* **2011,** *59* (23), 12598-12604.

68. Papiorek, S.; Junker, R. R.; Alves-dos-Santos, I.; Melo, G. A. R.; Amaral-Neto, L. P.; Sazima, M.; Wolowski, M.; Freitas, L.; Lunau, K., Bees, birds and yellow flowers: pollinator-dependent convergent evolution of UV patterns. *Plant Biology* **2016,** *18* (1), 46-55.

69. Cheynier, V.; Comte, G.; Davies, K. M.; Lattanzio, V.; Martens, S., Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry* **2013,** *72*, 1-20.

70. Mandal, S. M.; Chakraborty, D.; Dey, S., Phenolic acids act as signaling molecules in plant-microbe symbioses. *Plant Signaling & Behavior* **2010,** *5* (4), 359-368.

71. Caverzan, A.; Casassola, A.; Brammer, S. P., Reactive Oxygen Species and Antioxidant Enzymes Involved in Plant Tolerance to Stress. In *Abiotic and Biotic Stress in Plants - Recent Advances and Future Perspectives*, Shanker, A. K.; Shanker, C., Eds. InTech: Rijeka, **2016**; p Ch. 20.

72. Mittler, R., Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science 7* (9), 405-410.

73. Pham-Huy, L. A.; He, H.; Pham-Huy, C., Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science : IJBS* **2008,** *4* (2), 89-96.

74. Pereira, D.; Valentão, P.; Pereira, J.; Andrade, P., Phenolics: From Chemistry to Biology. *Molecules* **2009,** *14* (6), 2202.

75. Murkovic, M., *Phenolic compounds*. **2003**; Vol. 7, p 4507-4514.

76. Heleno, S. A.; Martins, A.; Queiroz, M. J. R. P.; Ferreira, I. C. F. R., Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. *Food Chemistry* **2015,** *173* (Supplement C), 501-513.

77. Mandave, P. C.; Pawar, P. K.; Ranjekar, P. K.; Mantri, N.; Kuvalekar, A. A., Comprehensive evaluation of in vitro antioxidant activity, total phenols and chemical profiles of two commercially important strawberry varieties. *Scientia Horticulturae* **2014,** *172*, 124-134.

78. Salminen, J.-P.; Karonen, M., Chemical ecology of tannins and other phenolics: we need a change in approach. *Functional Ecology* **2011,** *25* (2), 325-338.

79. Gasperotti, M.; Masuero, D.; Guella, G.; Palmieri, L.; Martinatti, P.; Pojer, E.; Mattivi, F.; Vrhovsek, U., Evolution of Ellagitannin Content and Profile during Fruit Ripening in Fragaria spp. *Journal of Agricultural and Food Chemistry* **2013,** *61* (36), 8597-8607.

80. Crozier, A.; Jaganath, I. B.; Clifford, M. N., Phenols, Polyphenols and Tannins: An Overview. In *Plant Secondary Metabolites*, Blackwell Publishing Ltd: **2007**; pp 1-24.

81. Chong, J.; Poutaraud, A.; Hugueney, P., Metabolism and roles of stilbenes in plants. *Plant Science* **2009,** *177* (3), 143-155.

82. Heldt, H.; Piechulla, B., *Phenylpropanoids comprise a multitude of plant secondary metabolites and cell wall components*. **2011**; p 431-449.

83. Wang, S. Y.; Chen, C.-T.; Wang, C. Y.; Chen, P., Resveratrol Content in Strawberry Fruit Is Affected by Preharvest Conditions. *Journal of Agricultural and Food Chemistry* **2007,** *55* (20), 8269-8274.

84. Saltveit, M. E., Synthesis and Metabolism of Phenolic Compounds. In *Fruit and Vegetable Phytochemicals*, Wiley-Blackwell: **2009**; pp 89-100.

85. Yeh, S.-Y.; Huang, F.-C.; Hoffmann, T.; Mayershofer, M.; Schwab, W., FaPOD27 functions in the metabolism of polyphenols in strawberry fruit (Fragaria sp.). *Frontiers in Plant Science* **2014,** *5* (518).

