March 2019

Biochemical and Proteomic Approaches to Determine the Impact Level of Each Step of the Supply Chain on Tomato Fruit Quality

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Biochemical and Proteomic Approaches to Determine the Impact Level of Each Step of the Supply Chain on Tomato Fruit Quality

by

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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

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Date of Approval:
March 07, 2019

Keywords: tomato, supply chain, abiotic stress, proteomics, carotenoids, ascorbic acid, sugars

DEDICATION

This thesis is dedicated to my family, especially my parents Penny and Dan Madden. My father is currently getting his Ph.D. and knew exactly what I was going through ever step of the way, from the stress of lab work to writing. To my mother for always making sure I stayed healthy and didn't always eat junk food. I would also like to dedicate this thesis to the rest of my family, and to my cousins for always asking how my citrus research is going, hopefully once they read this they will finally ask the right question. Finally, I would like to thank Jasmin D'Andrea for all the love in the world and all help she gave me when I came home late at night. I am certain this could not have been done without you and I hope that I can help you a tenth as much as you helped me when you write your thesis.

ACKNOWLEDGEMENTS

First, I would like to acknowledge Dr. Cecilia Nunes. I sent an application to USF not knowing how much it would change the course of my life. She has given me a concrete path forward and I never felt so sure about the future of my life as I do leaving this lab. All the skills that she has taught me both in and outside of the lab I will carry with me for the rest of my life and I cannot truly thank her for all of the hard work she has done helping me. I would also like to acknowledge my committee members Dr. Brant Burkhardt for helping me focus my energy more onto proteomics. Dr. Sandy Westerheide for joining my committee to replace Dr. Stanley Stevens moving to Vermont. I would like to thank Dr. Dale Chaput for helping me navigate the topic of proteomics, an area of expertise that was completely new to me. I would like to acknowledge my lab members Katrina Kelly for showing me how the lab was run and almost every technique that I have used here came from her knowledge and Alyssa Smith for spending the late nights with me extracting proteins for a tomato and taking over the lab responsibilities as I wrote my thesis. I would also like to thank Michelle Stein, Justin Engel, Kanika Saxena, Brittany Dudley, Oliva Curran, and Haley Inselberg.

Table of Contents

List of Tables

List of Figures

ABSTRACT

Fresh fruits and vegetables (FFVs) are the most frequently wasted foods because of their perishability and handling requirements. However, there is a lack of information on how much each step of the supply chain impacts FFVs quality, particularly on tomatoes, and what measures need to be taken for an immediate and effective impact on waste reduction. There is also no information on how the supply chain affects the proteome of the tomato and what proteins are differentially regulated by the most impactful steps of the supply chain. The objectives of the work presented on this thesis were to evaluate the decline in the overall quality and quantify tomato waste at each step of the supply chain, from the farm to consumer; and to determine what proteins are impacted by the decline in quality that is associated with temperature abuse. To determine overall quality and tomato waste, light-red tomatoes were exposed to an optimum temperature (13 °C) and eighteen different time-temperature scenarios, normally encountered during supply chain, and sensory and physicochemical attributes measured at each step. To determine the impact of chilling and non-chilling temperatures normally encountered during tomato supply chain, on the proteome, light-red tomatoes were exposed to an optimum temperature (13 °C) and to two time and temperature supply chain scenarios (2 °C and 25 °C) that showed the most negative impact on tomato overall quality, and physicochemical and proteomic attributes were measured at each step. For the first tomato harvest, the steps with the highest impact on quality and waste were shipping to distribution center (DC; 20°C), cooling at the grower (25°C) and storing at the consumer (4°C). For the second tomato harvest, shipping to the store (2°C), cooling at the farm (10°C) and displaying at the store (20°C) negatively impacted quality. High temperatures during cooling, shipping and

store display impacted sensory quality and resulted in increased weight loss, and decreased sugar, carotenoids, and ascorbic acid contents. Although low temperatures during shipping, cooling and consumer did not impact tomato sensory quality, they contributed to a decline in sugar, carotenoids and ascorbic acid contents. Overall, the most impactful steps on tomato quality and waste, regardless of the temperature, were shipping to DC, cooling, shipping to stores, displaying at the store, and consumer storage. Analysis of the differentially expressed proteins in the tomato showed that metabolic proteins were greatly impacted by temperature abuses such as phosphomannomutase, heme oxygenase 1, and MAP kinase; and that proteins regulating cellular membrane integrity such as vacuolar protein sorting-associated protein were also impacted.

CHAPTER ONE:

INTRODUCTION

Tomato fruit is culturally and economically a relevant crop that is seeing growth in its consumption across the United States (Gunders, 2012). In the United States, a focus on healthy dieting and cultural diversity has led to a large increase in the consumption of fresh fruit and vegetables (FFV) and the tomato is no exception (Lucier et al., 2006). Tomatoes are grown around the United States with hydroponics and green house predominantly in the north, however, the two largest growers in the U.S. are California and Florida. In the state of Florida, most tomatoes are grown to be served fresh rather than processed such as the ones grown in California. Due to Florida's geographic location, the farmers can grow out of season from the rest of the country and provide for most of the tomatoes sold nationwide off season. The biggest competitor to Florida grown tomatoes is Mexico that can out compete locally grown tomatoes due to lower production costs (USDA, 2018).

Florida produces tomatoes for both the fresh and processed industry but is mainly focused on growing table fresh tomatoes. Florida has been the second largest producer of fresh tomatoes since 1980 and contributes up to one-third of all tomato crop grown in the United States (USDA, 2018). The state of Florida alone receives $453 million in revenue from fresh tomatoes annually (USDA, 2018). The tomato is the fourth most consumed fresh produce in the United States and consumption has been steadily on the rise (Lucier et al., 2006).

In the process of getting tomatoes from farm to table, there are damages that inevitably occur as a regular part of the food supply chain (Kader et al., 1978). These can range from

minor bruising to serious temperature abuses that both result in lower quality fruit that is available to the consumer or may eventually result in tomato to be wasted at different steps along the supply chain. On average, 15 % of tomatoes are lost before they are made available to purchase for the consumer (Mena et al., 2014). The loss of quality occurs due to damages acquired in the supply chain (Mena et al., 2014). Briefly, the supply chain consists of seven standard steps, starting when the farmer picks the fruit off the plant and sends it to the farm storehouse for grading, sorting, packaging and cooling. After that the tomato fruit is shipped to the distribution center of local super markets and from there is shipped and displayed at the store. At the store the consumer will be able to buy tomatoes with purchasing decision being driven mostly by quality appearance (Bubzy et al., 2017).

Tomato fruit quality is based on the physical and chemical characteristics of the fruit. The physical characteristics are quality attributes that consumers can perceive at the supermarket such as color, any blemishes or damage on the skin, size of the fruit. The chemical qualities are underlying features of the fruit that consumers cannot see at the supermarket but will taste at home. Attributes such as sugars, ascorbic acid (Vitamin C), and the level of other antioxidants such as carotenoids make up the chemical and nutritional attributes that are important for the consumer (Oltman et al., 2014).

Florida contributes up to 40% of all tomatoes grown in the United States and as such, it is critical to maintain the postharvest quality for as long as possible to ensure that minimal amounts of fruit will be wasted (Parfit et al., 2010). Even though the supply chain results in a waste of 15% of the tomatoes that are sold, there is little information as to which step in the supply chain results in the largest loss of quality and how each step impacts tomato fruit quality (Mena et al., 2014). Therefore, the objectives of the work presented in this thesis were 1) to identify which steps along the tomato supply chain have the greatest impact on the quality of the fruit using traditional physical and chemical quality markers, 2) to use biochemical approaches to determine the impact of chilling and non-chilling temperatures encountered during supply

chain on tomato quality, and 3) measure the impact of two stress temperatures on tomato specific chemical attributes (i.e., vitamin C, carotene and sugars) and key proteins, using biochemical and proteomic approaches. Results from this study will provide insight on how the tomato overall quality is impacted by conditions encountered during supply chain and how the proteome is impacted by post-harvest temperature stress.

CHAPTER TWO:

REVIEW OF LITERATURE

History and Origin

Cultivated tomatoes have wild relatives that are native to western South America. From the coast to the high Andes, they can be found growing in almost all countries in South America that border the Pacific Ocean (Muler, 1940). The ancestor to today's modern tomato cultivars is most likely a wild cherry tomato (Rick and Holle, 1990). This variety has more recently been found in a larger distribution area going as far north as Mexico and as far south as Peru. Wild tomatoes grow in a wide habitat range from arid coastal lowlands to mesic upland in the Andes Mountains (Bergougnoux, 2011). They typically occupy valleys that drain into the Pacific and are characterized by their geographic isolation. These wild tomatoes are well adapted to the microclimates, the altitude, and the soil conditions present in each of the different valleys. This climate differences lead to a large diversity of wild tomatoes cultivars (Warnock, 1988). Wild tomatoes are perennial herbaceous plants, however, can be considered annuals as they often die due to frost or drought after the first growing season.

The origin of the domestication of the tomato has two prominent theories put forth. One theory states that the original domesticated tomato was developed in Peru and the other theory that tomato was originally domesticated in Mexico. The Peruvian defense is justified by DeCandolle (1886) botanical records which indicate that tomatoes have never been found outside of the Americas before initial domestication (Bauhin, 1623) and, historical evidence that the tomato was found mostly in Peru before being transported to Mexico (Harnadez, 1651). The hypothesis that tomato was originally domesticated in Mexico was first described by Jenkins (1948).

Jenkins found that before 1651 the terminology used for tomatoes was often confused for another plant like the original tomato plant but bore no fruit. This discrepancy on what was actually a tomato makes any of the records unclear before then and, because DeCandolle (1886) relied so heavily on these records, his argument that tomatoes are Peruvian in origin is weak casting doubt. Jenkins also argued that there was considerably more variation in tomato cultivars in Mexico than in Peru. Jenkins states that the tomato was transported to Mexico during pre-Columbian times and was a secondary center of diversity for the plant.

The history of the tomato in Europe is also unclear until late into the 16th century. The earliest known herbarium samples come from Italy, sometime between 1550 and 1560 (Jerna, 1947). The first recognized use of tomatoes in culinary occurred in Southern Europe during the 17th century (Ray, 1973). Reports indicated that in Italy there were three tomato varieties and instructions were given on how to cultivate them (Filippo, 1814). Tomato cultivation was not difficult however, there were never given a large economic importance until breeding programs were established in the late 19th century (Rick, 1973).

Today, tomatoes rank fourth in the world among vegetables with the top producing countries being the United States, China, India, Italy, Spain, Brazil, Mexico, Greece, and Chile. Over the past twenty years there has been an upward trend in tomato production around the world (Razdan, 2006). In the United States, tomatoes are ranked as the second leading vegetable with an annual economic value of 1.2 billion dollars. Florida and California are the two dominant states leading national production and sales. Florida alone accounts for 40% of all fresh market tomatoes grown in the United States (USDA, 2015). Since 1920, increase demand for tomato has been growing steadily. This increase can be attributed to population growth and changes in diets looking to increase the consumption of fresh fruits and vegetables (Razdan, 2006).

Morphology and Physiology

From a culinary standpoint tomatoes are considered a vegetable rather than a fruit. In 1893, a court case discussed the issue and ruled that the tomato is a vegetable and that to treat it differently would go against the rules of society and taxed it as such (Marmor, 2013). So despite the tomato being considered a vegetable, being taxed as a vegetable, and being added to meals as one, it is in fact a fruit. Botanically speaking, tomato fruit is classified as a berry because it develops from the ovary of the plant after the flower has been pollinated (Rick, 1990).

Tomatoes belong to a genus *Solanum* making them related to the nightshade plants. The plant is dicot, meaning that it grows as a series of branching stems with a terminal bud where all growth occurs. The flowers of the tomato plant appear on the apical meristem, have fused anthers which form a column around the pistil's style. This is important to note as, the major breakthrough of tomato domestication, was when breeding lead to changes in the position of the stigma from the anther tube (Rick, 1990).

The tomato is comprised of several tissue zones: the epidermis, the locular cavities, the placental tissue, the columella, and the pericarp (Figure 1). The fleshy part of the tomato is comprised of the pericarp and the placental tissue. The locular cavity of a tomato comprises a gelatinous membrane containing the seeds of the fruit (Razdan, 2006). The function of the locules is to hold the seeds. Surrounding the locular cavity is the pericarp which contains vascular bundles that control the flow of nutrients in the tomato fruit when still attached to the plant. Tomatoes can either be bilocular or multilocular. Most cultivars of tomatoes have around 4-5 locules with the exception of cherry and grape tomatoes, which are usually bilocular (Razdan, 2006).

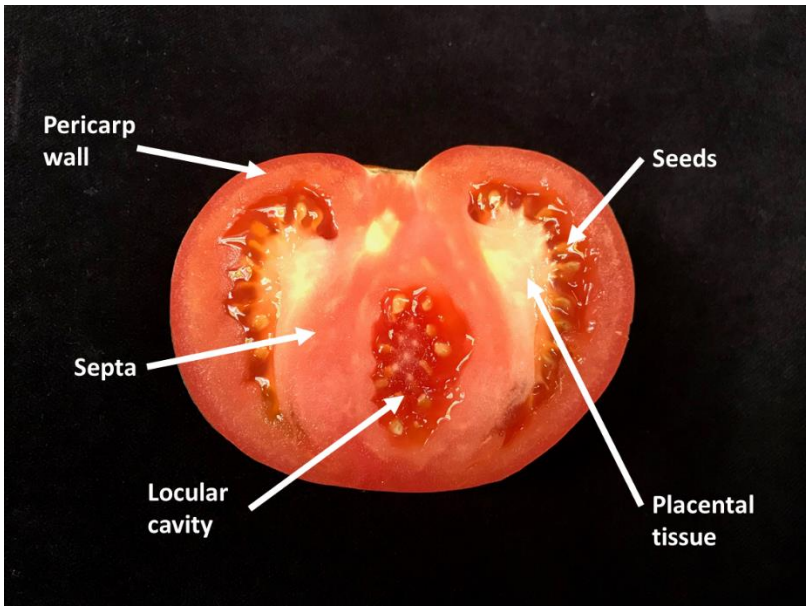

Figure 1: Internal anatomy of a tomato fruit.

Quality Attributes

The overall quality of tomato fruit is based on the sensory attributes (i.e., size, shape, color, texture, and flavor) and chemical profile (i.e., sugars, acids, bioactive compounds). Tomato appearance is a combination of factors such as color, and skin glossiness and smoothness. Consumer's surveys showed that a good quality tomato is one that is firm to the touch, dark-red like in color, and perfectly round in shape (Oltman et al., 2014). Consumers felt that these attributes reflect the freshness of the tomato and play an important role in purchase decision. In addition, these quality attributes are strongly related to the major chemicals compounds measured in the fruit such as sugars, organic acids, vitamins (i.e., ascorbic acid), and carotenoids (i.e., lycopene).

Appearance

Tomato appearance is an important quality attribute as, from a consumer's perspective, it relates to overall quality and freshness (Wolters and Gemert, 1970). Tomato external appearance correlates positively with the internal quality of the fruit (Wismer, 2014). Consumer preference is for a tomato with high quality appearance because external traits can be easily

measured at the supermarket. Since consumers cannot taste test tomatoes at the supermarket they must rely on external appearances to judge overall quality.

As a tomato matures, it undergoes distinct stages of ripeness, from immature green showing a light green coloration to mature green showing a deeper green coloration, followed by a breaker stage of ripeness. At the breaker stage, the chlorophyll starts to break down and synthesis of carotenoids begins resulting in a tomato with a combination of green and red coloration. Afterward, the tomato will enter a turning stage of maturity where there is still enough chlorophyll to impact tomato color. Following the turning stage, the tomato enters the pink stage of maturity where there is no remaining green coloration. After that, carotenoid synthesis increases and the tomato will transition from a light red color, to a red color, before finally reaching its final stage of maturity of fully ripe red (Arias et al., 2000). At this point, the tomato is fully red and is most desired by consumers. After the fully ripe red stage, the fruit will shortly start to decay in quality and begin to turn to a deep red color and can appear almost purple, a color undesired by consumers.

The characteristic red color of tomatoes is derived from the balance between the major pigment carotenoids and chlorophylls (Nisar et al., 2015). The pigment carotenoid with the biggest impact on tomato color is lycopene and comprises up to 88% of total carotene content (Fattore et al., 2016). Chlorophyll plays a role in tomato color by giving the fruit a green coloration when still immature. As the tomato develops and starts to ripen, chlorophyll breaks down and develops into carotenoids giving the tomato a distinctive red coloring. This process is highly dependent on environmental temperature, with dramatic changes in temperature resulting in tomato having abnormal levels of carotenoids. Higher temperatures are associated with redder color whereas lower temperatures are responsible for a green colored tomato. Carotenoids are found in all tissues of the tomato however, 50% are concentrated in the epidermis of the fruit (Toor and Savage, 2006). Concentration of carotenes in tomato skin is an important indicator of fruit ripeness with higher concentrations resulting in tomatoes with a deep

red color that is desired by consumers (Oliveria et al., 2010). This occurs due to increased levels of carotenes and decreased levels of chlorophyll as the fruit ripens (Fiedor, 2014). Tomatoes of different cultivars can have different shades of red due to varying levels of carotenes the fruit develops during ripening. The tomato will continue ripening and synthesis of carotenoids will progress until the point the fruit becomes overripe. The color at this stage of maturity is characterized by an undesirable red coloration, which can be described as dark red almost purple. This is a consequence of carotenoids breakdown and may be caused by many factors including temperature abuses, improper lighting, pH, and other chemical imbalances in the fruit. Carotenoid stability is also dependent on the pH of the soil and the temperature in which the tomato grows (Lopez et al., 2004).

Texture and Flavor

Tomato texture is determined by the ripeness of the fruit (Lunn et al., 2013). When the fruit is ripe, intact cells maintain a strong cellular wall, which allow for resistance against pressure, resulting in a firm fruit. Tomato softening results from a variety of factors; however, the most prominent cause seems to be related with cell wall modifying proteins, which are a complex web of enzymes associated with tomato softening (Lunn et al., 2013). In tomato specifically, polygalacturanase and expansin are the two primary enzymes responsible for cell wall degradation. These enzymes are upregulated as the fruit matures and when it reaches a stage of over ripeness, the fruit itself will become undesirably soft (Parfitt et al., 2010).

Characteristic tomato flavor is highly responsible for the popularity of the fruit worldwide. Sweetness is a desired trait but many people would describe tomatoes as having an umami or savory flavor (Hajeb and Jinap, 2013). The main chemical components responsible for tomato flavor are the sugars fructose, glucose, and organic acids such as citrate, malate, glutamate, and a mixture of volatile compounds (Tieman et al., 2012). Consumer preference is for a tomato that is sweet with a touch of acidity, meaning that the sugar/acid ratio of the fruit is important to consumers as it has the largest impact on the desired flavor traits. Tomato flavor is highly

dependent on the balance between sugars and acids as an increase in their levels results in an increase of perceived flavor (Kader et al., 1977).

Major Chemical Components

Sugar Content

Fructose and glucose are the two most abundant sugars in tomato, and, as mentioned in the previous sections, have the largest contribution towards flavor and sweetness. Fructose is the major sugar in tomato fruit followed by glucose. The total sugar content varies between cultivars and ripeness stages, with average levels ranging from 2 to 4% (Gautier et al., 2008; USDA, 2018). Tomato sugars are highly dependent on the ripening stage of the tomato; green tomatoes have low sugar content whereas high levels of sugar are associated with ripe red tomatoes (Gautier et al., 2008). At the green stage, sugars are mostly stored in the flesh of the tomato, with roughly 25% stored in the locules, but as the fruit ripens, it increases to 33%. The change in the morphological distribution of sugars is driven by a variation in fructose levels with glucose levels changing little at this time. As the tomato ripens, the levels of sugars increase by 0.5% as the tomatoes transitions from mature green to breaker to pink to red. Fructose and glucose levels in tomatoes vary depending on the cultivar but the average fructose value is 1.4 g / 100 g of tomato and glucose 1.5 g / 100 g (Beckles, 2012). Sucrose levels are especially low in tomatoes to the point that in most cultivars they are negligible or null due to this sugar being quickly hydrolyzed into fructose and glucose (Beckles, 2012).

In tomatoes, synthesis and flow of sugar is regulated by a set of genes known as Brix9-2-5 that encode the functional amino acid polymorphism of cell wall invertase. Cell wall invertase controls the flow of sugar into the cells by hydrolyzing sucrose into hexose in the apoplast, the region outside of the plasma membrane where materials are free to diffuse (Gear et al., 2000). However, it is important to note that this process and the physiological mechanisms are poorly understood (Kanayama, 2017).