86. Kumar, S.; Pandey, A. K., Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal* **2013,** *2013*, 16.

87. Buendía, B.; Gil, M. I.; Tudela, J. A.; Gady, A. L.; Medina, J. J.; Soria, C.; López, J. M.; Tomás-Barberán, F. A., HPLC-MS Analysis of Proanthocyanidin Oligomers and Other Phenolics in 15 Strawberry Cultivars. *Journal of Agricultural and Food Chemistry* **2010,** *58* (7), 3916-3926.

88. Agati, G.; Azzarello, E.; Pollastri, S.; Tattini, M., Flavonoids as antioxidants in plants: Location and functional significance. *Plant Science* **2012,** *196* (Supplement C), 67-76.

89. Harkamal, W.; Wilson, C.; Pascal, C.; Liu, X.; Abdelbagi, M. I.; Zeng, L.; Steve, I. W.; Jayati, M.; Xu, J.; Xinping, C.; Close, T. J., Comparative Transcriptional Profiling of Two Contrasting Rice Genotypes under Salinity Stress during the Vegetative Growth Stage. *Plant Physiology* **2005,** *139* (2), 822-835.

90. Paolacci, A. R.; D'Ovidio, R.; Marabottini, R.; Nali, C.; Lorenzini, G.; Abenavoli, M. R.; Badiani, M., Ozone induces a differential accumulation of phenyalanine ammonia-lyase, chalcone

synthase and chalcone isomerase RNA transcripts in sensitive and resistant bean cultivars. *Functional Plant Biology* **2001,** *28* (5), 425-428.

91. Winkel-Shirley, B., Biosynthesis of flavonoids and effects of stress. *Current opinion in plant biology* **2002,** *5* (3), 218-223.
92. Josuttis, M.; Dietrich, H.; Treutter, D.; Will, F.; Linnemannstöns, L.; Krüger, E., Solar UVB Response of Bioactives in Strawberry (Fragaria × ananassa Duch. L.): A Comparison of Protected and Open-Field Cultivation. *Journal of Agricultural and Food Chemistry* **2010,** *58* (24), 12692-12702.
93. Peer, W. A.; Murphy, A. S., Flavonoids and auxin transport: modulators or regulators? *Trends in Plant Science 12* (12), 556-563.
94. Schaart, J. G.; Dubos, C.; Romero De La Fuente, I.; van Houwelingen, A. M. M. L.; de Vos, R. C. H.; Jonker, H. H.; Xu, W.; Routaboul, J.-M.; Lepiniec, L.; Bovy, A. G., Identification and characterization of MYB-bHLH-WD40 regulatory complexes controlling proanthocyanidin biosynthesis in strawberry (Fragaria × ananassa) fruits. *New Phytologist* **2013,** *197* (2), 454-467.
95. da Silva, F. L.; Escribano-Bailón, M. T.; Pérez Alonso, J. J.; Rivas-Gonzalo, J. C.; Santos-Buelga, C., Anthocyanin pigments in strawberry. *LWT - Food Science and Technology* **2007,** *40* (2), 374-382.
96. Aaby, K.; Mazur, S.; Nes, A.; Skrede, G., Phenolic compounds in strawberry (Fragaria x ananassa Duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chemistry* **2012,** *132* (1), 86-97.
97. Nagpala, E. G.; Guidarelli, M.; Gasperotti, M.; Masuero, D.; Bertolini, P.; Vrhovsek, U.; Baraldi, E., Polyphenols Variation in Fruits of the Susceptible Strawberry Cultivar Alba during Ripening and upon Fungal Pathogen Interaction and Possible Involvement in Unripe Fruit Tolerance. *J Agric Food Chem* **2016,** *64* (9), 1869-78.
98. Fait, A.; Hanhineva, K.; Beleggia, R.; Dai, N.; Rogachev, I.; Nikiforova, V. J.; Fernie, A. R.; Aharoni, A., Reconfiguration of the Achene and Receptacle Metabolic Networks during Strawberry Fruit Development. *Plant Physiology* **2008,** *148* (2), 730.
99. Amil-Ruiz, F.; Blanco-Portales, R.; Muñoz-Blanco, J.; Caballero, J. L., The Strawberry Plant Defense Mechanism: A Molecular Review. *Plant and Cell Physiology* **2011,** *52* (11), 1873-1903.
100. Aaby, K.; Skrede, G.; Wrolstad, R. E., Phenolic Composition and Antioxidant Activities in Flesh and Achenes of Strawberries (Fragaria ananassa). *Journal of Agricultural and Food Chemistry* **2005,** *53* (10), 4032-4040.
101. Valentinuzzi, F.; Mason, M.; Scampicchio, M.; Andreotti, C.; Cesco, S.; Mimmo, T., Enhancement of the bioactive compound content in strawberry fruits grown under iron and phosphorus deficiency. *Journal of the Science of Food and Agriculture* **2015,** *95* (10), 2088-2094.
102. Keutgen, A. J.; Pawelzik, E., Quality and nutritional value of strawberry fruit under long term salt stress. *Food Chemistry* **2008,** *107* (4), 1413-1420.
103. Okutsu, K.; Matsushita, K.; Ikeda, T., Differential Anthocyanin Concentrations and Expression of Anthocyanin Biosynthesis Genes in Strawberry 'Sachinoka' during Fruit Ripening under High-temperature Stress. *Environmental Control in Biology* **2018,** *56* (1), 1-6.
104. Wang, S. Y.; Camp, M. J., Temperatures after bloom affect plant growth and fruit quality of strawberry. *Scientia Horticulturae* **2000,** *85* (3), 183-199.