Sucrose phosphosynthase and invertase are also important regulatory enzymes that control sugar levels. These enzymes control the flux of sucrose from the main body of the plant and the conversion into fructose and glucose (Yin et al., 2009). Sugar levels control ADP-glucose pyrophosphorylase (AGPase) encoding genes by helping create a promoter that binds to the gene sequence. AGPase controls starch biosynthesis during early stages of fruit development allowing for the accumulation of sugar for later consumption and use in metabolic processes during ripening (Preiss, 1988; Yin et al,. 2009). Fructose and glucose have many roles in the growing and ripening of tomato fruit and act in the initial oxidative step of glycolysis, the synthesis of pyruvate (Missio et al., 2015). Glucose to fructose ratios differ due to higher consumption of glucose during different metabolic pathways. (Patching et al., 1975)

Ascorbic Acid Content

Ascorbic acid (vitamin C) is a water-soluble compound with vitamin properties and is considered an essential micronutrient because humans lack the enzyme gulonolactone oxidase and cannot synthesize their own vitamin C. Ascorbic acid is an important cofactor for post-translational hydroxylation of collagen, in the biosynthesis of carnitine, is important for neuron health (Gropper et al., 2012) and it is also an important regulator of iron uptake. Lack of vitamin C can lead to scurvy and ultimately to death (Carr and Lykkesfeldt, 2018). Chemically, ascorbic acid is considered a reductone, which due to their chemical nature, are powerful antioxidants (Duarte et al., 2005). Therefore, ascorbic acid can easily donate two electrons to form dehydroascorbic acid and reacts with reactive oxygen species (ROS) protecting nucleic acids. The oxidized form of ascorbate is relative unreactive however the presence of free metal ions can trigger pro-oxidative compound formation (Wheeler et al., 1998).

Synthesis of ascorbic acid can be achieved via two pathways known as main and alternative pathways. In the main pathway, ascorbic acid is generated from the conversion of glucose into different sugars with the final resulting product being ascorbate. In this pathway, D-glucose-6-P is converted to D-fructose-6-P by the action of phosphoglucose isomerase (PGI)

(Tsuge et al., 2004). PGI functions by protonating the C5 oxygen group and deprotonating the C1 hydroxyl group, allowing the opening of the glucose ring at the C2 position. Once opened, PGI deprotonates the C2 allowing for PGI to donate a proton to C1 and then deprotonate C2 to form fructose. Finally, PGI closes the ring by forcing a rotation at the C3-C4 bond and deprotonates the C5 hydroxyl group (Read et al., 2001). D-fructose-6-P is then converted to D-mannose-6-P by the action of phosphomannose isomerase (Gao et al., 2005). This reaction is accomplished by adding a proton to the C1 oxygen group and removing a proton from C1. This allows for the fructose ring to open where two protons are added, one to C2 and one to Oxygen 1 (Gao et al., 2005). These chemical changes allow for the ring to re-form with a mannose structure, synthesizing D-mannose-6-P. Phosphomannose mutase (PMM) then binds D-mannose-6-P to convert it to D-mannose-1-P. D-mannose-1-P is next converted to GDP-D-mannose by the enzyme GDP mannose pyrophosphorylase (GMPase). GMPase acts by binding a GTP to the sugar group at the first carbon (Wolucka et al., 2001). GDP-D-mannose is then converted into GDP-L-galactose by the enzyme GDP mannose-3, 5-epimerase (GME) (Wolucka et al., 2001). GME functions by cleaving the alcohol group on C5 and flipping the chirality of the hydroxyl group on C3 (Loannidi et al., 2009). At this point the GDP-L-galactose could alternately be used in a pathway that helps maintain cell wall structure by acting as a precursor for cell wall polysaccharides (Loannidi et al., 2009). However, this is a very uncommon pathway, with studies showing that GDP-L-galactose is present only in minimal levels in plant cell walls (Wheeler et al., 1998). Therefore, GDP-L-galactose is mostly used in the ascorbate pathway, binding to the enzyme GDP-L- galactose phosphorylase (GGP) and converting it to L-galactose-1-P. The function of GGP is to attach a phosphate group onto the first carbon in order the reaction to proceed (Linster et al., 2008). L-galactose-1-P is then converted into L-galactose by the enzyme galactose - 1-P phosphatase (GPP) cleaving off the phosphate group (Ioannidi et al., 2009). The final step of the main ascorbate pathway is the conversion of L-galactone-1, 4-lactone into ascorbate. This is performed by the enzyme L-

galactono-1,4-lactone dehydrogenase which uses and oxidized PMS and reduces it to remove a proton and form a double bond in the galactone sugar ring (Schertl et al., 2012) which is then converted into L-galactone1,4-lactone and finally into ascorbate (Figure 2).

An alternative pathway involves the conversion of pectin into L-galactone1, 4-lactone or D-glucuronate into ascorbate. This pathway is used not only to synthesize ascorbic acid but the first part of the pathway is also used to provide precursors for the synthesis of polysaccharides that contain mannose, L-galactose, and L-fructose. The first part of the pathway is defined as the conversion of the sugars into L-galactose. Once this step occurs, the pathway is committed to the synthesis of ascorbate (Hancock and Viola, 2005). All steps of the ascorbate pathway, both main and alternative, are thought to take place in the cytoplasm, except for the conversation of L-galactone1,4-lactone which takes place on the inner mitochondrial membrane (Loannidi et al., 2009).

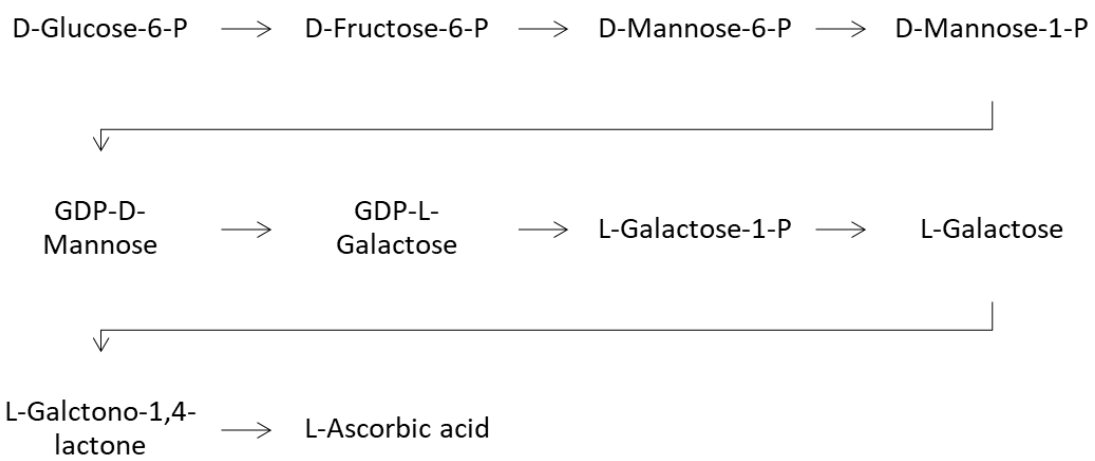

Figure 2: Ascorbic acid pathway (Pallanca and Smirnoff,1999).

There are 13 genes directly involved in the synthesis of ascorbate (Wheeler et al., 1998). These genes are associated with chromosomes 2, 8, 9, 10, and 12, with special emphasis on chromosome 9. The most important genes are a monodehydroascorbate reductase and a GDP-mannose epimerase both located on chromosome 9. These genes are responsible for the regulation of ascorbic acid precursors and, depending on their upregulation, have a direct influence on the levels of ascorbic acid (Stevens et al., 2007). In tomato fruit, the active genes in the first half of the main pathway of ascorbic acid synthesis are upregulated until the breaker stage of maturity. Once enough GDP-L-galactose is converted to ascorbate, these genes will be down-regulated and thus less active. Interestingly, the genes involved in the alternative pathway follow a similar pattern and become down-regulated before tomatoes reach the pink stage of maturity. The genes responsible for the conversion of GDP-L-galactose into ascorbate are up-regulated throughout tomato ripening and maintain high levels of RNA transcripts until the red ripe maturity stage (Ishikawa et al., 2006).

Carotenoids

Carotenoids are a diverse class of pigments found in tomato and are responsible for giving the fruit their characteristic red color. Carotenoids can be divided into two classes, xanthophylls and carotenes. Xanthophylls contain an oxygen group while carotenes do not contain oxygen in their chemical structure. In tomatoes, carotenes are the most common pigments, and among them, lycopene which comprises a large percentage of the total carotenes.

The carotenoid synthetic pathway, known as the 1-deoxy-D-xylulose 5-phosphate (DOXP) biosynthetic pathway, starts with the reaction of pyruvate with glyceraldehyde 3-phosphate dehydrogenase catalyzed by the enzyme DOXP synthase forming 1-deoxy-d-xylulose-5-phosphate (DOXY). In the following step, NADPH is added to DOXY to form 2-C-

methyl-D-erythritol-4-phosphate (MEP), which then binds to cytidylyl transfer phosphatase forming 4-diphosphocytidyl-2C-methylerythritol (CDP MEP), allowing ATP to bind and convert to 4-diphosphocytidyl-2C-methylerythritol 2-phosphate (CDP ME-2-P). CDP ME-2-P is then converted into 2C-methyl-D-erythritol 2, 4-cyclodiphosphate (MECDP) and then into dimethylallyl diphosphate (DMAPP). The final step of the DOXY pathway involves the conversion of DMAPP into isopentenyl diphosphate (IPP) by the enzyme IPP isomerase (Schwender et al., 1996; Lichtenthaler, 1999; Bramley, 2003).

IPP is the subtract to the next pathway, the isoprenoid biosynthetic pathway. This pathway is responsible for creating C_{20} geranylgeranyl diphosphate (GGPP). IPP bounds to other IPP molecules to become a 20-carbon molecule to form GGPP. GGPP will then bind to the enzyme phytoene synthase twice to become phytoene which is the first carotene product. Phytoene desaturase will then convert phytoene to phytofluene and then to ζ-carotene. ζ-carotene desaturase similarly acts on ζ-carotene and converts ζ-carotene into neurosporene and then into lycopene (Breitenbach et al., 2005; Isaacson et al., 2002). GGPP is also a precursor molecule and regulator for many classes of proteins. For example, GGPP regulate ubiquinones, tocopherols, polyterpenes, gibberellins, and plastoquinone (Joyard et al., 2009).

Lycopene is the most abundant carotenoid and constitutes up to 88% of all carotenoids found in the tomato fruit. However, lycopene is also a precursor for the cyclical α-carotene, ε-carotene, and β-carotene. Of these carotenes, β-carotene is the most common, making up to 5% of the total carotenoid content. The cyclization process of lycopene results in the synthesis of a set of carotenoids that are either α-carotene or ε-carotene. Lycopene β-cyclase (LCY-B) synthesizes β-carotene while lycopene ε-cyclase (LCY-E) synthesizes ε-carotene. The intermediates for α-carotene and ε-carotene are δ-carotene and γ-carotene respectively. Both can be converted into α-carotene by lycopene β-cyclase (Ronen, 2000).

Carotenoids play a diverse roll in plant metabolism. In the chloroplast, they participate in light harvesting activities while also protecting the chloroplasts from excessive light energy. This is due to the inevitable accumulation of reactive oxygen species (ROS) that are generated during photosynthesis. Carotenoids act as antioxidant agents by actively binding and quenching ROS. They can also indirectly help by acting as a signaling molecules when activated by ROS (Shumbe et al., 2016).

In human health, carotenoids are also important bioactive molecules and precursors of vitamin A. The antioxidant effect of carotenoids is well known as ROS sequesters, and in human health, ROS have been associated with DNA damage and aging. Studies have shown a correlation between diets rich in antioxidant foods and lower cancer rates (Engelmann, 2011; Fiedor and Burda, 2014; Maria et al., 2015; Fattore et al., 2016). Carotenoids also play a role in maintaining cardiovascular health via oxidation and elimination of low-density lipoproteins, which are found in the blood vessels and lead to the development of atherosclerotic lesions. Carotenoids may also help reducing pro-inflammatory cytokines as well as help with the delivery of insulin to muscles and the liver. In addition, carotenoids are also an important precursor to vitamin A which is important for growth, the immune system, and vision (Engelmann, 2011).

Impact of Postharvest Conditions on Tomato Quality

Postharvest handling and storage conditions have a direct and significant impact on the quality of tomato fruit. Inadequate storage conditions throughout the supply chain can cause tomato fruit to lose quality and spoil prematurely, ultimately resulting in waste because consumers will not purchase the fruit (Kader et al., 1978). Thus, maintain a proper temperature throughout the supply chain from the field to the consumer is paramount in maintaining tomato quality. Tomato storage temperature is dependent on ripening stage of the fruit but red ripe tomatoes should be optimally stored at 13 °C (Kader, 1984). Mature green tomatoes should be handled between 12.5 to 15 °C, light red tomatoes between 10 to 12.5 °C, and firm ripe stored between 7 and 10 °C (Cantwell, 2009). These ranges of temperature are considered ideal for

maintaining tomato overall quality, preventing fruit senescence and development of chilling injury (CI) symptoms. Other contributing factors for ideal tomato storage is the relative humidity of the surrounding environment, which should be maintained at 90% (Kader, 1984).

Temperature

Temperature abuses during tomato supply chain from the field to consumer inevitably occur and contribute to premature deterioration of overall quality, reduced shelf life and increased waste. For example, if temperature is kept too high (i.e., above 20 °C) surface color will not develop properly as there will be uneven ripening that will ultimately reduce the quality and salability of tomato fruit (Porat et al., 2017). The severity of the symptoms related to exposure to high temperatures are directly related to time and temperature exposure, with higher temperatures causing accelerated senescence and faster decline in tomato quality. Exposure to high temperature results mostly in uneven ripening and blotchy appearance. Other effects of exposure to high temperatures are lower levels of sugars, carotenoids, ascorbic acid, and several volatiles that affect flavor quality (Kader et al., 1984).

Exposure to low temperatures also result in decreased overall tomato quality and are notable for having a large impact on chemical compounds (Aghdam, 2013). Exposure of tomato fruit to temperature below 13 °C, results in chilling injury (CI) (Li et al., 2016). As with high temperature, the symptoms of CI will be more severe the longer and the lower the exposure temperature. Exposure to lower temperatures require shorter exposure times before symptoms develop (Aghdam et al., 2012) and symptoms vary between tomato cultivars and maturity stage of the fruit. For example, mature green tomatoes usually develop CI symptoms faster and with higher degree of severity. As tomato fruit matures, resistance to CI increases with red ripe fruit being much less sensitive to exposure to chilling temperature than mature green tomatoes. Chilling injury greatly affects ripening of the tomato fruit, but if the tomato is already ripe, there will be less of an impact on the overall quality (Saltveit, 1992).

Mature green tomatoes stored at 4 °C start decaying after only 15 days of storage while red color development was irreversible impaired and inhibited after 34 days of cold storage (Cheng et al., 1988). Besides symptoms associated with discoloration, tomatoes also experience other external quality deteriorations. For example, depending on the temperature and exposure time, tomato skin may show shows pitting and shriveling and, water soaking damage may also occur due to breakdown of cell wall membranes and leakage of cell contents (e.g., water and electrolytes) (Hobson, 1993). Tomato metabolism also increases due to CI, resulting in increased rates of respiration, ethylene production, and solute leakage into tissues (Saltveit, 2001; Luengwilai et al., 2012). The occurrence of CI can be explained by loss of membrane integrity and an increase in membrane permeability (Saltveit, 1992). The loss of membrane stability is the direct cause of tissue pitting and water-soaking damage observed when tomato fruit is exposed to chilling temperatures. Another significant symptom of CI is uneven ripening during maturation. Typically, it takes a short amount of time for the fruit to change from one ripening stage to the next (e.g., from light pink to red). However, when under chilling stress, each ripening stage will be delayed and ripening phase is arrested. The tomato fruit will fail to mature and attain the full ripe red stage. Because color development is dependent on carotenoid synthesis, the impact of CI on carotenoid levels will vary depending on the maturity stage. However, the overall effect is that there will be delayed carotenoid synthesis and consequently lower levels of carotenoids than expected. If the fruit is removed for chilling conditions and placed at a higher temperature, then the tomato may resume ripening, however, it will still retain large amounts of chlorophyll and remain splotchy in appearance.

Humidity

Impact of surrounding relative humidity is not as detrimental to tomato quality as temperature, nonetheless it is also an important factor to take into consideration during handling and storage. During tomato handling and storage, relative humidity (RH) should be kept at approximately 90% as lower levels have been shown to increase water loss (Paull, 1999). By

maintaining high RH levels in the surrounding environment, water is less likely to evaporate and pull moisture out of the tomato fruit.

The maximum acceptable level of water loss before a tomato is considered unacceptable for sale due to compromised quality was establish between 1% and 2% (Robinson et al., 1975; Hruschka, 1977; Nunes et al., 2007). At low temperatures, weight loss is considerably lower in tomatoes regardless of cultivar. For example, tomatoes that have been stored at temperatures below 15 °C with 90% RH lost less than 1% of their weight (Proulx et al., 2001) whereas tomatoes stored at 20 °C with a 65% RH lost a total of 15% of their weight after storage period of 12 days. Tomatoes stored at 2 °C for the same amount of time lost only 2.3% of the total weight (Syamal, 1989).

Tomato Supply Chain and its Significance

Supply chains are a fundamental part of the food distribution systems, which bring foods from the farm or processing plants to the consumers' tables. However, abuse conditions during handing, shipping and distribution inevitable occur with tomatoes experiencing fluctuations in temperature and humidity conditions throughout the supply chain. Tomatoes, as most produce, must go through a supply chain to get from the farm to the consumer. This is a lengthy process and loss of quality can occur at different steps throughout the supply chain. Mechanical and physiological damage can occur during handling and transportation leading to loss of quality and if there is severe enough damage the fruit can be wasted and never consumer (Hall et al., 2006). For example, growers can leave the fruit on the field too long in the sun after harvesting, when shipped from the farm to the distribution center and from there to the stores, tomatoes can also experience temperature abuse which will cause decline in the overall quality of the fruit and it will significantly reduce its shelf life. This represents an economic challenge as $60 million is wasted each year for Florida tomatoes alone. Overall, about 70% of food waste occurs at the consumer level where people will buy fruits and vegetables and then throw them away. The remaining 30% of waste occurs at all levels of the supply chain (Parfit et al., 2010). At the retail

level a value of up to $1.7 billion worth of tomatoes are wasted in the United States each year (Bubzy et al., 2012).

Farm

The largest percentage of loss for the tomato outside of consumer waste occurs at the farm level (Hall et al., 2009). Most of the loss is due to environmental factors or economic forces that cannot reasonably be prevented (Hall et al., 2009). Examples of this include hurricanes, drought, and competing produce from other countries. The loss can be placed into two categories, which are crops that are never harvested and crops that are lost between harvest and sale (Hall et al., 2009). Farms typically harvest tomatoes and place them into storage containers until they are ready to be brought to a farm storehouse (Sargent et al., 2005). Once it is there, the tomato will be made ready to ship to its next destination (Sargent et al., 2005). Farm fresh tomatoes will be usually shipped to a packaging plant (Sargent et al., 2005).

At the farm, before tomatoes are sent to the next stage, several measures can be taken to ensure that the quality will not drop and fruit will not be wasted (Hall et al., 2009; Sargent et al., 2005). Farmers will use either a workforce or an automated picker to remove the tomatoes from the vine into trucks (Sargent et al., 2005). The trucks travel along with the pickers until the truck bed is full. Then the truck will travel to the farm storehouse (Sargent et al., 2005). These trucks, for the most part, are unprotected and allow the fruit to sit out in the sun longer than the fruit can tolerate (Sargent et al., 2005). At this point during harvest is where the fruit will be exposed to high abuse temperatures. The exposure to the sun and the weight of other tomatoes can cause bruising and heat stress compromising the quality of the fruit (Sargent et al., 2005). Ideally, tomatoes should be taken directly from the field to the storehouse and graded under shaded conditions. If left in the sun, the fruit can reach up to 35 °C causing heating damage to the unripen fruit.

Although not very common with field tomatoes, some farms may also cool the tomatoes to appropriate temperatures. The goal of cooling the tomatoes is to lower the field temperature

of the fruit to the optimum temperature of 13 °C as fast as possible. However, common abuse temperatures found are cooling the tomato at 2 °C resulting in severe chilling injury or room-cooling the tomatoes at 25 °C leading to accelerated ripening or uneven ripening. After the tomato is cooled, farmers will store the fruit until it is ready to be sold or shipped to distributors. The optimum storage temperature for tomatoes is 13 °C but temperature abuses such as exposure to 2 °C and 25 °C have been observed. While this entire process of grading, cooling, and storage usually takes place on the same day, random error happens, and the fruit can be stored over night or for longer periods of time (Hall et al., 2009).

Farms in the United States harvest tomatoes when they are at a mature-green stage and will ripen them using ethylene gas. This is done to trigger the tomato ripening process. Ethylene works by acting as a biological trigger for the ripening process and causes the proteins in control of ripening to be upregulated (Saltveit, 1999). This optimally is done at 12.5 °C and then the tomatoes are shipped to stores or usually to distribution centers (Saltveit, 1999).

Shipping and distribution

After harvesting and grading, tomatoes are shipped from the farm to the distribution center (DC). Depending on the distance between the farm and the DC, this step in the supply chain can take up to 72 hours when shipping from Florida to New York, and at temperatures close to 13 °C. However, exposure to low temperatures around 10 °C and high temperatures of 20 °C can also be found during shipping from the farm to the DC (Nunes et al., 2011). Because shipping from the farm to the DC can take several days, it is critical that temperatures are maintained as close as possible to optimum.

The second shipping step is transporting tomatoes from the DC to the store. When the DC sends produce to the supermarkets, many varieties of fruits and vegetables are sent at once (mixed loads) as to fulfill the needs of the supermarket. This leads to tomatoes often being shipped at temperatures that can range from 2 °C to 8 °C. The average shipping time from the DC to the store is approximately 8 hours and, if tomato fruit is exposed to very low temperatures

that can lead to CI (Nunes et al., 2011). At the DC, usually tomatoes are temporarily stored in a large warehouse before being shipped to the supermarket (Hall et al., 2009). This time and temperature delay can result in 13% of tomato being wasted before reaching the consumers (Hall et al., 2009). To prevent waste DCs try to follow the guidelines set by governmental agencies however because there are so many types of fresh fruits and vegetables, each with different temperature requirements keeping each of them at optimal storage conditions is expensive and unpractical (Hall et al., 2009; Sargent et al., 2005). Guidelines set by the FDA give general storage practices but they do not set a standard for temperatures and humidity (Sargent et al., 2005). This lack of standard means that tomato fruit can be subjected to abuse temperatures resulting in either CI or accelerated ripening.

Retail

At the retail level is where up to one-seventh of all of the food is wasted (Lucier et al., 2006). For tomatoes, retail stores may lose $1.7 billion worth of tomatoes after being received due to poor appearance quality (Gustavsson et al., 2011). Because consumers demand for the freshest and “best-looking” fruit is high, supermarkets are forced to discard significant amounts of fresh fruits and vegetables on a daily basis (Bubzy et al., 2011; Gunders et al., 2017). In addition, at the supermarket ripe red tomatoes are often displayed inside non-refrigerated displays with an average temperature of 20 °C, which is 7 degrees higher than the optimum storage temperature (13 °C). Tomatoes that are not immediately displayed are stored in the backstore of the supermarket along with other produce where temperatures can be as low as 2 °C (Nunes et al., 2011). These abuse conditions along with consumers demand for tomato with good appearance will cause large amounts of fruit to be wasted.

Impact of Postharvest Conditions on Tomato Key Chemical Compounds

Sugars

Levels of sugar in tomato fruit are closely linked to the postharvest environmental conditions (i.e., temperature) in which the tomato is kept. It is well-established that tomatoes

should be ideally kept at 13 °C as for each 10 °C increase in temperature above 13 °C, there is a doubling of the rate of fruit deterioration (Saltveit, 2003). Below 13 °C, and depending on the maturity of the fruit, chilling injury occurs contributing to a significant decline in the overall quality of the fruit. Chilling temperatures also affect the normal metabolism of the fruit contributing to changes in the accumulation of sugars. For example, Gomez et al. (2009) reported that when tomato fruit is stored at 6 °C for two weeks the final sugar content is reduced by almost 25% compared to fruit stored at optimum temperatures. On the other hand, tomatoes stored in a continuous elevated temperature (above 13 °C) show negligible change in sugar content when compared to those stored at optimum temperatures (Lu et al., 2010). However, sugar content increases in tomato fruit that has been exposed to a temperature of 20 °C before being stored at 13 °C (Lu et al., 2010). Water loss has also been shown to negatively affect sugars levels in tomatoes (Beckles, 2012). It has been shown that perceived sweetness of the fruit is lower as well as sugar levels after tomatoes were exposed to very low RH (5 to 10%) (Cantwell et al, 2009).

Ascorbic Acid (AA)

The levels of AA in harvested fruits are highly correlated with the temperature at which the fruit is stored and handled (Lee and Kader, 2000). At high temperatures, there is an increase in the breakdown of AA, with the longer times having a larger impact (Lee and Kader, 2000). However, because of tomato natural higher acidity levels, AA oxidations seems to be delayed as acidity it creates a stable environment for ascorbic acid (Stevens et al., 2008). Bruising also significantly affects the levels of AA because of the disruption of cell walls, promoting the contact between oxidizing enzymes (e.g., ascorbate oxidase) and AA. For example, AA levels were up to 15% lower in bruised tomatoes tissue when compared to unbruised tissue (Moretti et al., 1998). Maintaining water content of tomato is also important to maintaining the levels of AA as water loss negatively impacts the content of AA in tomatoes (Lee and Kader, 2000). In strawberries, it has been shown that water loss plays a more

significant role than temperature in AA levels of the fruit, with water loss contributing directly to lower AA levels (Nunes et al., 1998).

Carotenoids

As tomato fruit ripens, chlorophyll breakdown and the level of carotenoid and lycopene increase giving the fruit its characteristic red coloration. Temperature during growing and during postharvest have a significant impact on the levels of carotenoids. Temperature above 20 °C, accelerate ripening are lead to an increase in levels of carotenoids. Tomatoes that have been exposed to temperatures above 13 °C for extended periods show levels of carotenoids 10% higher than those stored at 13 °C temperatures. Temperatures between 21 to 26 °C lead to an increase in carotenoid content but do not considerably change the amount of lycopene. A significant increase in lycopene content in the tomato was reported only after exposure to temperatures as high as 27 to 32 °C (Gautier et al., 2008). On the other hand, storage at temperatures that will cause chilling injury usually inhibit the normal ripening process, reducing synthesis and the levels of carotenoids in the fruit. Low temperatures have a negative impact on the content of both carotenoids and lycopene as well as any of the associated chemical precursors (Niasr et al., 2015). Temperatures below 10 °C are especially damaging and can result in a 50% reduction in carotenoid and lycopene contents (Toor and Savage, 2006).

Effects of Postharvest Conditions on Tomato Proteome

Studies on tomato proteome are scarce and most of them focus on ripening stages or on the impact of disease on protein expression. Few studies have investigated the impact chilling injury on tomato proteome. To our knowledge there are no data published on the impact of time-temperature abuse conditions normally encountered during supply chain on tomato proteome and the relationship between biochemical markers.

For example, a study conducted on the impact of low temperature on tomato fruit proteome, showed that when chilling injury develops, two major groups of proteins are

impacted. These proteins comprise defensive proteins, that are associated with small heat shock, and late embryogenesis, and those related to the uncoupling of photosynthetic processes and protein degradation machinery (Sanchez-Bel et al., 2011; Page et al., 2010). Within this group of proteins, the majority are associated to energy metabolism and to a lesser degree, stress response. A sizable minority of the proteins identified, were related to the maintenance of the plant cellular wall as well.

As the tomato fruit ripens, there are also changes in the protein levels associated with ripening. The most significant and highly expressed of these proteins are the ripening inhibiting proteins (RIN), which are the main gateway that regulates fruit ripening. RIN proteins regulate ripening-related genes by binding directly to the promoter regions of these genes. There are many targets of RIN proteins including genes related to cell wall metabolism, ethylene biosynthesis, carotenoid biosynthesis, and sucrose metabolism (Martel et al., 2011; Fujisawa et al., 2013; Qin et al., 2016; Cai et al., 2018;). As ripening progresses, the proteome shows that RIN is down regulated allowing the transcription and translation of the ripening related genes (Cai et al., 2018).

CHAPTER THREE:

BIOCHEMICAL APPROACHES TO DETERMINE THE IMPACT LEVEL OF EACH STEP ALONG THE SUPPLY CHAIN ON TOMATO FRUIT QUALITY

Introduction

Tomato fruit is an important crop in the United States with an economic return of two billion dollars annually (USDA, 2017). Tomato production is divided into two markets, fresh and processed. In the United States, tomato is the fourth most consumed fresh produce and consumption has been steadily on the rise (Lucier et al., 2006). Florida produces tomatoes for both the fresh and processed markets but production focus mainly on fresh field tomatoes. Since 1980, Florida has been the second largest producer of fresh tomatoes contributing up to one-third of all tomatoes grown in the United States and providing an annual revenue of approximately $453 million (USDA, 2017). In Florida, 50 different varieties of tomato fruit are grown year-round, providing the consumer with fruit with different sensorial and chemical characteristics.

At the consumer level, appearance of tomato (i.e., size, maturity, color, texture, decay, and presence of defects) is the most important quality criteria as it greatly influences purchase. In general, consumers expect tomatoes to have a bright red color and may reject green and overripe fruit. Besides, consumers also prefer tomatoes that are neither too soft nor too hard (Oltman et al., 2014). After color and texture, flavor is an important quality attribute and will influence consumer repeated purchases. Tomato flavor is influenced by the amount of sugars and organic acids present and, in general, the higher the sugar content the sweeter the fruit. Tomato fruit is also a good source of vitamin C and, the levels present in the fruit are equally

important to consumers because this vitamin is considered a powerful antioxidant and an essential micronutrient (Oltman et al., 2014). In addition, carotenoids in tomato are responsible for the bright red color of the fruit. Some carotenoids also are precursors of vitamin A and have been associated with the decrease of some degenerative diseases (Khachik et al., 2002). Together sugars, vitamin C, and carotenoids are considered important biochemical markers of tomato fruit overall quality and good indicators of flavor and nutritional quality (Oltman et al., 2014). However, during handing from the farm to the consumer tomato quality can be compromised due to exposure to inadequate environmental conditions (i.e., temperature), resulting in a significant decline in these important attributes and contributing to increased waste (Yelle et al., 1989).

At the farm level, tomato is typically harvested mature-green and placed into bulk containers until it is ready to be brought to the growers' facilities (Sargent et al., 2005). At the growers' level, mature-green tomatoes are usually sanitized by immersing the fruit into a water-chlorine solution in large tanks, then fruit is sorted and packaged into cardboard cartons. At the grower, tomatoes are usually stored at room temperature and, before shipping to the distribution center (DC), mature-green tomatoes can be treated with ethylene gas to trigger ripening and color development (Sargent et al., 2005). From the DC, tomato is shipped to the stores and usually displayed for sale at ambient conditions. Delays between harvest, sorting and storing or handling at inadequate temperatures often occur from the farm to the consumer resulting in loss of quality and waste due to the extensive abuse the fruit may receive (Yelle et al., 1989). Although some studies have shown that tomato quality deterioration and waste begin at the farm and accumulates throughout the supply chain, there is no information available regarding the impact of each step along the supply chain on tomato quality, and on how to prioritize actions along the supply chain to achieve an immediate and effective impact on waste reduction. Therefore, the objectives of this study were to determine the impact level of each step

along the supply chain, from the farm to the consumer, on the quality of tomatoes, and to identify critical supply chain steps where decline in tomato quality was highest.

Materials and Methods

Plant material and supply chain simulation

For the first experiment (first harvest), 250 tomatoes (cv. Rebelska) were harvested at the light red color stage from Red Farm Hydroponics in Dover, Florida on April 5, 2017. For the second experiment (second harvest), 250 tomatoes (cv. Beefsteak) were harvested from Hydro Harvest Farms in Ruskin, Florida on November 29, 2017. Tomatoes were brought to the laboratory within one hour after harvest. Upon arrival to the laboratory, tomato fruit were selected based on color and freedom from defects. Nine of these fruit were used for initial quality evaluations. Nine fruits per treatment (control plus 18 supply chain conditions) were carefully distributed to clamshells and used for non-destructive analysis (i.e., subjective appearance and weight loss). For destructive analysis (color and texture analysis, and chemical analysis), nine fruit each per treatment (control plus 18 supply chain conditions) were carefully distributed to clamshells. The clamshells containing the tomato for both non-destructive and destructive quality evaluations were then stored for specific periods of time inside temperature and humidity-controlled chambers (Forma Environmental Chambers Model 3940 Series, Thermo Electron Corporation, OH, USA) set at 2.0 ± 0.3 °C, 4.0 ± 0.6 °C, 8.0 ± 0.2 °C, 10.0 ± 0.2 °C, 13.0 ± 0.1 °C, 15.0 ± 0.2 °C, 20.0 ± 0.3 °C, 25.0 ± 0.2 °C and approximately 80 % RH (Figure 3). Quality of the fruit was evaluated, at each step individually, after a total supply chain length of 278 hours (≈ 12 days). The total time (278 h) was chosen based on a typical supply chain for tomato: harvest → grading → room cooling → commercial ripening (ethylene applications) → storage at grower → transport from grower to distribution center (DC) →

storage at DC → transport from DC to stores → display at the store → purchase by consumer. Since light-red tomatoes were used for this work, the ethylene treatment step was omitted. Simulated supply chain conditions within each step were selected based on time-temperature profiles previuously measured. Within each supply chain step, a best and worst time-temperature scenario was tested. Before and after each time-temperature treatment, within each supply chain step, tomatoes were kept at constant optimum conditions (i.e., 13 °C and 90 % RH) so only the specific segment within each step was different from one step to the other (Figure 3). Tomato optimum storage conditions (13.0 °C and 90 % RH) were selected based on data from from Gross et al. 2004 and Nunes et al., 2008. Field temperatures (35 °C) were selected based on the average field temperatures measured in Florida between April and November (https://fawn.ifas.ufl.edu/). The time (72 h) used to simulate shipping from the grower to the DC was chosen based on the farthest distance in time from Florida to U.S. Midwestern States or Eastern Canada. Conditions used for consumer handling (4 and 20 °C for 24 h) were chosen based on common household refrigerator (Godwin, 2007) and countertop temperatures.

Temperature and Relative Humidity (RH) Monitoring

The temperature inside temperature and RH-controlled rooms was monitored throughout the study using HOBO® brand U12 data loggers (Onset Computer Corporation, Pocasset, MA, USA), which records within an accuracy of ± 0.35 °C. The RH was monitored using HOBO® brand U12 data loggers (Onset Computer Corporation, Pocasset, MA, USA), which records within an accuracy of ± 2.5 % from 10 to 90 % RH.

Visual Quality

Subjective quality attributes, namely color, shriveling and decay were determined subjectively using a 1 to 5 visual rating scale, and firmness was determined subjectively based on the whole specialty crop resistance to slight applied finger pressure and recorded using a 1 to 5 tactile rating (Table 1). These data were primarily used to determine the end of shelf life due to loss of sensory quality, and to quantify waste. Thus, for each treatment, a limiting quality

factor(s) will be established considering the rating value of 3 as the minimum acceptable quality before the specialty crop becomes unmarketable (Nunes, 2015).

Instrumental Color

A total of two color measurements were taken on the opposite sides of the tomato fruit at the equatorial region. A hand-held tristimulus reflectance colorimeter (Model CR-400, Minolta Co., Ltd., Osaka, Japan) equipped with a glass light-projection tube (CR-A33f, Minolta Co., Ltd., Osaka, Japan) was used. Color was recorded using the CIE-L*a*b* uniform color space (CIE-Lab), L* (lightness), a* (redness), and b* (yellowness) values. Numerical values of a* and b* were converted into hue angle using the Konica Minolta CR-400 Utility software CR-S4w (2002-2010 Konica Minolta Sensing, Inc., Osaka, Japan) (Nunes, 2015).

Texture Analysis

Firmness of each individual specialty crop was measured using a TA.XT plus Texture Analyzer (Texture Technologies Corp., NY, USA) equipped with a 50 kg load cell. A tomato was placed on the flat surface of the texture analyzer with stem-end down, so the pressure was applied on the blossom-end part of the fruit. The instrument was fitted with a 76.2 mm diameter stainless compression plate and the probe will then be driven with a crosshead speed of 1 mm s^{-1}, and the compression force was recorded at 10.0 mm deformation (Nunes, 2015).

Weight Loss and Moisture Content

The weight of the tomato was measured using a precision balance with an accuracy of ± 0.01 g (Denver Instruments, Timberline Series Model TP-3102, CO, USA) as described by Proulx et al. (2011). Moisture content was determined by the standard gravimetric method. A 10 g homogenized sample was spread evenly over the bottom of a metal dish, weighed, and dried 24 h at 80 °C in a laboratory oven (Model 40GC, Quincy Lab Inc., Chicago, IL). Dry samples

were cooled in desiccators then weighed, and the final weight was subtracted from the initial weight to obtain the moisture content.

pH, Acidity and Soluble Solids Content (SSC)

A 50-g aliquot of the tissue slurry was centrifuged at 6,000 × g for 20 min. The clear juice was decanted and the pH, titratable acidity and SSC of the clear juice was determined as described by Nunes et al. (1995)

Total and Individual Sugar Contents

Total sugar analysis was conducted using a Hitachi HPLC system with an RI- refractive index detector and a 300 mm × 8 mm Shodex SP0810 column (Shodex, Colorado Springs, CO) with an SP-G guard column (2 mm x 4 mm). Isocratic solvent delivery of water will be set at 1.0 mL min^{-1}. Standards including sucrose, glucose and fructose were used to identify sample peaks. After comparison of retention time with the standards, the peaks were identified. The amount of total sugar in tomatoes was quantified using calibration curves obtained from different standard concentrations (Chilson and Nunes, 2011).

Total Ascorbic Acid Content

Homogenized tomato (4g) was mixed with 20 ml metaphosphoric acid mixture (6 % HPO_3 containing 2 *N* Acetic acid) and filtered through a 0.22 µm filter prior to HPLC analysis. Ascorbic acid analysis was conducted using a Hitachi LaChromUltra UHPLC system with a diode array detector and a LaChromUltra C18 2 µm column (2 × 50 mm) (Hitachi, Ltd., Tokyo, Japan). The analysis was performed under isocratic mode at a flow rate of 0.5 mL min^{-1} with a detection of 254 nm. Mobile phase was buffered potassium phosphate monobasic (KH_2PO_4, 0.5 %, w/v) at pH 2.5 with metaphosphoric acid (HPO_3, 0.1 %, w/v). After comparison of retention time with the ascorbic acid standard, the peak was identified. The amount of total ascorbic acid content in tomato was quantified using calibration curves obtained from different concentrations of ascorbic acid standards (Chilson and Nunes, 2011).

Total carotenoid content

Total carotenoids were extracted by mixing 2 g of homogenized tomato tissue with 25 mL of a solution containing acetone: ethanol (1:1) and 200 mg L^{-1} BHT as described by Talcott and Howard (1999). Samples were extracted in the dark, filtered through Whatman No. 4 paper filter, and washed until the residue was colorless. Samples were adjusted to 100 mL, and the absorbance was measured using a microplate reader (Biotek Instruments, Inc., Highland Park, VT, USA). Total carotenoids were calculated according to Gross (1991) using the following equation: µg carotenoid $g^{-1} = (A \times V \times 10^6) / (A^{1\,\%} \times 100 \times G)$, where *A* is the absorbance at 470 nm, *V* is the total volume of extract, *A*1 % is the extinction coefficient for a mixture of solvents arbitrarily set at 2500, and *G* is the sample weight in grams (Gross, 1991).