105. Palmieri, L.; Masuero, D.; Martinatti, P.; Baratto, G.; Martens, S.; Vrhovsek, U., Genotype-by-environment effect on bioactive compounds in strawberry (Fragaria x ananassa Duch.). *Journal of the Science of Food and Agriculture* **2017,** *97* (12), 4180-4189.

106. Wang, S. Y.; Zheng, W.; Galletta, G. J., Cultural System Affects Fruit Quality and Antioxidant Capacity in Strawberries. *Journal of Agricultural and Food Chemistry* **2002,** *50* (22), 6534-6542.

107. Pincemail, J.; Kevers, C.; Tabart, J.; Defraigne, J. O.; Dommes, J., Cultivars, Culture Conditions, and Harvest Time Influence Phenolic and Ascorbic Acid Contents and Antioxidant Capacity of Strawberry (Fragaria x ananassa). *Journal of Food Science* **2012,** *77* (2), C205-C210.

108. Crecente-Campo, J.; Nunes-Damaceno, M.; Romero-Rodríguez, M. A.; Vázquez-Odériz, M. L., Color, anthocyanin pigment, ascorbic acid and total phenolic compound determination in organic versus conventional strawberries (Fragaria×ananassa Duch, cv Selva). *Journal of Food Composition and Analysis* **2012,** *28* (1), 23-30.

109. Fernandes, V. C.; Domingues, V. F.; de Freitas, V.; Delerue-Matos, C.; Mateus, N., Strawberries from integrated pest management and organic farming: Phenolic composition and antioxidant properties. *Food Chemistry* **2012,** *134* (4), 1926-1931.

110. Xuebin, Z.; Mingyue, G.; Chang-Jun, L., Arabidopsis Kelch Repeat F-Box Proteins Regulate Phenylpropanoid Biosynthesis via Controlling the Turnover of Phenylalanine Ammonia-Lyase. *The Plant Cell* **2013,** (12), 4994.

111. Ferrer, J. L.; Austin, M. B.; Stewart, C.; Noel, J. P., Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. *Plant Physiology and Biochemistry* **2008,** *46* (3), 356-370.

112. Vogt, T., Phenylpropanoid Biosynthesis. *Molecular Plant* **2010,** *3* (1), 2-20.

113. Zhang, X.; Liu, C.-J., Multifaceted Regulations of Gateway Enzyme Phenylalanine Ammonia-Lyase in the Biosynthesis of Phenylpropanoids. *Molecular Plant* **2015,** *8* (1), 17-27.

114. Fraser, C. M.; Chapple, C., The Phenylpropanoid Pathway in Arabidopsis. *The Arabidopsis Book / American Society of Plant Biologists* **2011,** *9*, e0152.

115. McKegney, G. R.; Butland, S. L.; Theilmann, D.; Ellis, B. E., Expression of poplar phenylalanine ammonia-lyase in insect cell cultures. *Phytochemistry* **1996,** *41* (5), 1259-1263.

116. Maeda, K.; Kimura, S.; Demura, T.; Takeda, J.; Ozeki, Y., DcMYB1 Acts as a Transcriptional Activator of the Carrot Phenylalanine Ammonia-lyase Gene (DcPAL1) in Response to Elicitor Treatment, UV-B Irradiation and the Dilution Effect. *Plant Molecular Biology* **2005,** *59* (5), 739-752.