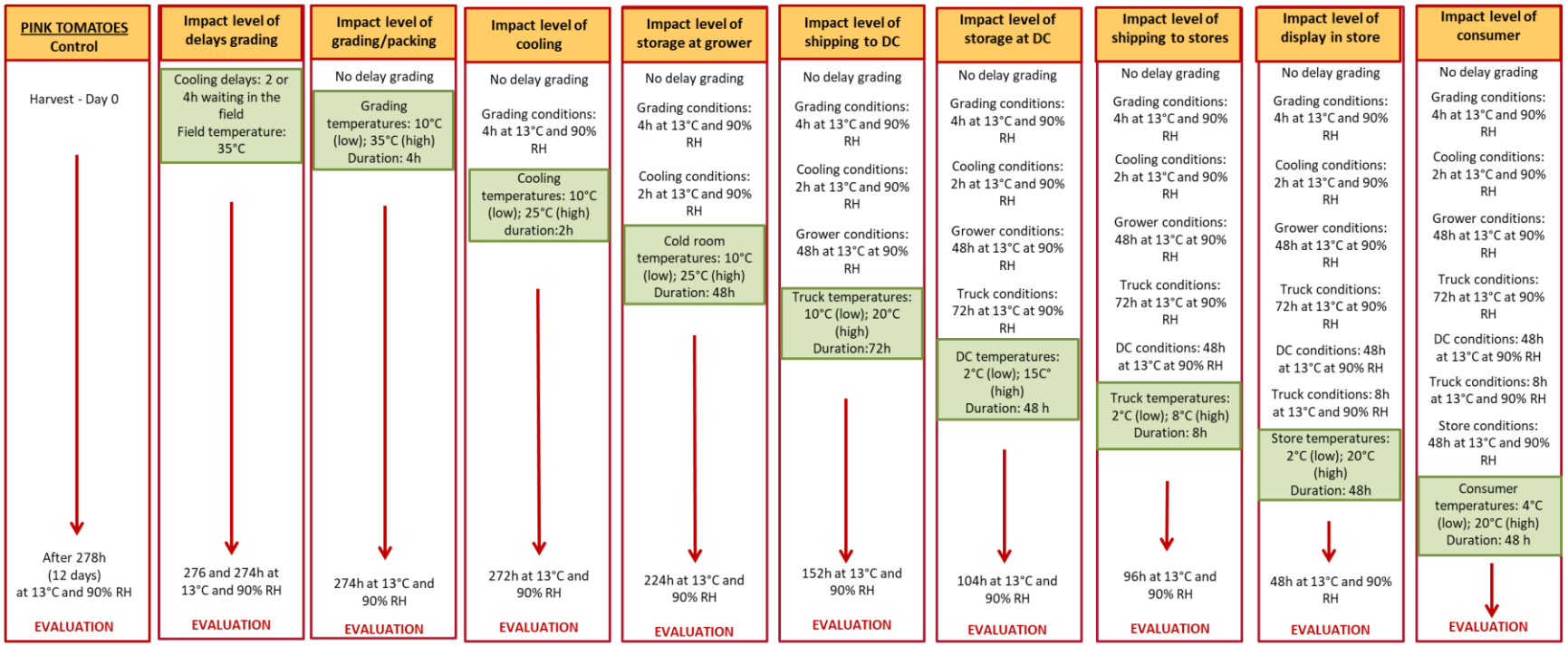

Figure 3. Tomato supply chain simulations from the field to the consumer. Each section represents a supply chain step and within each step a best and worst time-temperature scenario were tested. DC = distribution center.

Table 1. Visual quality scores and descriptors for tomato fruit.

	Scores and description				
	1	2	3[a]	4	5
	Very poor	Poor	Acceptable	Good	Excellent
Color	Very dark red, overripe	Dark red; dull color	Vivid red; loss of glossiness	Light red but less glossy	Light red with no trace of green; very glossy
Firmness	Extra-soft, overripe, fruit yields very readily to slight pressure	Soft, fruit yields readily to slight pressure	Firm, fruit yields slightly to moderate pressure	Hard, fruit yields only slightly to considerable pressure	Extra hard, fruit does not yield to considerable pressure
Shriveling	Extremely shriveled and dry; fruit appears old and deteriorated	Severe shriveling	Shriveling evident, but not serious	Slight signs of shriveling	Field-fresh, no signs of shriveling
Chilling injury[b]	Severe injury	Moderate injury	Slight injury	Trace (small pits)	No abnormality
Decay	76-100 % decay, severe to extreme decay (the fruit is either partial or completely rotten).	51-75 % decay, moderate to severe decay	26-50 % decay, slight to moderate decay (spots with decay and some mycelium growth);	1-25 % decay, probable decay (brownish/grayish sunken minor spots);	0 %, no decay

[a] Score of 3 was considered to be the minimum acceptable quality before cluster tomatoes become unmarketable.

[b] Symptoms of chilling injury include: failure to ripening and to develop full color and flavor, irregular (blotchy) color development, premature softening, surface pitting, browning of the seeds, and increased decay.

Statistical Analysis

The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used for the analysis of the data. Data from the two harvests are shown separately because there was a significant difference between harvests for several of the physicochemical attributes measured. The data was treated by two-way analysis of variance (ANOVA) with harvest and supply chain step as main effects. Significant differences between harvests and between initial, control and supply chain steps were detected using the least significant difference (LSD) at the 5 % level of significance.

Identification of Critical Supply Chain Steps

Based on data from statistical analysis, the steps in the simulated tomato supply chain that consistently showed the greatest decline in quality attributes, compared to the control, were selected. The three supply chain steps that contributed to the highest decline in tomato quality, in comparison to the control, are discussed.

Results and Discussion

Sensory Quality

Color. Tomato color perception was negatively impacted by the time-temperature treatments used, regardless of the supply chain step. Color of tomato from the first harvest significantly decreased when compared to the control, meaning that the fruit became redder after supply chain (Figure 4A). Overall, four supply chain steps showed a large negative impact on tomato color. That is, when compared to the control, storage at the grower at 25 °C resulted in a 26 % decrease in color-quality, shipping to DC at 20 °C resulted in a 28 % decrease, and both shipping to the store at 8 °C and consumer storage at 20 °C resulted in a 21 % decrease (Figure 4A). In the second harvest, supply chain steps with temperature abuse also contributed to a decline in perceived color quality and correlated strongly with the most negative steps in the first harvest, where loss of color quality was significant (Figure 4B). Therefore, shipping to the

DC at 20 °C and displaying at the store at 20 °C contributed to decrease of 23 % in tomato color quality compared to the control; storage at the grower at 20 °C and displaying at the store at 2 °C and consumer storage at 20 °C both contributed to a 17 % decreased in color quality when compared to the control.

Overall, in both harvests, the supply chain steps that contributed to significant tomato color development, from a consumer perception, were storage at the grower at 25 °C, shipping to the DC at 20 °C, and consumer storage at 20 °C. High temperature abuses in the supply chain were observed as more negatively impactful on tomato color perception than colder temperature abuses. A previous work has also shown that temperatures higher than 13 °C contribute to accelerated ripening and red color development of tomato fruit (Pek et al., 2010). Verheul et al. (2015) provided evidence that temperatures lower than 12 °C have only limited effects on tomato color quality. Although accelerated ripening at high temperatures gives tomato a healthy appearance, the flavor profile is not as good as when the fruit is ripened at optimal conditions (Sargent et al., 2005). Therefore, exposure of tomato fruit to high temperatures during supply chain, will accelerate red color development and most likely result in a dark and overripe fruit at the consumer level. Tomato fruit with objectionable color, leads to consumer rejection and waste at the retail and home levels (Buzby et al., 2012).

Firmness. In the first harvest, the effect of supply chain on tomato firmness was not as consistent as after supply chain, some fruit were firmer than the control despite the temperature abuse during supply chain simulations (Figure 5). Such discrepancy may have resulted from the fact that the initial quality of tomato from the first harvest was irregular with some fruit showing better overall quality than others. Nonetheless, there were some tomatoes that became softer than the control fruit and were negatively impacted by conditions used during the supply chain (Figure 5A). When compared to the control, shipping to the DC at both 10 and 20 °C caused a 10 and 12 % decrease in fruit firmness, respectively, whereas displaying at the store at 20 °C

cause a decrease of 5 % in fruit firmness. In the second harvest, several of the supply chain steps had a significant negative impact on firmness of the fruit, contributing to softening after supply chain (Figure 5B). For example, impact cooling at the growers at 10 °C caused a 10 % decrease in fruit firmness when compared to the control, storage at the grower at 25 °C caused a 17 % decrease, and shipping to the DC at 20 °C caused a 10 % decrease in tomato fruit when compared to the control.

Temperature fluctuations during handling have been shown to negatively impact tomato fruit firmness (Kader, 1984). Previous studies have shown that high temperatures accelerate tomato ripening and cause the fruit to soften prematurely. On the other hand, temperatures below 13 °C delay the ripening process and softening of tomato than when fruit stored in optimal temperatures (Tadesse et al., 2015). In the present study, the steps with the largest impact on firmness were the steps where tomato was exposed to higher temperatures. High temperature abuses in the supply chain caused fruit to ripen unevenly and caused undesirable softening. In general, consumer preference is for a tomato that is firm and gives a little to the touch while not being so firm and hard to slice (Oltman er al., 2014).

Shriveling. Shriveling followed the same pattern as firmness, with several of the supply chain steps in the first harvest showing less marked impact on tomato shriveling than the control (Figure 6). Nonetheless, impact cooling at 25 °C and shipping to the DC at 20 °C caused more shriveling on the fruit (7 and 12 % more shriveling, respectively) when compared to the control (Figure 6A). Unlike in the first harvest, in tomato fruit from the second harvest, development of shriveling was much more impacted by the time-temperature treatments with more than three supply chain steps contributing equally to increased shriveling compared to the control (Figure 6B). A four-hour delay in cooling and impact cooling at 10 °C caused more shriveling in the fruit than the control (9 and 8 %, more shriveling, respectively) whereas when compared to the control, impact cooling at 25 °C, storage at the grower at 10 °C, and displaying at the stores at 2

°C all impacted shriveling equally (7 % more shriveling than the control).

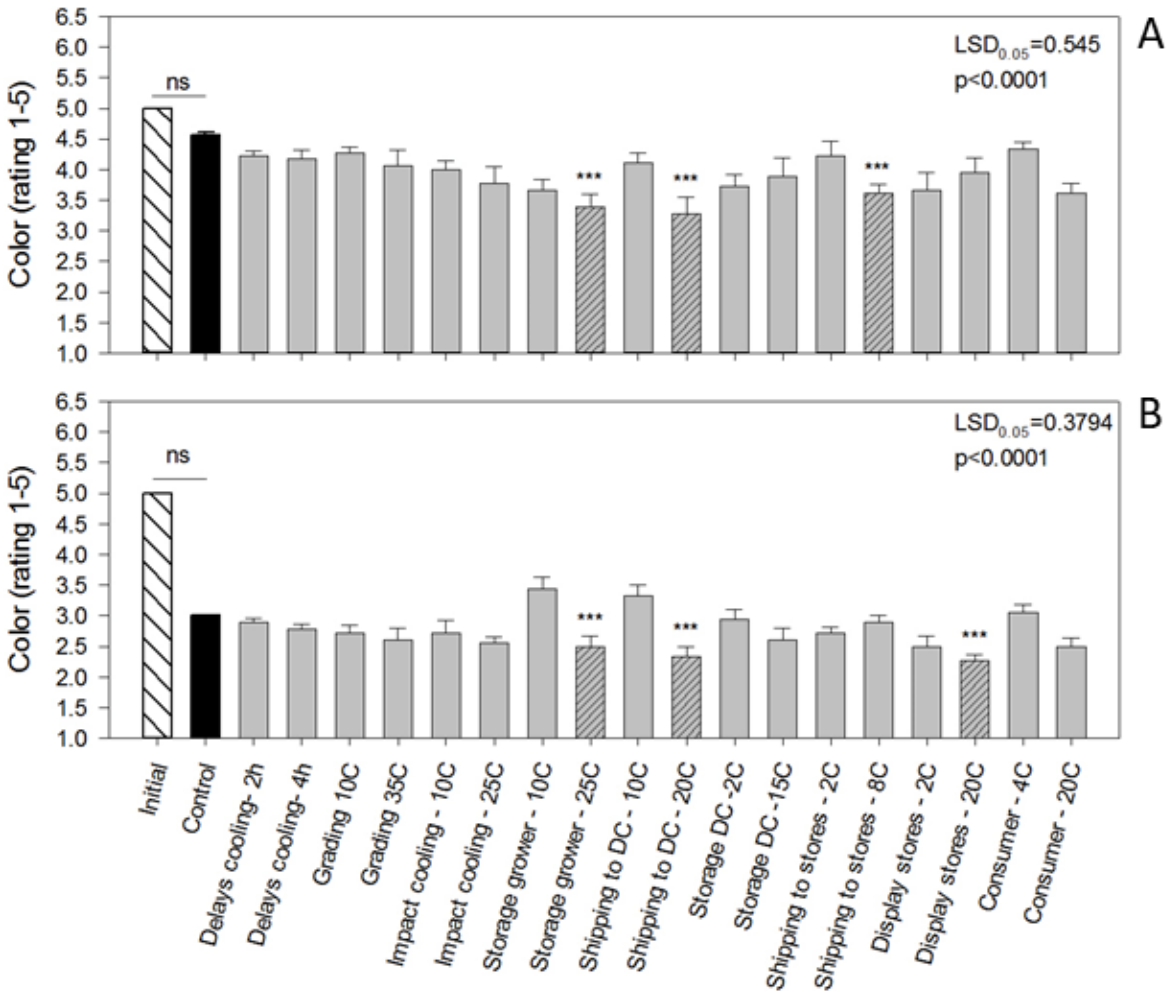

Figure 4: Impact level of each step along the supply chain on tomato color. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ***p≤ 0.0001).

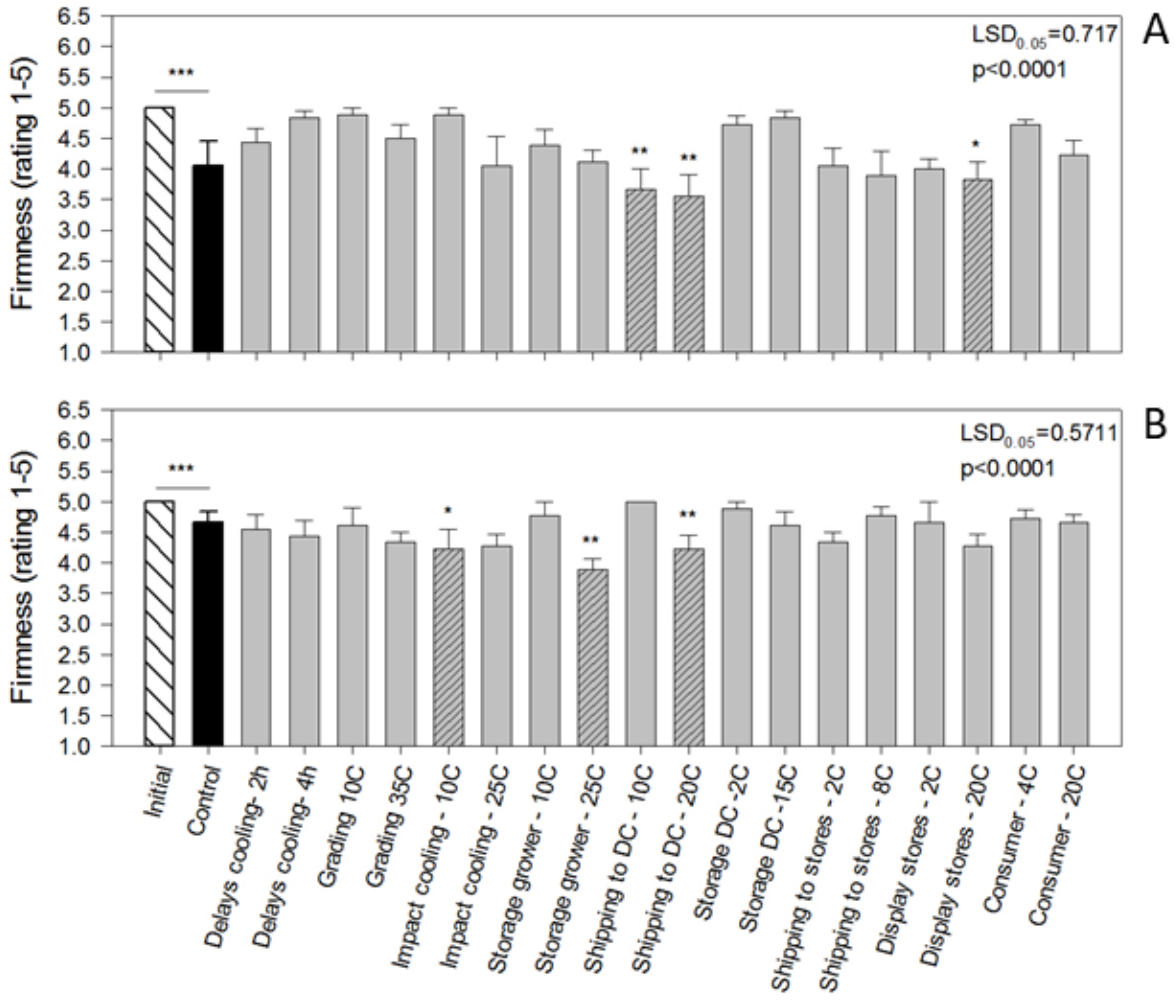

Figure 5: Impact level of each step along the supply chain on tomato firmness. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (*p≤ 0.05; ** p≤ 0.001; ***p≤ 0.0001).

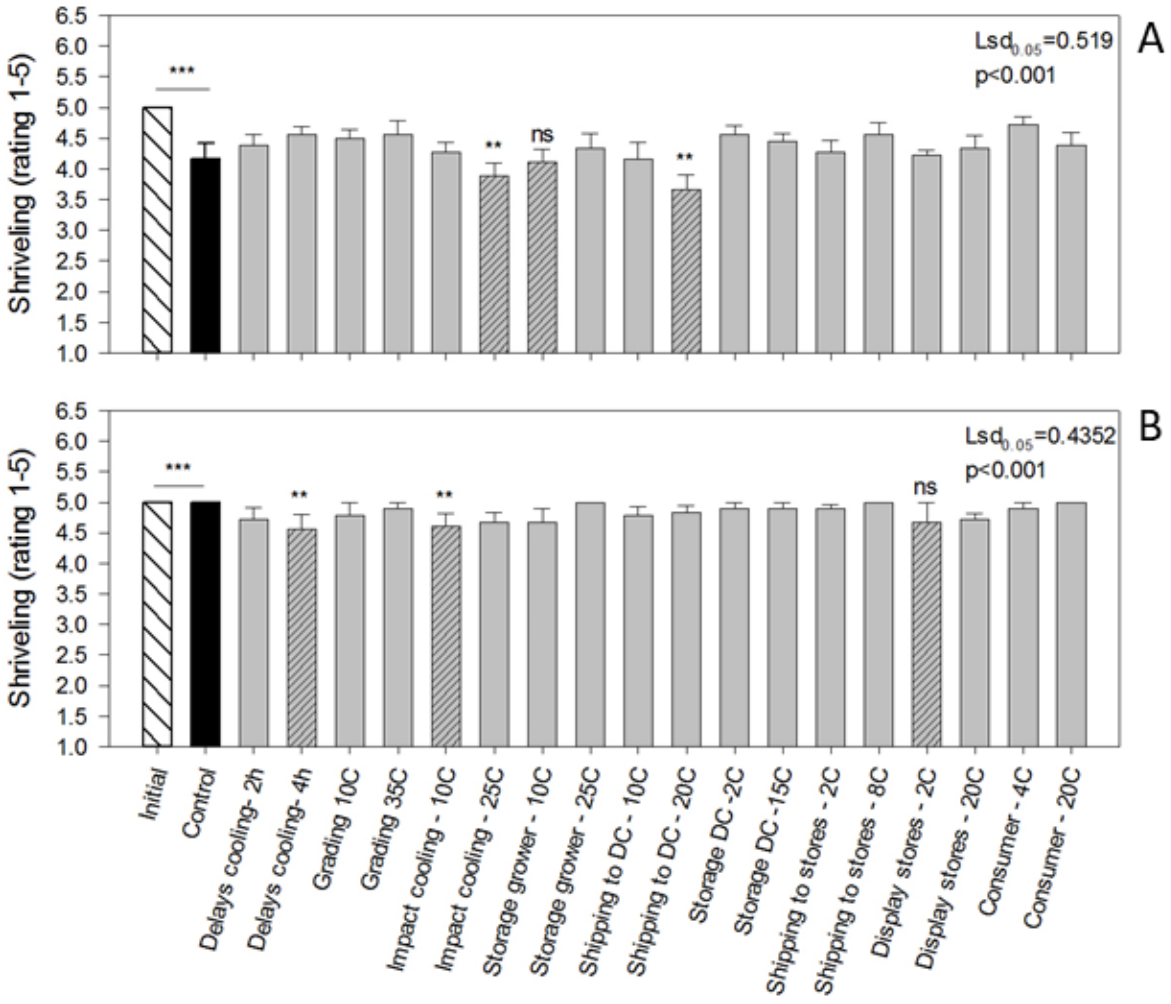

Figure 6: Impact level of each step along the supply chain on tomato shriveling. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ** p≤ 0.001; ***p≤ 0.0001).

Shriveling in tomatoes can be caused by a variety of factors but it is most often attributed to weight loss (Jois et al., 2016). Shriveling is an important contributor to the overall tomato appearance quality as it has a large influence on consumer decision to purchase. While shriveled tomatoes are perfectly edible, their appearance is undesirable. Tomatoes must have a

smooth not shriveled skin as consumers will not purchase the fruit otherwise (Kader et al., 1978). In this study, fruit shriveling was minimally impacted by any supply chain steps used.