117. Liu, J.; Osbourn, A.; Ma, P., MYB Transcription Factors as Regulators of Phenylpropanoid Metabolism in Plants. *Molecular Plant* **2015,** *8* (5), 689-708.

118. Aharoni, A.; De Vos, C. H. R.; Wein, M.; Sun, Z.; Greco, R.; Kroon, A.; Mol, J. N. M.; O'Connell, A. P., The strawberry FaMYB1 transcription factor suppresses anthocyanin and flavonol accumulation in transgenic tobacco. *The Plant Journal* **2001,** *28* (3), 319-332.

119. Edwards, K.; Cramer, C. L.; Bolwell, G. P.; Dixon, R. A.; Schuch, W.; Lamb, C. J., Rapid Transient Induction of Phenylalanine Ammonia-Lyase mRNA in Elicitor-Treated Bean Cells. *Proceedings of the National Academy of Sciences of the United States of America* **1985,** *82* (20), 6731-6735.

120. Jian-ye, C.; Li-hong, H.; Yue-ming, J.; Yong, W.; Joyce, D. C.; Zuo-liang, J.; Wang-jin, L., Role of phenylalanine ammonia-lyase in heat pretreatment-induced chilling tolerance in banana fruit. *Physiologia Plantarum* **2008,** *132* (3), 318-328.

121. Xu, F.; Cao, S.; Shi, L.; Chen, W.; Su, X.; Yang, Z., Blue Light Irradiation Affects Anthocyanin Content and Enzyme Activities Involved in Postharvest Strawberry Fruit. *Journal of Agricultural and Food Chemistry* **2014,** *62* (20), 4778-4783.

122. Paul Bolwell, G., A role for phosphorylation in the down-regulation of phenylalanine ammonia-lyase in suspension-cultured cells of french bean. *Phytochemistry* **1992,** *31* (12), 4081-4086.

123. Jeong, M.-J.; Choi, B. S.; Bae, D. W.; Shin, S. C.; Park, S. U.; Lim, H.-S.; Kim, J.; Kim, J. B.; Cho, B.-K.; Bae, H., Differential expression of kenaf phenylalanine ammonia-lyase (PAL) ortholog during developmental stages and in response to abiotic stresses. *Plant OMICS* **2012,** (4), 392.

124. Huang, J.; Gu, M.; Lai, Z.; Fan, B.; Shi, K.; Zhou, Y.-H.; Yu, J.-Q.; Chen, Z., Functional Analysis of the Arabidopsis PAL Gene Family in Plant Growth, Development, and Response to Environmental Stress. *Plant Physiology* **2010,** *153* (4), 1526.

125. Cheng, G. W.; Breen, P. J., Activity of Phenylalanine Ammonia-Lyase (PAL) and Concentrations of Anthocyanins and Phenolics in Developing Strawberry Fruit. *Journal of the American Society for Horticultural Science* **1991,** *116* (5), 865-869.

126. Yadav, S. K., Cold stress tolerance mechanisms in plants. A review. *Agronomy for Sustainable Development* **2010,** *30* (3), 515-527.

127. Hasanuzzaman, M.; Nahar, K.; Alam, M.; Roychowdhury, R.; Fujita, M., Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. *International Journal of Molecular Sciences* **2013,** *14* (5), 9643.

128. Ortega-García, F.; Peragón, J., The response of phenylalanine ammonia-lyase, polyphenol oxidase and phenols to cold stress in the olive tree (Olea europaea L. cv. Picual). *Journal of the Science of Food and Agriculture* **2009,** *89* (9), 1565-1573.

129. Rivero, R. M.; Ruiz, J. M.; García, P. C.; López-Lefebre, L. R.; Sánchez, E.; Romero, L., Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Science* **2001,** *160* (2), 315-321.

130. Zoratti, L.; Karppinen, K.; Luengo Escobar, A.; Häggman, H.; Jaakola, L., Light-controlled flavonoid biosynthesis in fruits. *Frontiers in Plant Science* **2014,** *5*, 534.

131. Miao, L.; Zhang, Y.; Yang, X.; Xiao, J.; Zhang, H.; Zhang, Z.; Wang, Y.; Jiang, G., Colored light-quality selective plastic films affect anthocyanin content, enzyme activities, and the expression of flavonoid genes in strawberry (Fragaria×ananassa) fruit. *Food Chemistry* **2016,** *207*, 93-100.

132. Guo, J.; Wang, M.-H., Ultraviolet A-specific induction of anthocyanin biosynthesis and PAL expression in tomato (Solanum lycopersicum L.). *Plant Growth Regulation* **2010,** *62* (1), 1-8.

133. Teklemariam, T. A.; Blake, T. J., Phenylalanine ammonia-lyase-induced freezing tolerance in jack pine (Pinus banksiana) seedlings treated with low, ambient levels of ultraviolet-B radiation. *Physiologia Plantarum* **2004,** *122* (2), 244-253.

134. Vanitha, S. C.; Niranjana, S. R.; Umesha, S., Role of Phenylalanine Ammonia Lyase and Polyphenol Oxidase in Host Resistance to Bacterial Wilt of Tomato. *Journal of Phytopathology* **2009,** *157* (9), 552-557.

135. Chaman, M. E.; Copaja, S. V.; Argandoña, V. H., Relationships between Salicylic Acid Content, Phenylalanine Ammonia-lyase (PAL) Activity, and Resistance of Barley to Aphid Infestation. *Journal of Agricultural and Food Chemistry* **2003,** *51* (8), 2227-2231.

136. Jukanti, A., *Polyphenol oxidases (PPOs) in plants*. Singapore : Springer, **2017**.

137. Mayer, A. M., Review: Polyphenol oxidases in plants and fungi: Going places? A review. *Phytochemistry* **2006,** *67*, 2318-2331.

138. Wesche-Ebeling, P.; Montgomery, M. W., Strawberry Polyphenoloxidase: Purification and Characterization. *Journal of Food Science* **1990,** *55* (5), 1315-1319.

139. Serradell, M. d. l. A.; Rozenfeld, P. A.; Martínez, G. A.; Civello, P. M.; Chaves, A. R.; Añón, M. C., Polyphenoloxidase activity from strawberry fruit (Fragaria ananassa, Duch., cv Selva): characterisation and partial purification. *Journal of the Science of Food and Agriculture* **2000,** *80* (9), 1421-1427.

140. Friedman, M., Food Browning and Its Prevention: An Overview. *Journal of Agricultural and Food Chemistry* **1996,** *44* (3), 631-653.

141. Shahidi, F.; Naczk, M., *Phenolics in Food and Nutraceuticals*. 2nd ed.; CRC Press: Boca Raton, **2003**.

142. Jukanti, A., Polyphenol Oxidase(s): Importance in Food Industry. In *Polyphenol Oxidases (PPOs) in Plants*, Springer Singapore: Singapore, **2017**; pp 93-106.

143. Terefe, N. S.; Yang, Y. H.; Knoerzer, K.; Buckow, R.; Versteeg, C., High pressure and thermal inactivation kinetics of polyphenol oxidase and peroxidase in strawberry puree. *Innovative Food Science & Emerging Technologies* **2010,** *11* (1), 52-60.

144. Pourcel, L.; Routaboul, J.-M.; Cheynier, V.; Lepiniec, L.; Debeaujon, I., Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Trends in Plant Science* **2007,** *12* (1), 29-36.

145. Terefe, N.; Matthies, K.; Simons, L.; Versteeg, C., Combined high pressure-mild temperature processing for optimal retention of physical and nutritional quality of strawberries (Fragaria×ananassa). *Innovative Food Science & Emerging Technologies* **2009,** *10* (3), 297-307.

146. Chisari, M.; Barbagallo, R. N.; Spagna, G., Characterization of Polyphenol Oxidase and Peroxidase and Influence on Browning of Cold Stored Strawberry Fruit. *Journal of Agricultural and Food Chemistry* **2007,** *55* (9), 3469-3476.

147. Nunes, M. C. N.; Emond, J. P.; Rauth, M.; Dea, S.; Chau, K. V., Environmental conditions encountered during typical consumer retail display affect fruit and vegetable quality and waste. *Postharvest Biology and Technology* **2009,** *51* (2), 232-241.