Chilling Injury. In tomato fruit, chilling injury (CI) results from exposure to temperatures below 13 °C. The lower the temperature and the longest the exposure time the more severe the symptoms of CI will be. For example, the impact of CI is apparent after two weeks at 10 °C or for longer than one week at 5 °C. Symptoms of CI include uneven ripening, improper color development, water spots, surface pitting, browning of the seeds, failure to ripen and develop full flavor, and increases in decay (Aghdam, 2013).

Chilling injury was apparent in tomato from both harvests and negatively impacted the quality of the fruit after supply chain. In the first harvest, CI symptoms were significant in tomato exposed to several supply chain steps (Figure 7A). However, compared to the control, the supply chain steps that contributed the most to development of CI in tomato fruit were impact cooling at 25 °C (25 % more CI than the control), shipping to the DC at 20 °C (23 % more CI than the control), and storage at the DC at 2 °C and impact cooling at 10 °C (15 %). The reason why high temperatures may have resulted in CI was most likely due to the poor initial quality of tomato fruit from the first harvest. Besides the possibility that the grower might have stored the fruit at too low temperatures prior to the beginning of our experiments, signs of senescence may also have been confused for symptoms of CI such as uneven ripening and tissue damage. Although not severe, tomato from the second harvest, showed development of CI symptoms only when the fruit were exposed to cold temperatures (Figure 7B). Therefore, compared to the control, grading at 10 °C and storage at the DC at 2 °C showed CI symptoms (10 % more CI than the control) as well as storage at the grower at 10 °C and shipping to the DC at 10 °C (11 % more CI than the control) and impact cooling at 10 °C (12 % more CI than the control).

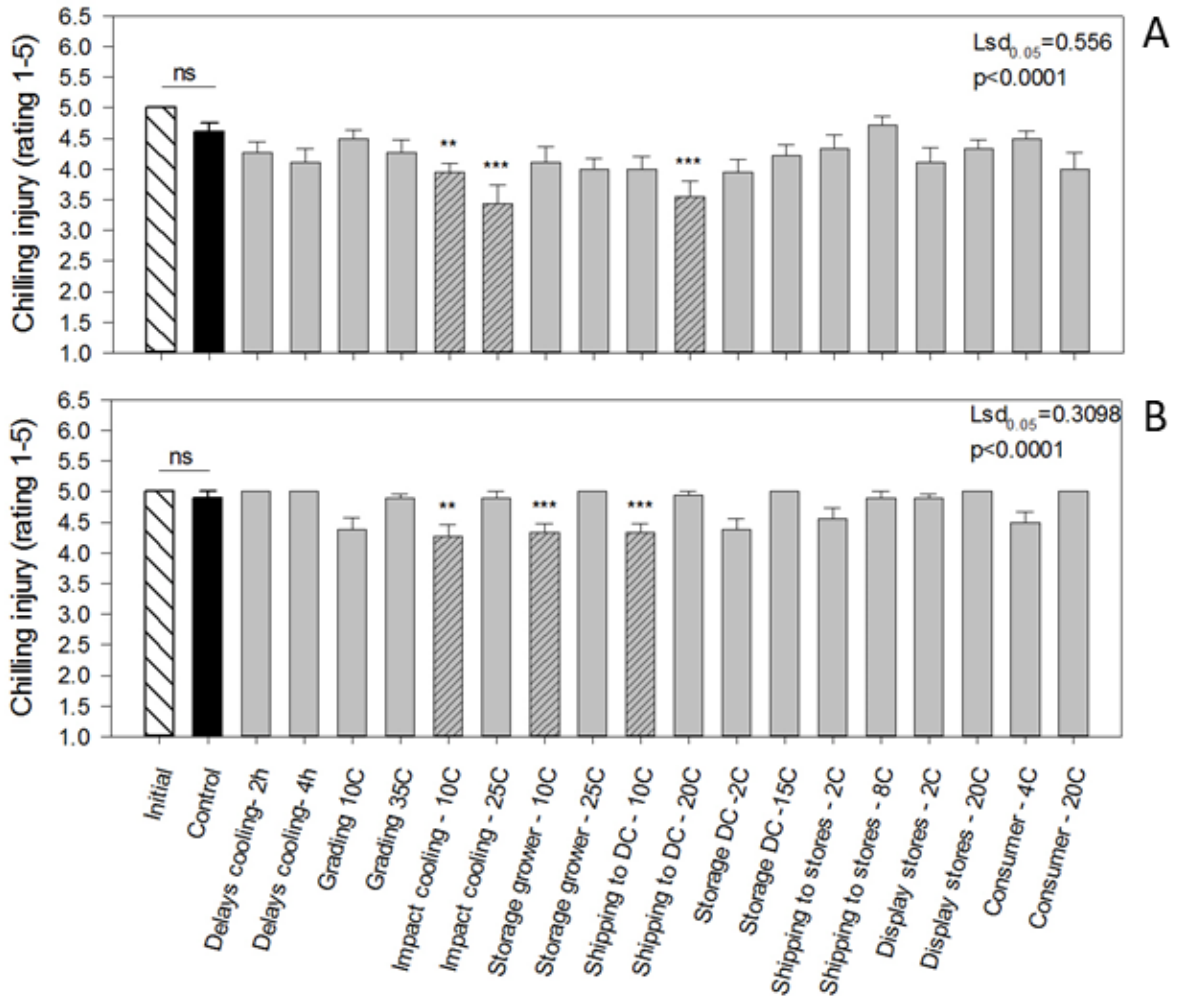

Figure 7: Impact level of each step along the supply chain on development of chilling injury in tomato. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ** p≤ 0.001; ***p≤ 0.0001).

Previous work done by Lurie and Sabehat (1997) showed that tomatoes stored at temperatures below 13 °C do not ripen as quickly or at all when compared to tomatoes stored at higher temperatures. Tomatoes stored at low temperatures for a week begin to show signs of rotting during post-storage ripening and after several weeks, tomatoes are unable to develop red coloring (Li et al., 2016). In the present study, tomatoes from the first harvest showed signs of

CI even at high temperature abuses, this can be due to the tomato fruit showing signs of decay that are typical of CI but not exclusive, such as tissue collapse or rot. Most of the fruit from the first and second harvests exposed to high temperature during supply chain were rated as having a higher quality than the control whereas tomatoes exposed to low temperatures were rated as having lower quality than the control. Most of the CI symptoms observed were small pits across the tomato surface with only few signs of water damage or tissue collapse.

Decay. Development of decay in tomato was minor and not significantly impacted by the time-temperature scenarios used to simulate the supply chain (Figure 8). In the first harvest, higher temperatures seemed to have contributed to an increase in fruit decay compared to the control (Figure 8A). For example, grading at 35 °C, impact cooling at 25 °C, and storage at the grower at 25 °C contributed to more signs of decay than the control (3, 16, and 4 % more decay than the control, respectively). In the second harvest, development of decay was minimally impacted by the supply chain time-temperature scenario with only one step causing a negative impact on the development of decay (Figure 8B). Compared to the control, shipping to the stores at 8 °C had a negative impact of development of decay (1 % more decay than the control).

From a consumer stand point, the supply chain steps that contributed to significant development of decay in both harvests were those that simulated high temperature abuse in the supply chain. In fact, higher temperatures were observed as more negatively impactful on tomato decay perception than colder temperature abuses (Sargent et al., 2005). Higher temperatures allow pathogens to proliferate and damage the exterior of the tomato making them appear undesirable to consumers (Olson and Freeman, 2016). In this study, decay development was minor and thus it would have passed consumer inspection (Bubzy, 2012).

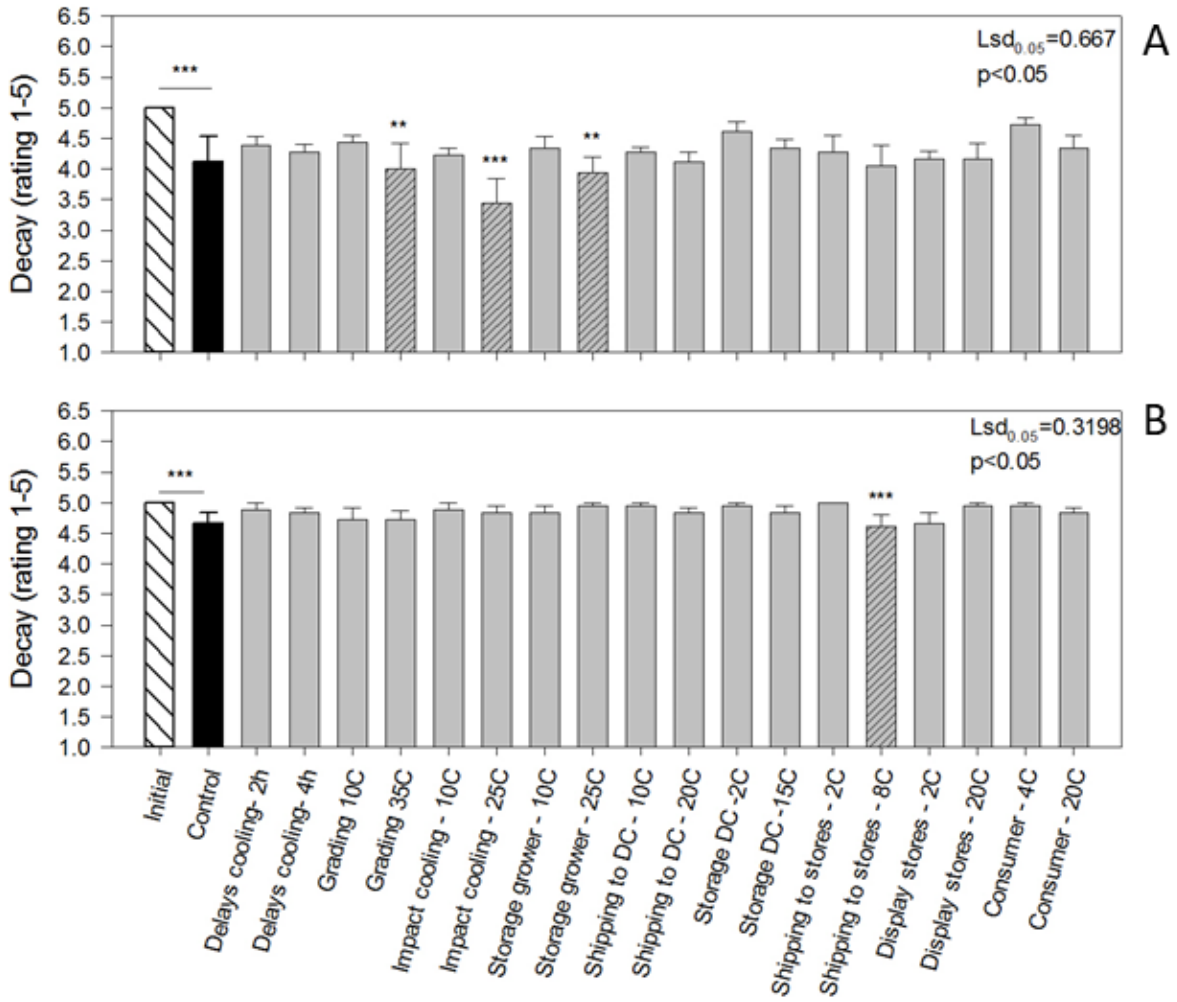

Figure 8: Impact level of each step along the supply chain on the development of decay in tomato. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (** p≤ 0.001; ***p≤ 0.0001).

Overall Quality. Overall quality of tomato fruit from both harvest was negatively impacted regardless of the supply chain steps (Figure 9). In the first harvest, overall quality of tomato significantly decreased during supply chain, regardless of the step, when compared to the control (Figure 9A). The supply chain steps that contributed to the largest decline in tomato overall quality were impact cooling at 25 °C, storage at the grower at 25 °C, and shipping to the DC at 20 °C (23, 27, and 33 % decrease compared to the control). In the second harvest, only

three steps showed a larger negative impact on tomato overall quality compared to the control (Figure 9B). Compared to the control, grading at 10 and 35 °C contributed to a decline of 9 and 12 %, respectively on tomato overall quality while storage at the grower at 10 °C contributed to a 7 % drop in tomato overall quality.

Overall, decline in the sensorial quality of tomato observed in this study agrees with previous published data (Kader et al., 1978). Decline in tomato sensory quality can be attributed to loss of water and accelerated ripening as a result from exposure to temperature abuse. Temperatures below 13 °C can lead to CI, whose symptoms include water damage, uneven ripening, and can lead to tissue collapse, which further diminishes the sensory quality of the tomato (Aghdam, 2012). Temperatures higher than 13 °C may result in accelerated ripening and senescence leading to a poor-quality tomato as well. Temperature abuses (either below or above optimum temperatures) have a significant impact on ripening causing the development of undesirable quality traits in tomato fruit. In this study, temperature abuses lead to a decline in sensory quality, which consumers rely upon to make their purchasing decisions. Objectionable tomato quality can lead to consumers avoiding purchasing, or throwing away fruit, that appears to be undesirable but otherwise edible. Consumers avoiding edible tomatoes because of poor appearance quality is important and may result in increased waste at the retail or consumer levels (Porat et al., 2018).

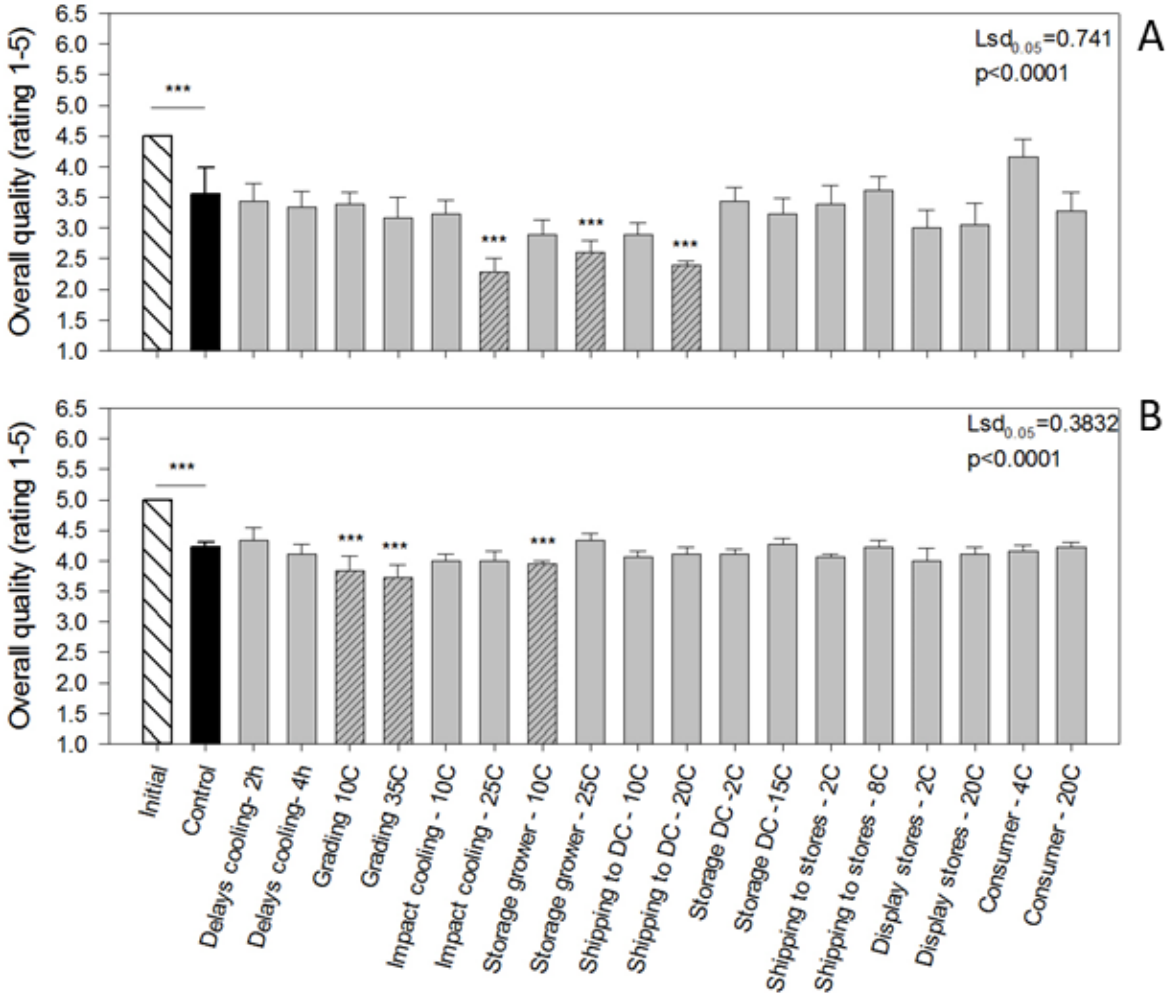

Figure 9: Impact level of each step along the supply chain on tomato overall quality. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (***p≤ 0.0001).

Instrumental Color

In the first harvest, several supply chain steps did not have a significant impact on the brightness of tomatoes, with in some instances fruits remaining brighter (higher L*) than the control (Figure 10A). This may have been due to the quality and the ripening stage of the fruit from the first harvest which had a redder color. Nonetheless, there were three steps that were

negatively impacted by the supply chain. When compared to the control, impact cooling at 25 °C and consumer storage at 4 °C caused a decrease of 6 % in brightness (lower L*; darker fruit), while shipping to the stores at 8 °C caused a 2 % decrease in L* values. Unlike in the first harvest, in the second harvest brightness (L*) of tomato fruit was less impacted by the supply chain (Figure 10B). However, there were three steps negatively impacted by the supply chain; impact cooling at 10 °C (2 % decrease compared to the control), storing at the grower at 25 °C (2 % decrease compared to the control), and displaying at the store at 20 °C (3 % decrease compared to the control). When compared to the control, impact cooling at 10 °C and storing at the grower at 25 °C caused a 2 % decrease in brightness, whereas displaying at the store at 20 °C caused a 3 % decrease in L* values.

In the first harvest, the effect of the supply chain on development of tomato red color (a* value) was minimal, as most fruit retained a less red color when compared to the control (Figure 11A). This could have been a result of tomatoes being at an already well developed red color making the changes in redness subtler. Despite this, there were some supply chain steps that had a negative impact on a* value of the fruit. When compared to the control, displaying tomatoes in the store at 20 °C increased redness (higher a* value) of the fruit by 3 % whereas consumer storage at 4 °C caused an increase of 18 % in redness of the fruit. Color of tomato fruit from the second harvest was more impacted by the supply chain showing more red coloring (higher a* values). Therefore, in the second harvest, several supply chain steps had a significant negative impact on redness on the fruit (Figure 11B). For example, compared to the control, storing tomato at the grower at 10 °C increased a* values by 12 %, shipping to the DC at 10 °C increased redness (higher a* values) of tomato by 10 %, and shipping to the store at 2 °C increased redness by 7 %.

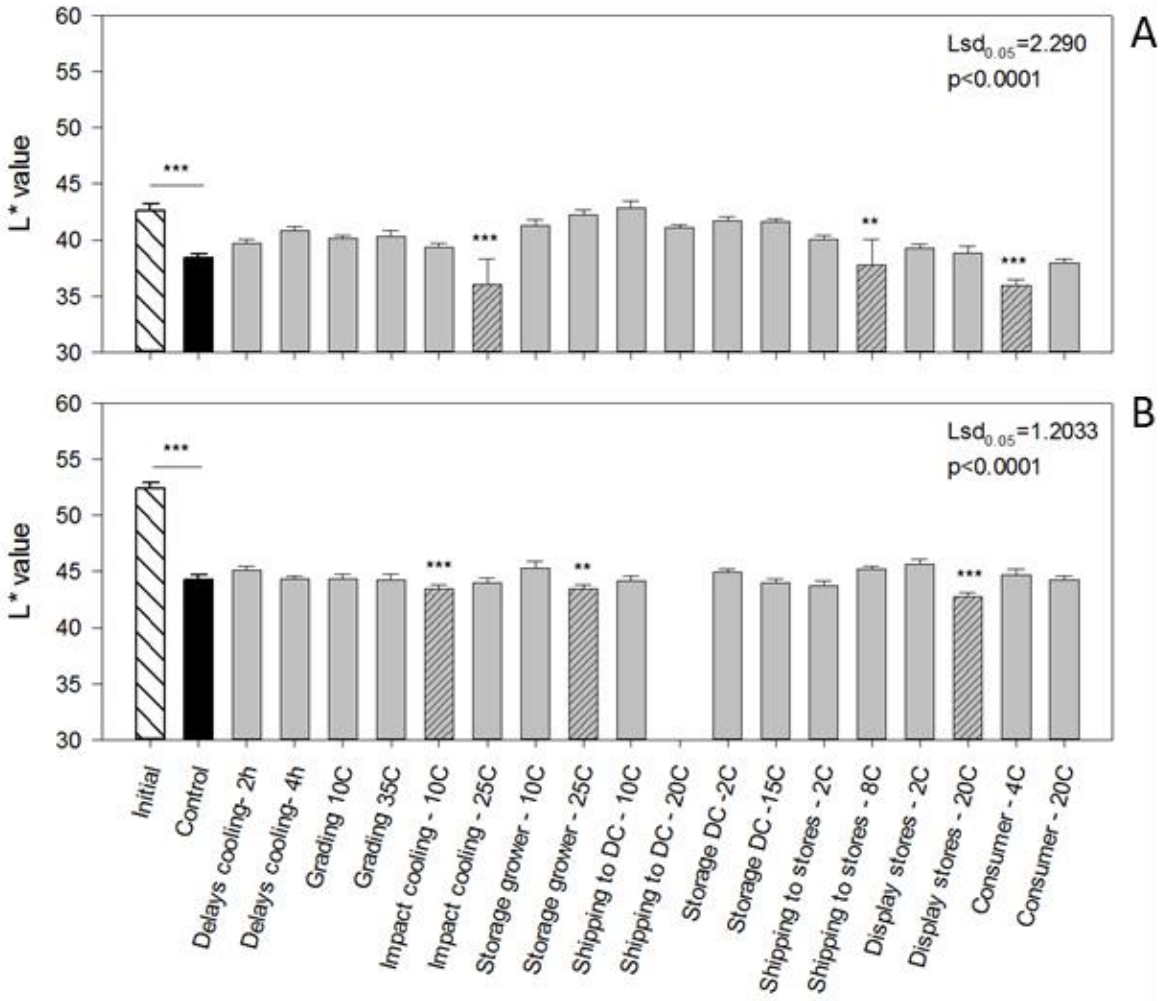

Figure 10: Impact level of each step along the supply chain on tomato L* value. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (**p≤ 0.001***p≤ 0.0001).