148. Nunes, M. C. N.; Brecht, J. K.; Sargent, S. A.; Morais, A. M. M. B., Effects of delays to cooling and wrapping on strawberry quality (cv. Sweet Charlie). *Food Control* **1995,** *6* (6), 323-328.

149. Vicente, A. R.; Martínez, G. A.; Chaves, A. R.; Civello, P. M., Effect of heat treatment on strawberry fruit damage and oxidative metabolism during storage. *Postharvest Biology and Technology* **2006,** *40* (2), 116-122.

150. Adyanthaya, I.; Kwon, Y.-I.; Apostolidis, E.; Shetty, K., APPLE POSTHARVEST PRESERVATION IS LINKED TO PHENOLIC CONTENT AND SUPEROXIDE DISMUTASE ACTIVITY. *Journal of Food Biochemistry* **2009,** *33* (4), 535-556.

151. Sanchez-Rangel, J. C.; Benavides, J.; Heredia, J. B.; Cisneros-Zevallos, L.; Jacobo-Velazquez, D. A., The Folin-Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Analytical Methods* **2013,** *5* (21), 5990-5999.

152. Nunes, M. C. N.; Dea, S. In *Bioactive compounds in strawberry fruit exposed to optimum and sub-optimum relative humidity*, International Society for Horticultural Science (ISHS), Leuven, Belgium: **2016**; pp 231-236.

153. Araji, S.; Grammer, T. A.; Gertzen, R.; Anderson, S. D.; Mikulic-Petkovsek, M.; Veberic, R.; Phu, M. L.; Solar, A.; Leslie, C. A.; Dandekar, A. M.; Escobar, M. A., Novel Roles for the Polyphenol Oxidase Enzyme in Secondary Metabolism and the Regulation of Cell Death in Walnut. *Plant Physiology* **2014,** *164* (3), 1191-1203.

154. Yoshida, Y.; Koyama, N.; Tamura, H., Color and anthocyanin composition of strawberry [Fragaria] fruit; changes during fruit development and differences among cultivars, with special reference to the occurrence of pelargonidin 3-malonylglucoside. *Journal of the Japanese Society for Horticultural Science (Japan)* **2002,** (3), 355.

155. Nour, V.; Trandafir, I.; Cosmulescu, S., Antioxidant Compounds, Nutritional Quality and Colour of Two Strawberry Genotypes from Fragaria × Ananassa. *Erwerbs-Obstbau* **2017,** *59* (2), 123-131.

156. Del Pozo-Insfran, D.; Duncan, C. E.; Kristine, C. Y.; Talcott, S. T.; Chandler, C. K., Polyphenolics, ascorbic acid, and soluble solids concentrations of strawberry cultivars and selections grown in a winter annual hill production system. *Journal of the American Society for Horticultural Science* **2006,** *131* (1), 89-96.

157. Määttä-Riihinen, K. R.; Kamal-Eldin, A.; Törrönen, A. R., Identification and Quantification of Phenolic Compounds in Berries of Fragaria and Rubus Species (Family Rosaceae). *Journal of Agricultural and Food Chemistry* **2004,** *52* (20), 6178-6187.

158. Gasperotti, M.; Masuero, D.; Mattivi, F.; Vrhovsek, U., Overall dietary polyphenol intake in a bowl of strawberries: The influence of Fragaria spp. in nutritional studies. *Journal of Functional Foods* **2015,** *18*, 1057-1069.

159. Hernanz, D.; Recamales, Á. F.; Meléndez-Martínez, A. J.; González-Miret, M. L.; Heredia, F. J., Assessment of the Differences in the Phenolic Composition of Five Strawberry Cultivars (Fragaria × ananassa Duch.) Grown in Two Different Soilless Systems. *Journal of Agricultural and Food Chemistry* **2007,** *55* (5), 1846-1852.

160. Timberlake, C. F.; Bridle, P., Distribution of Anthocyanins in Food Plants. In *Anthocyanins As Food Colors*, Academic Press: 1982; pp 125-162.

161. López-Serrano, M.; Barceló, A. R., Histochemical Localization and Developmental Expression of Peroxidase and Polyphenol Oxidase in Strawberries. *Journal of the American Society for Horticultural Science* **2001,** *126* (1), 27-32.