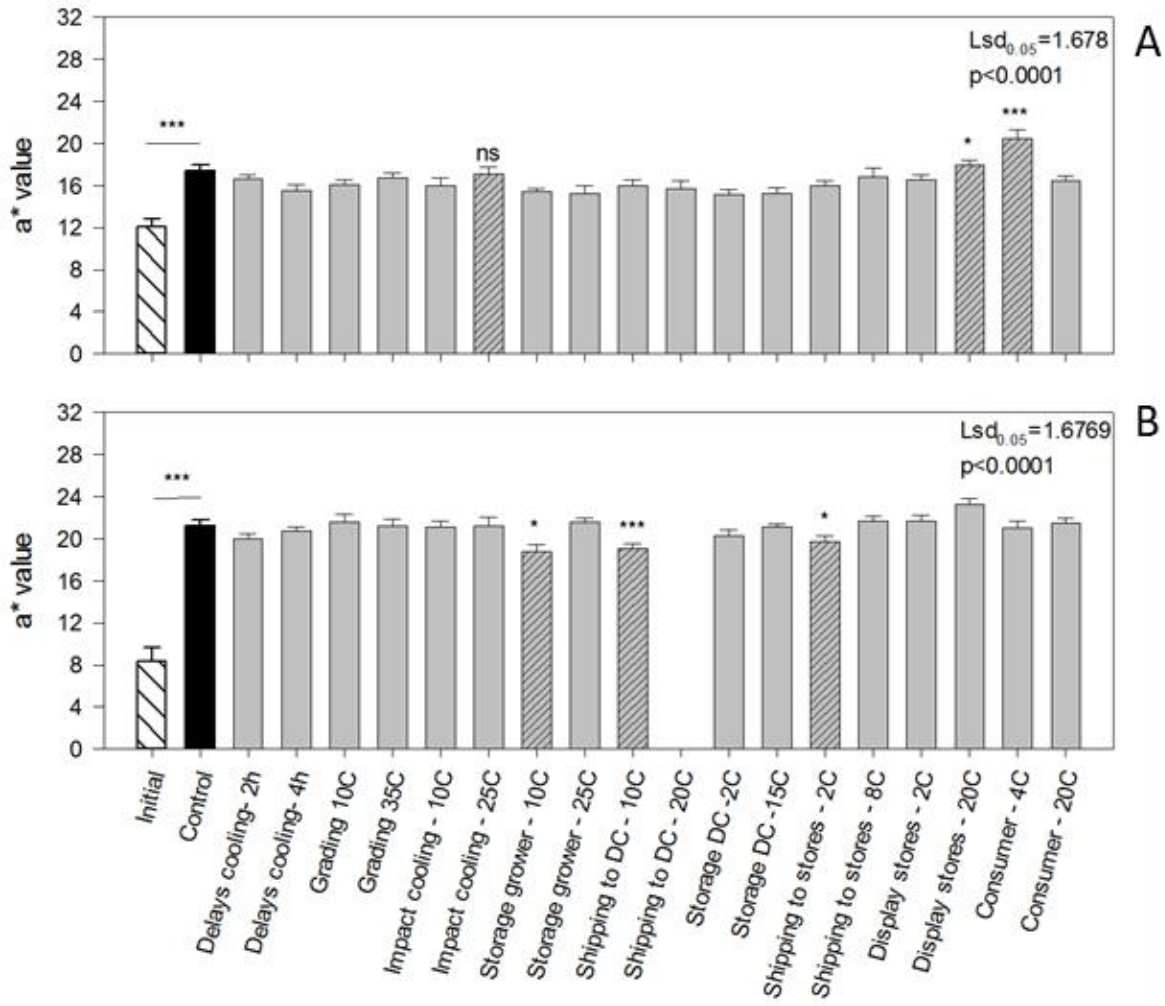

Figure 11: Impact level of each step along the supply chain on tomato a* value. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; *p≤ 0.05***p≤ 0.0001).

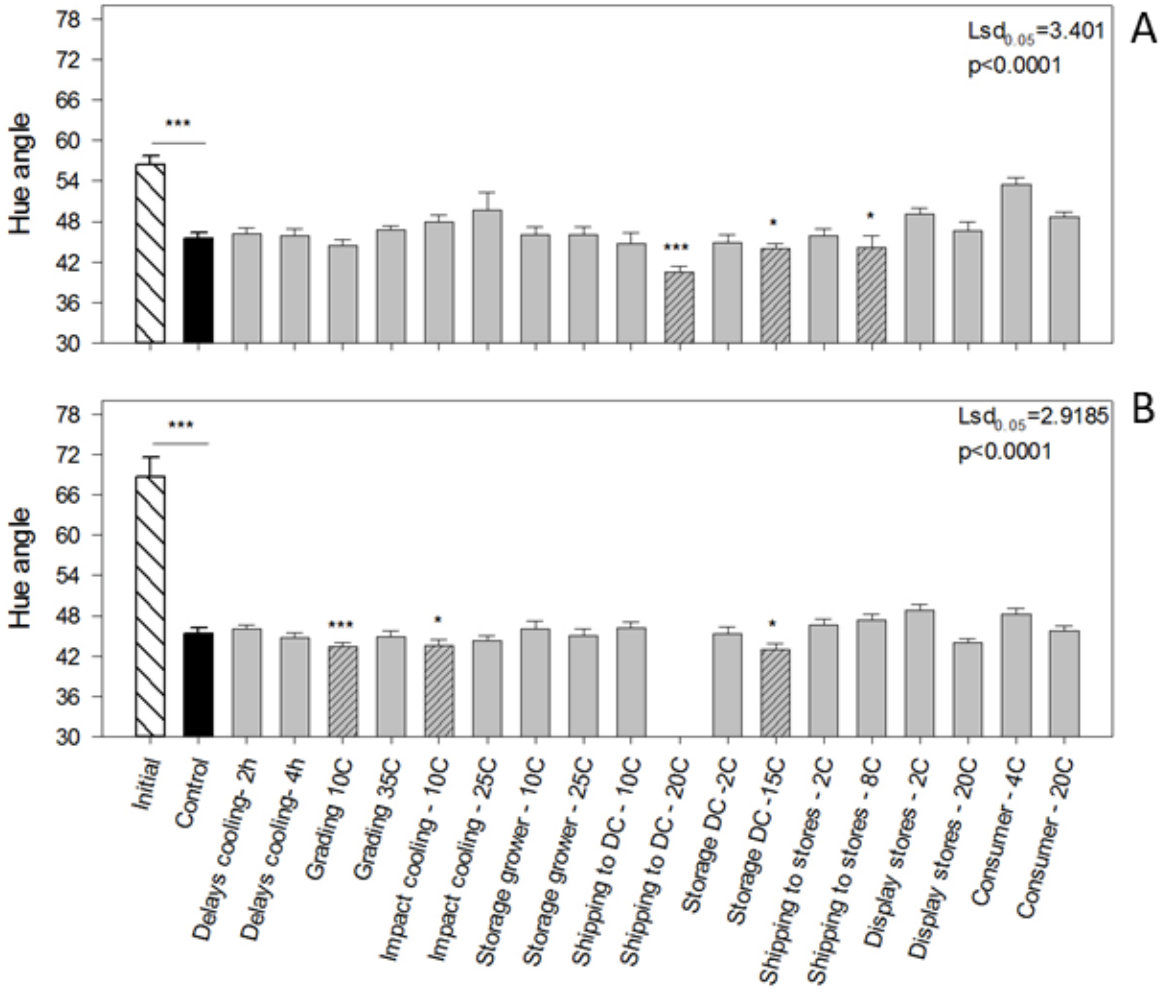

Figure 12: Impact level of each step along the supply chain on tomato hue angle. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (*p≤ 0.05; ***p≤ 0.0001).

In both harvests the effect of supply chain on hue angle was consistent and showed smaller deviations from the control (Figure 12). However, there were several supply chain steps that caused tomatoes to turn darker red (lower hue values compared to the control). In the first harvest, shipping to the DC at 20 °C decreased hue angle by 11 % (redder), and both storage at the DC at 15 °C and shipping to the stores at 8 °C decreased hue angle by 3 % when compared to the control (Figure 12A). Results from the second harvest followed the same trend of the first

harvest with smalls deviations (Figure 12B). When compared to the control, grading at 10 °C and impact cooling at 10 °C resulted in a 4 % decrease in hue angle whereas storage at the DC caused a 5 % decrease in hue angle (redder).

Overall, in both harvests, supply chain condition seemed to have had a slight impact on color of tomato fruit. While there was a greater variability in the brightness (L* value) of the fruit, there seemed to be a less rapid evolution of red coloring (a* and hue values). For each step of the supply chain, tomato fruit exposed to low temperatures during supply chain had higher a* and lower hue values (less red) than fruit exposed to high temperature abuse within the same step. Previous work showed that lower temperatures impact color development in tomatoes by slowing the production of lycopene and other carotenoids resulting in a less red tomato (Farneti et al., 2012). Conversily, tomatoes stored at high temperatures develop a deep red color due to an accelerated synthesis of lycopene (Getinet et al., 2008). In general, consumers prefer a red ripe tomato and do not value tomatoes with a pink or deep red color as these are percieved as under or overripe, respectively (Oltman et al., 2014).

Texture Analysis

In the first harvest, the texture of the tomatoes was not negatively impacted by the supply chain. That is, there was no significant difference between texture of tomato from the control treatment and that of fruit exposed to supply chain regardless of the step (Figure 13A). Texture of tomato fruit from the second harvest showed a different trend compared to that of the first harvest (Figure 13B). Overall, when compared to the control, the supply chain steps that caused the largest decline in firmness were impact cooling at 25 °C and consumer storage at 20 °C which resulted in 8 % decrease in firmness, and storage at the growers at 25 °C which resulted in a 15 % decrease in firmness.

Tomato firmness is an important attribute because it allows consumers to determine quality of a tomato and drives purchasing decisions (Oltman et al., 2014). Tomato texture is

strongly corelated to ripeness; as tomatoes ripen, significant changes to the cell walls are made by hydrolytic enzymes resulting in a softer tomato (Lunn et al., 2013). In this study, tomatoes that underwent low temperature abuses during supply chain scored as consistently firmer that those stored at high temperatures. The most critical supply chain steps in the second harvest were those where temperatures were above 13 °C which agrees with results published by others literature (Brashlyanova et al., 2014).

Weight Loss

In this study, there was a significant loss in tomato weight regardless of the supply chain step (Figure 14). Weight loss of the tomato fruit increased in most steps when compared to the control, meaning that the fruit lost a larger percentage of its weight after supply chain. In the first harvest, three steps showed a significantly larger negative impact on tomato weight loss (Figure 14A). That is, tomato fruit exposed to shipping to the DC at 20 °C lost 87 % more weight compared to the control, displaying in the store at 2 °C lost 54 % more weight, and fruit exposed to consumer storage at 20 °C lost 53 % more weight when compared to the control. In the second harvest, temperature abuses resulted in an overall increase in weight loss as well, with three steps showing the largest impact (Figure 14A). Impact cooling at 10 °C resulted in 87 % increase in tomato weight loss compared to the control, shipping to the DC at 20 °C had a 73 % increase compared to the control, and shipping to the stores at 2 °C resulted in a 134 % increase of weight loss when compared to the control.

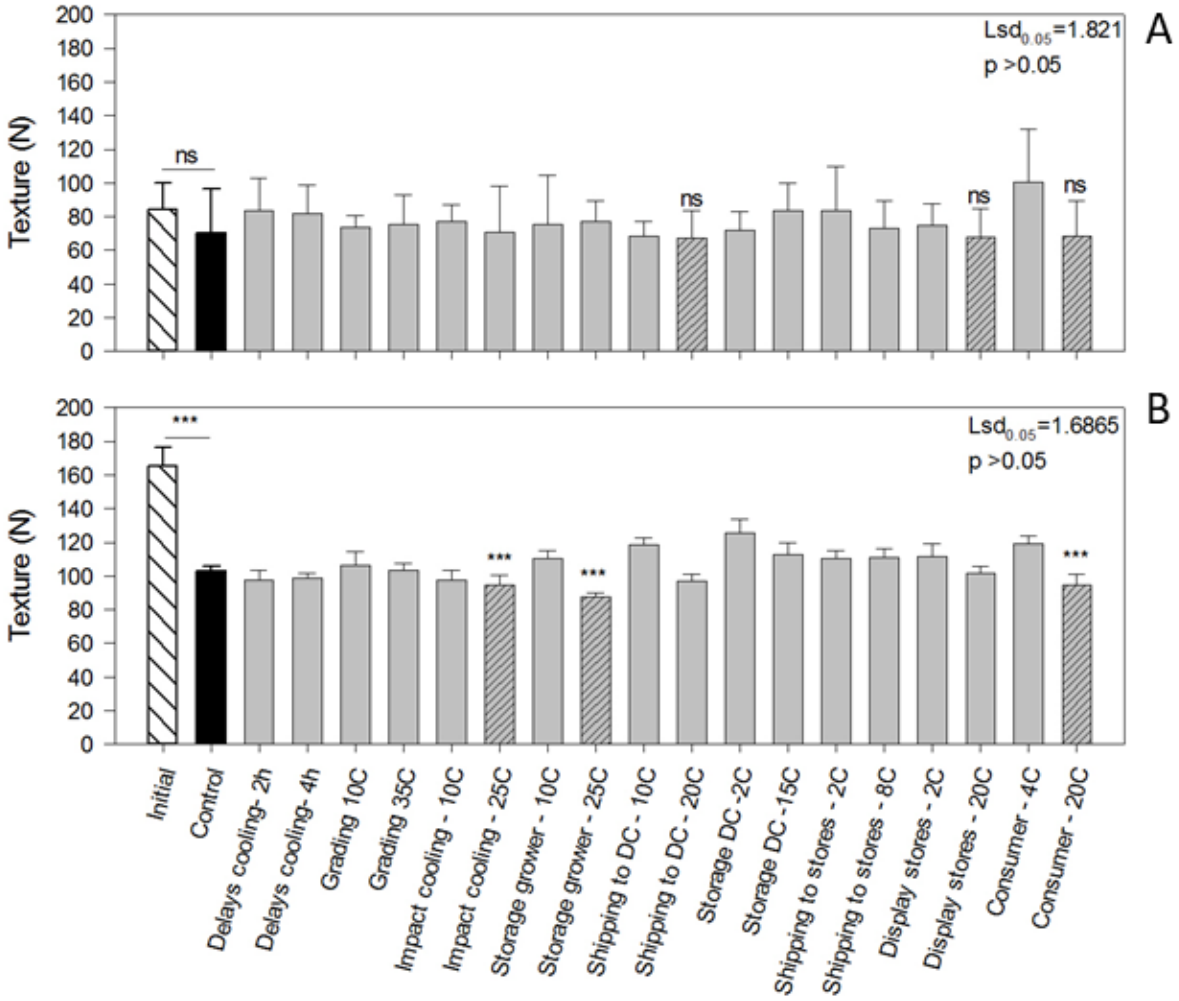

Figure 13: Impact level of each step along the supply chain on tomato texture. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ***p≤ 0.0001).

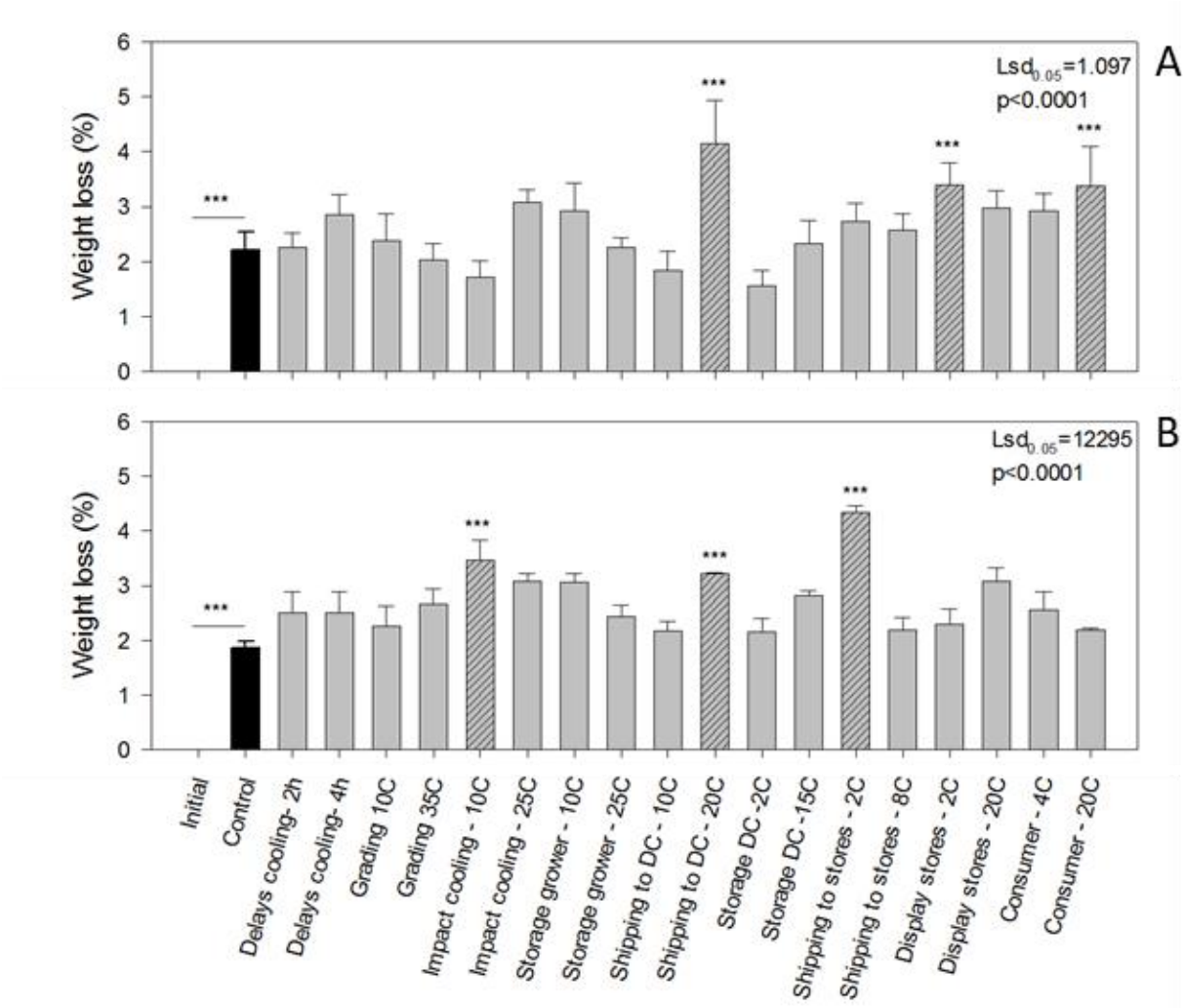

Figure 14: Impact level of each step along the supply chain on tomato weight loss. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ***p≤ 0.0001).