162. Tonnessen, B. W.; Manosalva, P.; Lang, J. M.; Baraoidan, M.; Bordeos, A.; Mauleon, R.; Oard, J.; Hulbert, S.; Leung, H.; Leach, J. E., Rice phenylalanine ammonia-lyase gene OsPAL4 is associated with broad spectrum disease resistance. *Plant Molecular Biology* **2015,** *87* (3), 273-286.

163. Shadle, G. L.; Wesley, S. V.; Korth, K. L.; Chen, F.; Lamb, C.; Dixon, R. A., Phenylpropanoid compounds and disease resistance in transgenic tobacco with altered expression of l-phenylalanine ammonia-lyase. *Phytochemistry* **2003,** *64* (1), 153-161.

164. Way, H. M.; Kazan, K.; Mitter, N.; Goulter, K. C.; Birch, R. G.; Manners, J. M., Constitutive expression of a phenylalanine ammonia-lyase gene from Stylosanthes humilis in transgenic tobacco leads to enhanced disease resistance but impaired plant growth. *Physiological and Molecular Plant Pathology* **2002,** *60* (6), 275-282.

165. Civello, P. M.; Martínez, G. A.; Chaves, A. R.; Añón, M. C., Heat Treatments Delay Ripening and Postharvest Decay of Strawberry Fruit. *Journal of Agricultural and Food Chemistry* **1997,** *45* (12), 4589-4594.

166. Sulaiman, A.; Silva, F. V. M., High pressure processing, thermal processing and freezing of 'Camarosa' strawberry for the inactivation of polyphenoloxidase and control of browning. *Food Control* **2013,** *33* (2), 424-428.

167. Flurkey, W. H.; Jen, J. J., Peroxidase and Polyphenol Oxidase Activities in Developing Peaches. *Journal of Food Science* **1978,** *43* (6), 1826-1828.

168. Nigro, F.; Ippolito, A.; Lattanzio, V.; Di Venere, D.; Salerno, M., EFFECT OF ULTRAVIOLET-C LIGHT ON POSTHARVEST DECAY OF STRAWBERRY. *Journal of Plant Pathology* **2000,** *82* (1), 29-37.

169. Spadoni, A.; Guidarelli, M.; Sanzani, S. M.; Ippolito, A.; Mari, M., Influence of hot water treatment on brown rot of peach and rapid fruit response to heat stress. *Postharvest Biology and Technology* **2014,** *94*, 66-73.

170. Tsao, R.; Yang, R.; Young, J. C.; Zhu, H., Polyphenolic Profiles in Eight Apple Cultivars Using High-Performance Liquid Chromatography (HPLC). *Journal of Agricultural and Food Chemistry* **2003,** *51* (21), 6347-6353.

171. Gil, M. I.; Holcroft, D. M.; Kader, A. A., Changes in Strawberry Anthocyanins and Other Polyphenols in Response to Carbon Dioxide Treatments. *Journal of Agricultural and Food Chemistry* **1997,** *45* (5), 1662-1667.

172. Casañal, A.; Zander, U.; Muñoz, C.; Dupeux, F.; Luque, I.; Botella, M. A.; Schwab, W.; Valpuesta, V.; Marquez, J. A., The Strawberry Pathogenesis-related 10 (PR-10) Fra a Proteins Control Flavonoid Biosynthesis by Binding to Metabolic Intermediates. *Journal of Biological Chemistry* **2013,** *288* (49), 35322-35332.

173. Oszmiański, J.; Wojdyło, A., Comparative study of phenolic content and antioxidant activity of strawberry puree, clear, and cloudy juices. *European Food Research and Technology* **2009,** *228* (4), 623-631.

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

175. Kosar, M.; Kafkas, E.; Paydas, S.; Baser, K. H. C., Phenolic Composition of Strawberry Genotypes at Different Maturation Stages. *Journal of Agricultural and Food Chemistry* **2004,** *52* (6), 1586-1589.

176. Mahmood, T.; Anwar, F.; Abbas, M.; Saari, N., Effect of Maturity on Phenolics (Phenolic Acids and Flavonoids) Profile of Strawberry Cultivars and Mulberry Species from Pakistan. *International Journal of Molecular Sciences* **2012,** *13* (4), 4591.

177. da Silva Pinto, M.; Lajolo, F. M.; Genovese, M. I., Bioactive compounds and quantification of total ellagic acid in strawberries (Fragaria x ananassa Duch.). *Food Chemistry* **2008,** *107* (4), 1629-1635.