Differences in weight loss between supply chain steps may have been a result of variations in morphological characteristics of the fruit (Nunes and Edmond, 2007). Most weight loss of stored fruit is caused by transpiration that results in tomato fruit losing water resulting in a decline in chemical compounds namely sugars, and bioactive compounds such as vitamin C (ascorbic acid) and carotenoids. Since bioactive compounds such as ascorbic acid are water soluble,

when water loss increases above a certain level the amounts of these compounds significantly decrease.

pH, Acidity and Soluble Solids Content (SSC)

Tomato pH was not significantly impacted by any of the time-temperature treatments used to simulate the supply chain step. Fruit pH from both harvests did not significantly change when compared to the control meaning that the tomatoes keep a consistent pH after supply chain (Figure 15).

In the first harvest, tomato acidity did not significantly change when compared to the control with the exception of displaying at the store at 2 °C which resulted in a 21 % decrease in tomato acidity when compared to the control (Figure 16A). In the second harvest, compared to the control, storage at the growers at 10 °C resulted in 36 % decrease in tomato acidity, shipping to the DC at 20 °C and shipping to the stores at 2 °C caused a 32 and 34 % decrease in tomato acidity, respectively (Figure 16B).

Tomato SSC was negligibly impacted by time-temperature abuses during supply chain, with only two step having significant negative impact on SSC of tomato fruit from both harvests (Figure 17). In the first harvest, impact cooling at 25 °C resulted in a 5 % decrease in tomato SSC when compared to the control (Figure 17A) whereas in the second harvest shipping to stores at 2 °C caused a 16 % decrease in tomato SSC (Figure 17B).

Overall in both harvests, the supply chain had a negligible impact on pH, and minor impact on tomato acidity and SSC. These chemical attributes are closely related to ripening of tomato fruit. For example, pH has been shown to increase as tomato ripens while acidity and SSC have been shown to decrease as ripening advances (Anthon et al., 2011). Temperatures that induce chilling injury in tomato are known to negatively impact tomato acidity with higher temperatures causing an equivalent degree of damage to the flavor profile (Tigist et al., 2013).

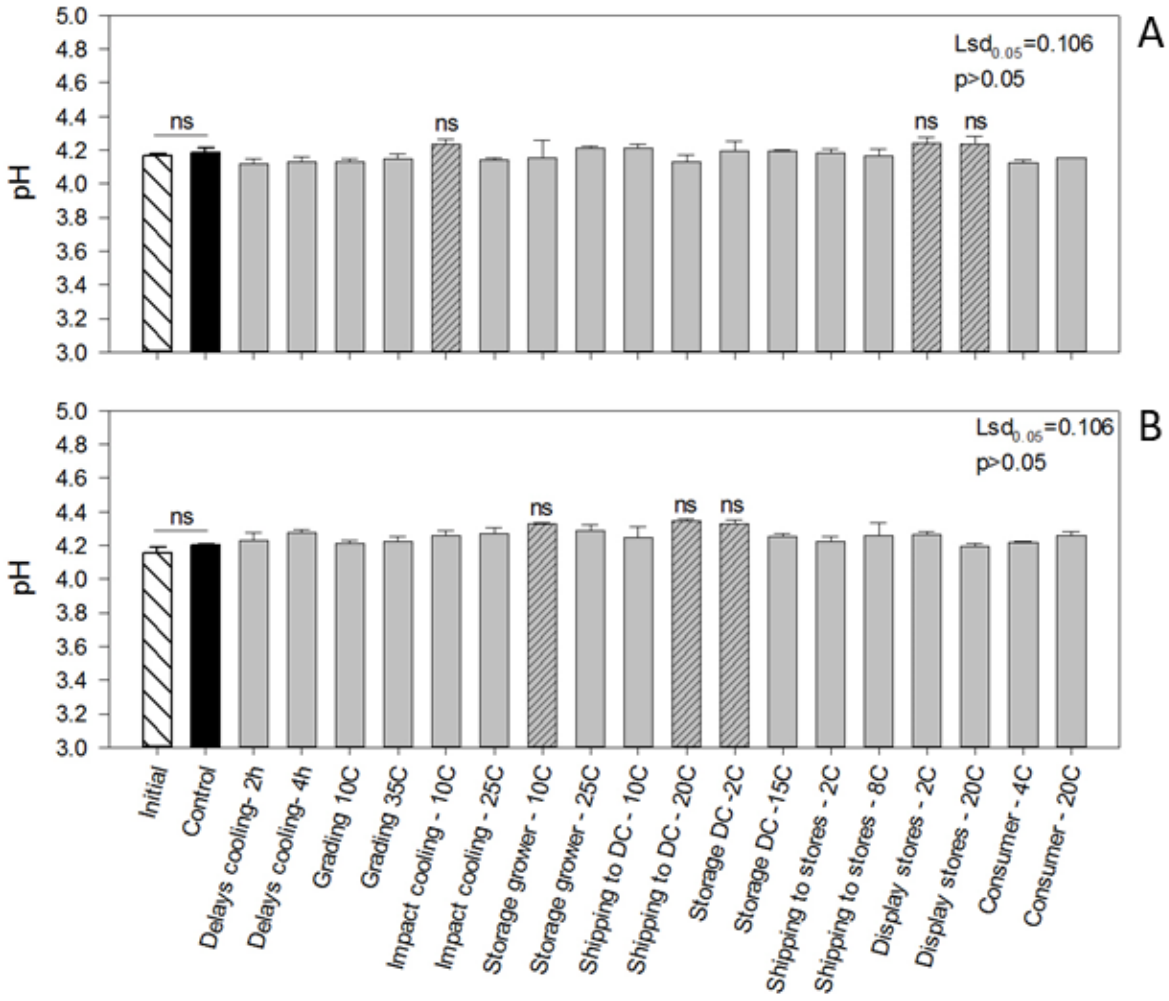

Figure 15: Impact level of each step along the supply chain on tomato pH. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant).

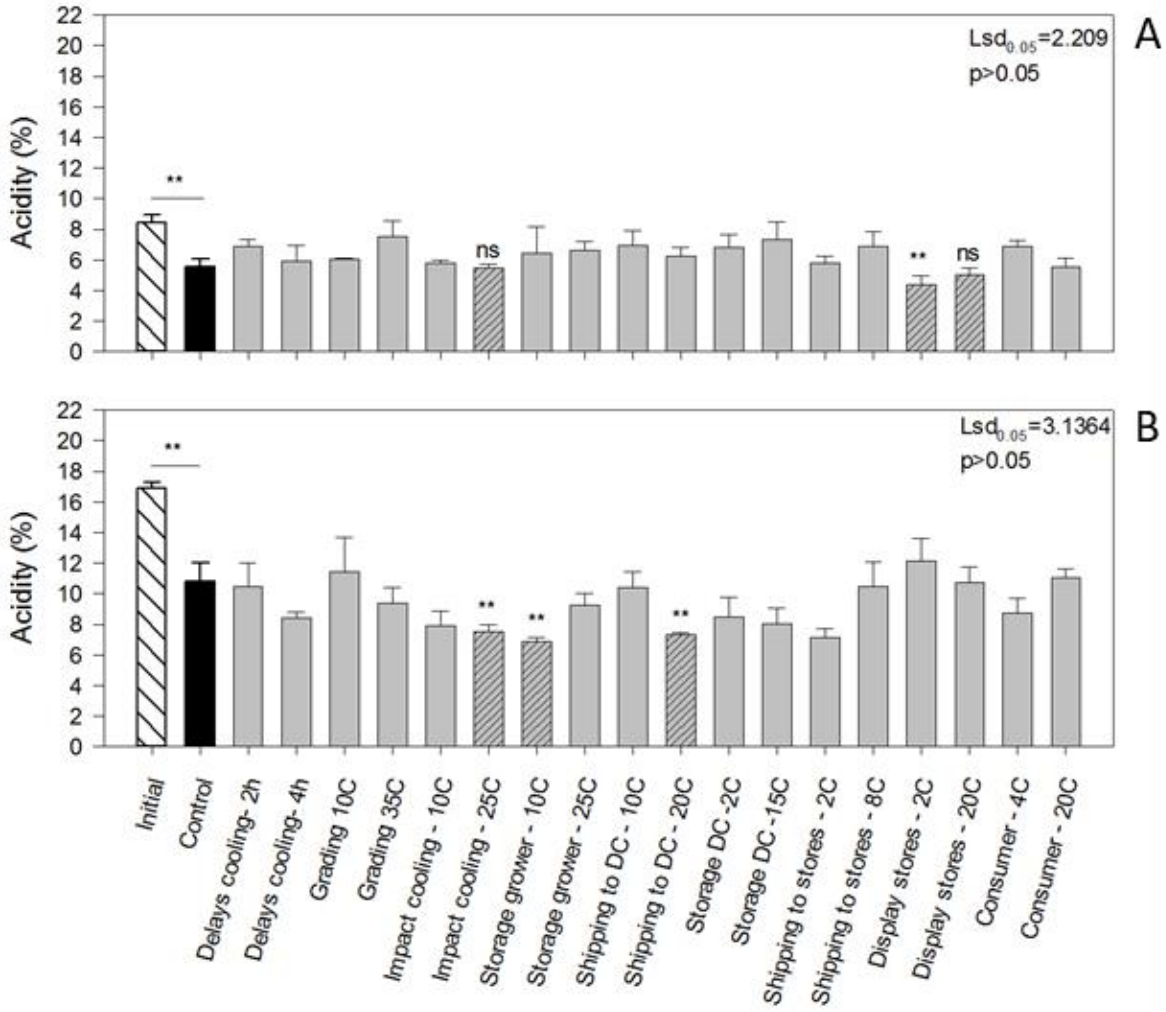

Figure 16: Impact level of each step along the supply chain on tomato acidity. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; **p≤ 0.001).

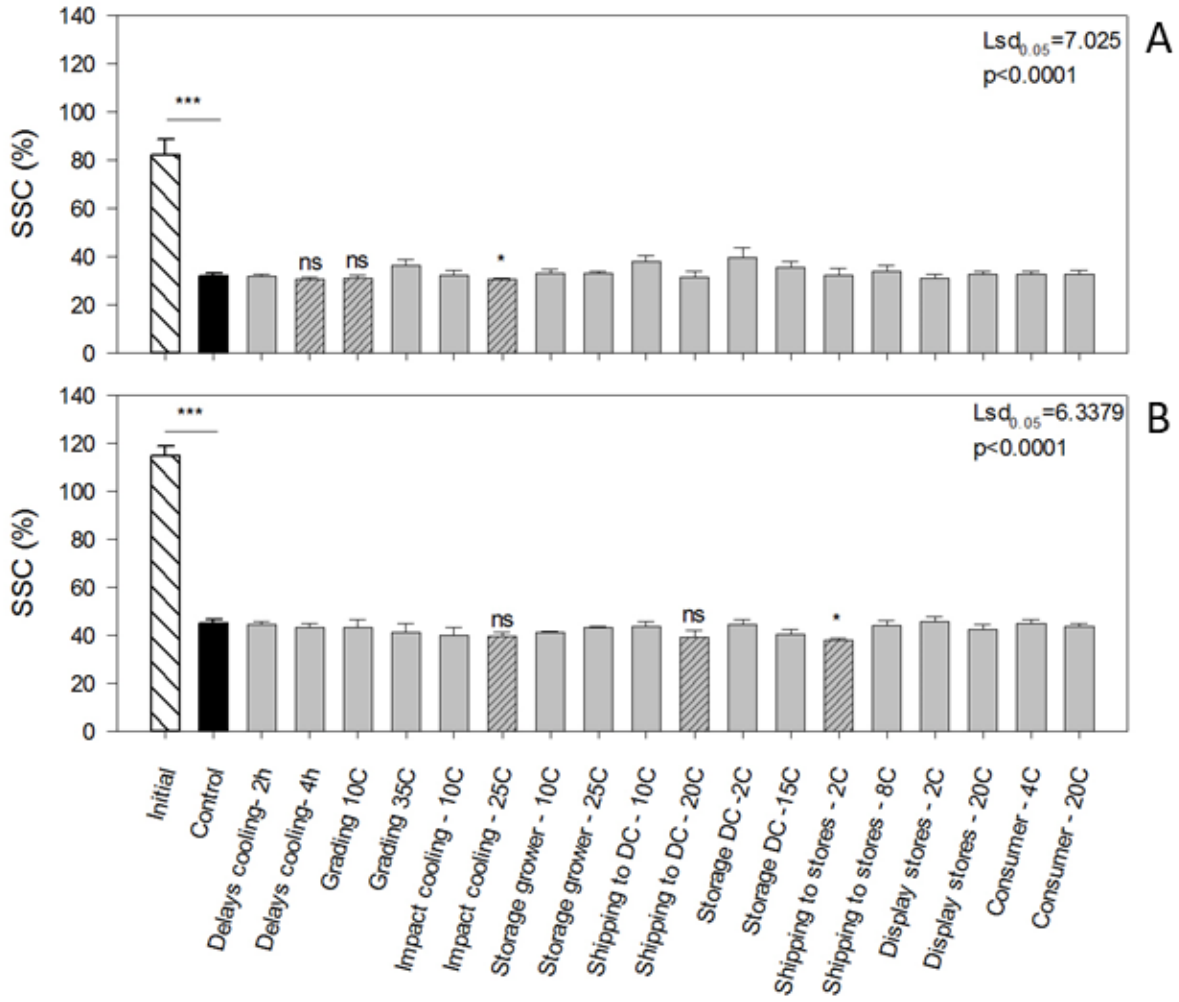

Figure 17: Impact level of each step along the supply chain on tomato soluble solids content (SSC). (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; *p≤ 0.05).

Total and Individual Sugar Contents

In general, in the first harvest, tomato fructose content decreased after supply chain (Figure 18A). When compared to the control, shipping tomato fruit to the DC at 20 °C caused a 14 % decrease in fructose levels whereas consumer storage at 4 and 20 °C resulted in a

decline of 32 %. In second harvest, compared to the control, the steps that showed major impact on tomato fructose levels were impact cooling at 25 °C (15 % decrease), shipping to the stores at 2 °C (19 % decrease), and displaying at the stored at 20 °C (23 % decrease) (Figure 18B).

Glucose content of tomatoes was negatively impacted by temperature abuses during supply chain (Figure 19). In the first harvest, shipping to the DC at 20 °C caused a 20% decrease in tomato glucose content when compared to the control (Figure 19A). In addition, consumer storage at 4 and 20 °C caused an 18 and 16 % decrease in tomato glucose content. In the second harvest, the three supply chain steps with most negative impact in tomato glucose content were impact cooling at 25 °C (18 % decrease), shipping to the stores at 2 °C (21 % decrease) and displaying at the stored at 20 °C (17 % decrease) (Figure 19B).

Total sugar content (i.e., the sum of fructose and glucose) of tomato fruit was also affected by supply chain conditions (Figure 20). That is, in the first harvest, shipping to the DC at 20 °C resulted in a loss of 17 % of tomato total sugars, and consumer storage at 4 and 20 °C resulted in a drop of 26 % and 24 %, respectively (Figure 20A). In the second harvest, compared to the control, impact cooling at 25 °C caused a 17 % decrease in total sugars whereas shipping to stores at 2 °C and displaying at the store at 20 °C both caused a 20 % decrease in the total sugar content of tomato fruit (Figure 20B).

Overall, in this study decline in tomato sugar content was consistent with previously published results. For example, Beckles et al. (2011) showed that there was a steady decline in tomato sugar content when fruit were exposed to 10 °C. In this study it was found that exposure of tomato fruit to high temperature during supply chain resulted in a more significant decrease in sugar content when compared to exposure to low temperatures. This can be attributed to tomatoes ripening faster and consuming more sugar in the process due to accelerated respiration rate (Walker and Ho, 1977). Temperatures below 13 °C slow down tomato ripening and thus reducing the rate of sugar breakdown (Kader et al., 1978). Low sugar content in

tomato fruits can lead to consumer rejection as a highly desirable tomato is one that is sweet and flavorful (Tieman et al., 2012).

Total Ascorbic Acid Content

Exposure of tomatoes to supply chain conditions had an unexpected effect on the levels of total ascorbic acid (AA) with some steps causing an increase when otherwise an increase in AA would be expected (Figure 21). Nonetheless, when compared to the control, AA levels in tomato fruit from the first harvest were significantly reduced by cooling tomato at 25 °C and storage at the growers at 10 °C (15 % decrease) whereas shipping to the stores at 2 °C lead to a 25 % decrease in AA content (Figure 21A). In the second harvest, shipping tomato fruit to the stores at 2 °C caused a decrease of 16 % in AA levels while consumer storage at both 4 and 20 °C caused a 4 and 5 % decrease, respectively (Figure 21B).

Unlike in the present study, Toor and Savage (2005) reported that storing tomato fruit for one week at 7 or 25 °C did not affect AA levels. Stevens et al. (2008) found similar results at 4 °C. The authors suggested that tomatoes are high in titratable acidity resulting in a favorable, non-oxidative environment for AA. Mellidou et al. (2012) found similar results with AA levels remaining stable when tomato was exposed to temperature fluctuations. On contrary, results from the present study showed that certain steps of the supply chain have a significant impact on the AA levels in tomato fruit. It is also known that AA levels are tightly correlated to the ripening of the tomato fruit (Ye et al., 2015). Time-temperature conditions used during tomato supply chain simulation may have cause a disruption in the normal ripening process and possibly explain the variability in AA levels.

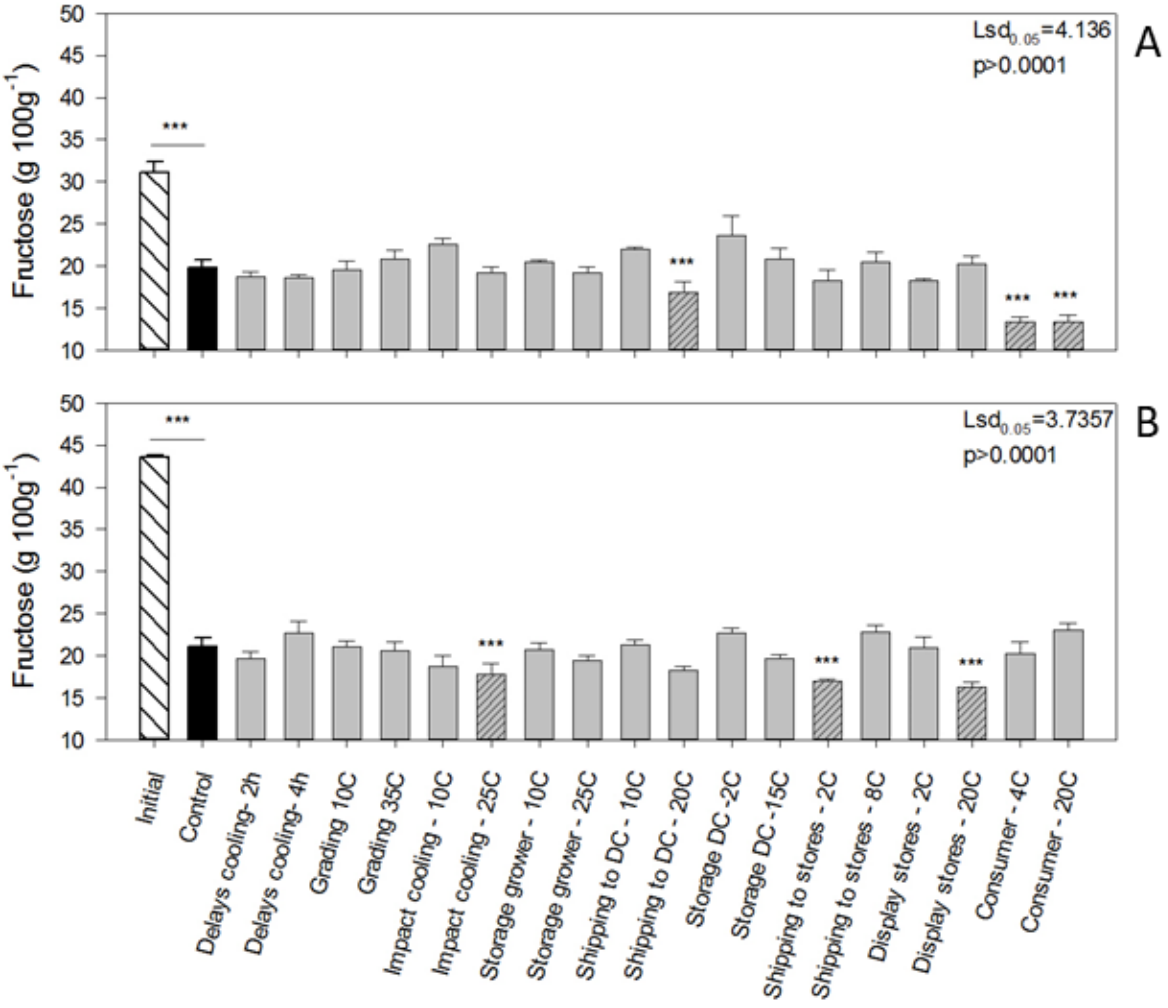

Figure 18: Impact level of each step along the supply chain on tomato fructose content. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (***p≤ 0.05).

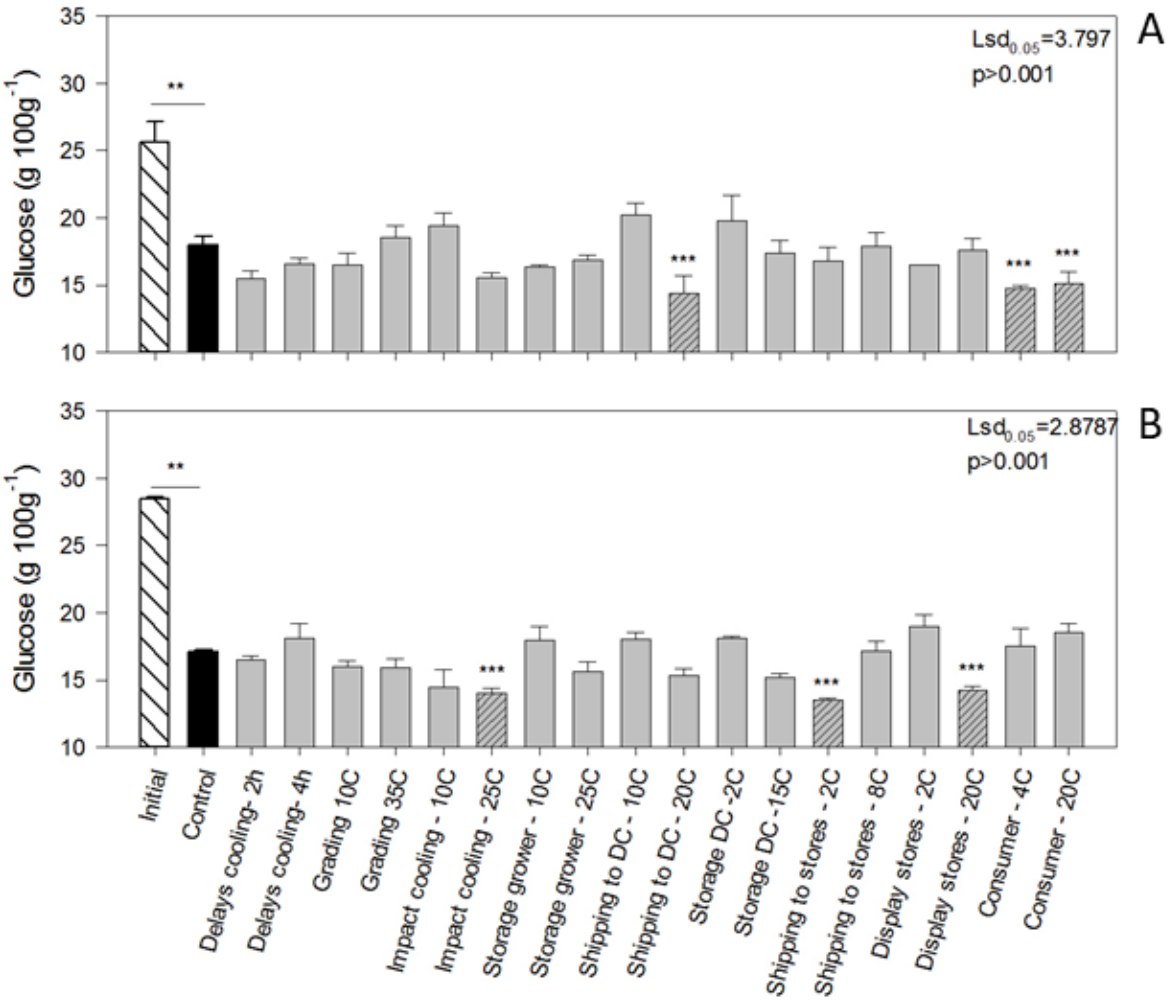

Figure 19: Impact level of each step along the supply chain on tomato glucose content. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (**p≤ 0.001; ***p≤ 0.0001).

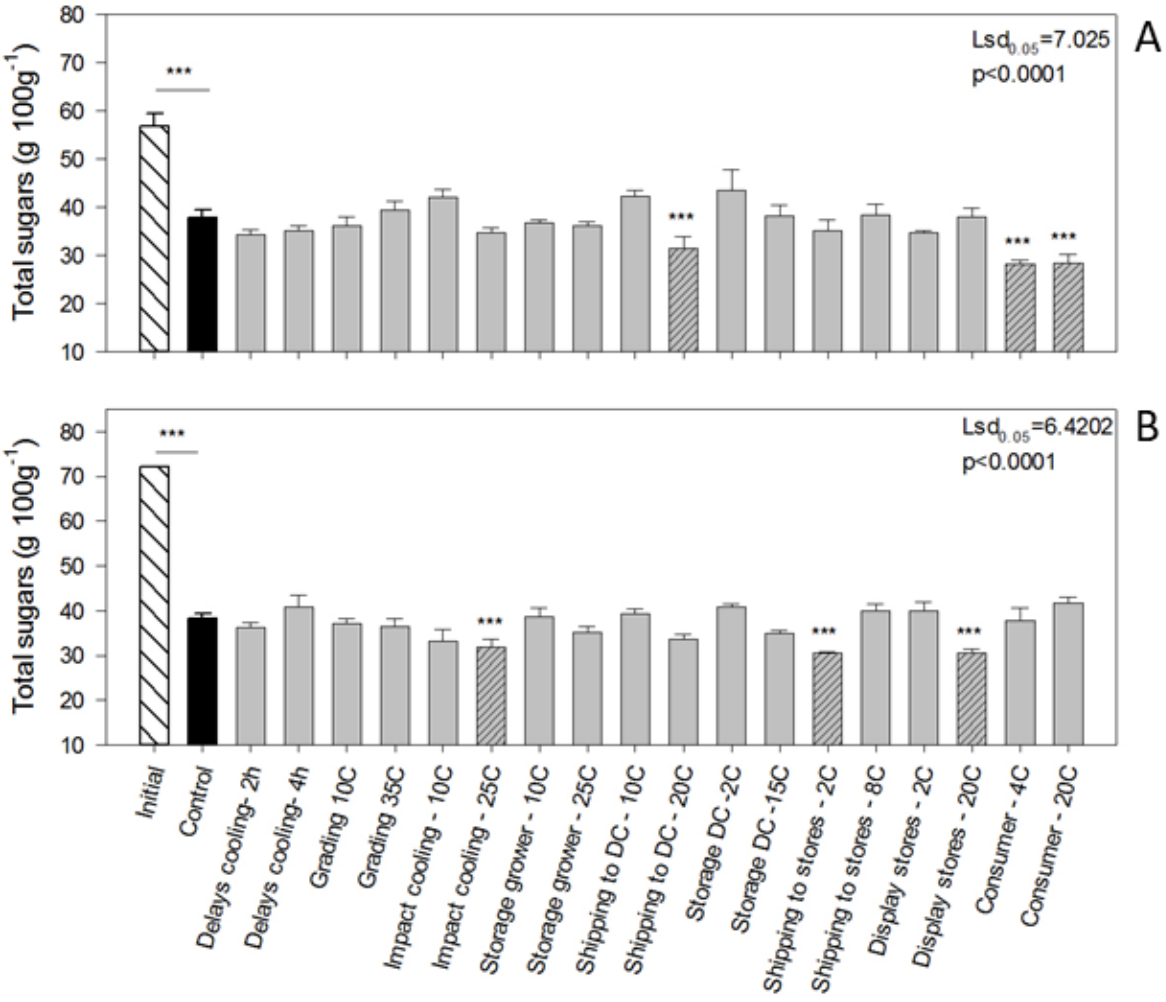

Figure 20: Impact level of each step along the supply chain on tomato total sugar content. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (***p≤ 0.0001).

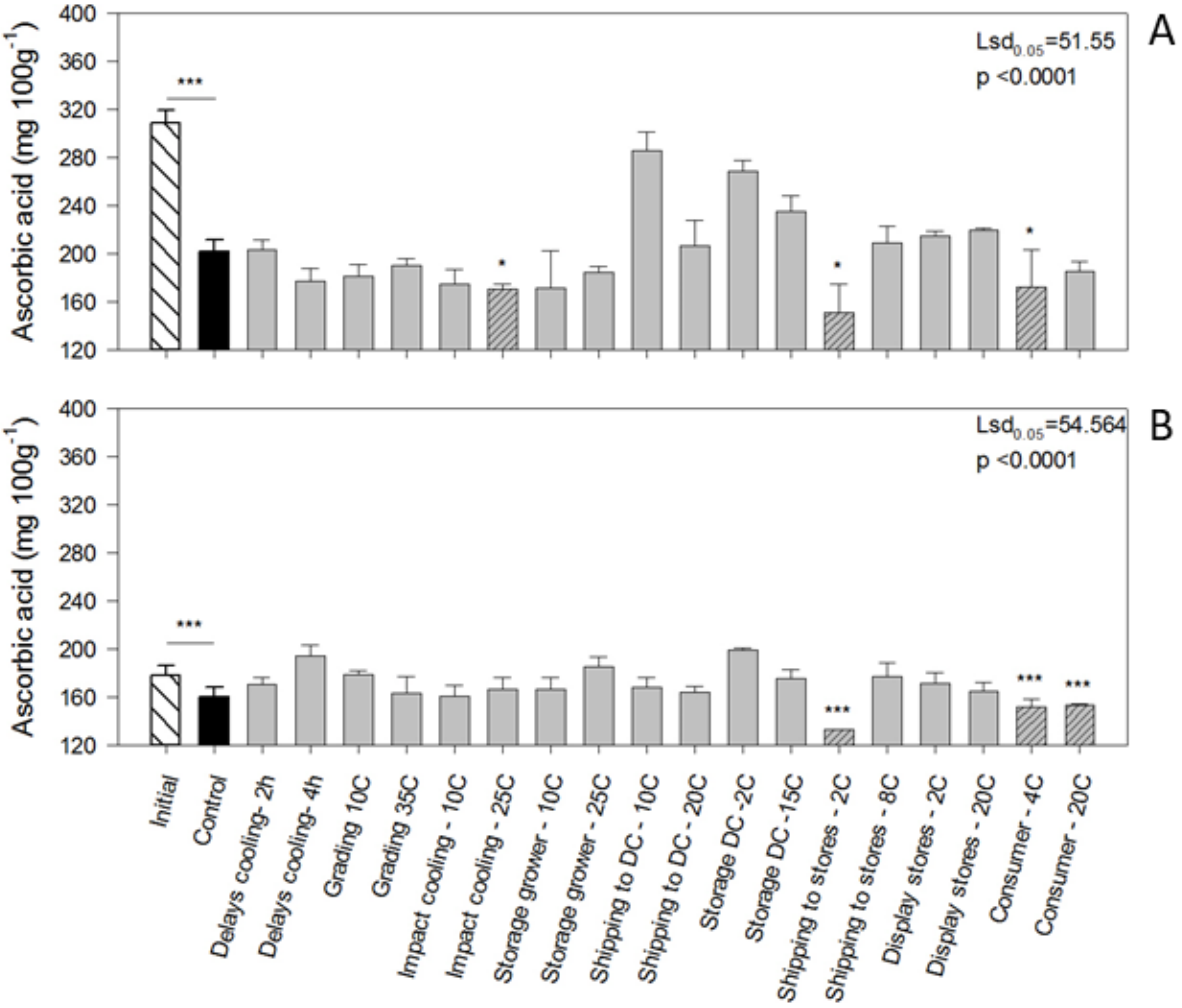

Figure 21: Impact level of each step along the supply chain on tomato ascorbic acid content. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (*p≤ 0.05; ***p≤ 0.0001).

Total carotenoid content

Carotenoid content in tomato fruit from the first harvest was variably impacted by the supply chain (Figure 22A). That is, compared to the control, several supply chain time-temperature treatments resulted in higher levels of carotenoid content. Nevertheless, impact cooling at 25 °C reduced carotenoid content by 8 % when compared to the control and consumer storage at 4 °C reduced carotenoid content by 21 %. In the second harvest, storage

at the DC at 15 °C caused a 28 % decrease in carotenoid content, shipping to the stores at 2 °C caused a 43 % decrease, and consumer storage at 4 °C caused a decrease of 31 % (Figure 22B).

Overall in both harvests, tomato total carotenoid levels were impacted by the conditions used to simulate supply chain. In the first harvest there was a lower impact from supply chain on carotene contents, however, this was most likely a result of the tomatoes being received at a more advanced ripening stage. Carotene content is directly tied to ripeness in tomatoes and in general increase as tomato ripens (Getinet et al., 2008). Previous studies have shown that in tomato fruit, temperature abuses have a negative impact in carotene synthesis (Farneti et al., 2012). Temperatures that result in CI significantly impact carotenoid synthesis, resulting in substantially lower carotene levels than tomatoes stored in optimal temperature conditions (Farneti et al., 2012; Fattore et al., 2016; Zhang et al., 2016). In this study, carotene levels were lower in tomatoes exposed to low temperatures during supply chain compared to that exposed to high temperature abuse.

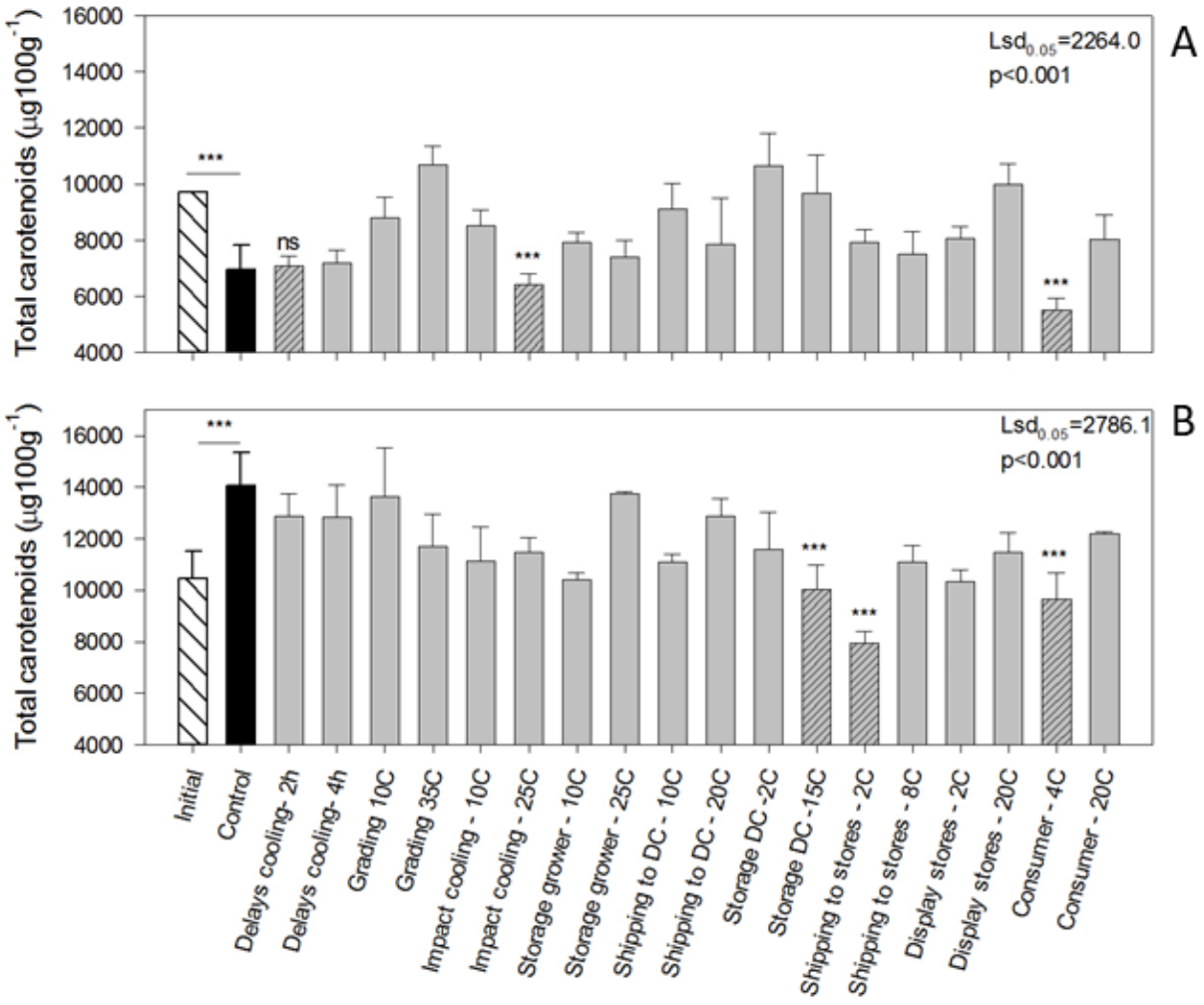

Figure 22: Impact level of each step along the supply chain on tomato total carotenoid content. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ***p≤ 0.0001).

Critical Supply Chain Steps

To determine the critical steps along the supply chain, where quality of tomatoes showed the largest decline, the number of times a supply chain step cause a decline in a quality attribute was recorded. In the first harvest the top three most negatively impactful supply chain steps were shipping to the DC at 20 °C (step 12), impact cooling at the growers at 25 °C (step 10) and

storing at the consumer at 4 °C (step 19). In the first harvest shipping to the DC at 20 °C had a negative impact on 12 quality attributes (color, firmness, shriveling, CI, overall, chroma, hue, texture, weight loss, fructose, glucose, and total sugars) of the 21 attributes measured in this study. Impact cooling at 25 °C had a negative impact on 9 quality attributes (shriveling, CI, decay, overall, L*, a*, acidity, SSC, and carotenoids) of the 21 attributes measured in this study. Consumer storage at 4 °C had a negative impact on 6 quality attributes (L*, a*, carotenoids, fructose, glucose, and total sugars) of the 21 attributes measured.

In the second harvest, the top three critical steps were displaying at the stores at both 2 °C (step 17) and 20 °C (step 18), and shipping to the stores at 2 °C (step 15). Displaying at the stores at 20 °C negatively impacted 6 quality attributes (color, L*, a*, chroma, glucose, and fructose) of the 21 attributes. Displaying at the store at 2 °C negatively impacted 5 quality attributes (shriveling, decay, a*, b*, and chroma) of the 21 attributes. Finally, shipping to the store at 2 °C impacted 6 quality attributes (SCC, acidity, carotene, glucose, fructose, and total sugars) of the 21 attributes.

Overall, shipping to the stores at 2 °C and impact cooling at 25 °C had a negative impact in tomato quality in both harvests and were therefore considered the most impactful steps where decline in tomato quality was highest. Previous reports on estimated losses of tomatoes during supply chain showed that grading causes a loss of 7 %, packing causes a loss of 3 to 5 %, and retail amounts to 2 to 3 % loss of all tomatoes (Mena et al., 2011). This adds up to be 15 % of all tomatoes produced. Finally, it was suggested that as an effort to reduce food waste, all individuals involved in the food supply chain, particularly retailers and consumers, would benefit for educational programs on how to handle, store, purchase, and use FFV.

CHAPTER FOUR:

USING BIOCHEMICAL APPROACHES TO DETERMINE THE IMPACT OF CHILLING AND NON-CHILLING TEMPERATURES ENCOUNTERED DURING SUPPLY CHAIN ON TOMATO QUALITY

Introduction

Tomatoes are an important crop in the United States because they generate a significant income of approximately 1.2 billion dollars annually (USDA, 2015). Consumers preference is for tomatoes with the highest appearance quality (i.e., size, shape, color, texture and absence of defects) and, the most important quality traits are a fruit with a bright red color and no signs of decay, shriveling or over ripeness (Oltman et al., 2014). Flavor is impacted by the levels of sugars and organic acids and, is the next most important quality attribute after appearance. Tomatoes are also a good source of vitamin C (ascorbic acid) and carotenoids. Ascorbic acid is considered a powerful antioxidant and an essential micronutrient (Oltman et al., 2014) while carotenoids are responsible for the red coloration of the tomato and are important precursors to other vital compounds such as vitamin A (Khachik et al., 2002). Together sugars, vitamin C, and carotenoids are considered important biochemical markers of tomato fruit overall quality and good indicators of flavor and nutritional quality (Oltman et al., 2014).

Impact cooling, sometimes referred to as postharvest cooling, occurs after the farmer has harvested the crop and cleaned the tomato fruits. This cooling time and the temperature during cooling are important factors as tomatoes are sensitive to chilling temperatures thus should not be exposed to temperatures below 13 °C (Sargent et al., 2005). Conversely, at this stage, tomatoes can be cooled off at a temperature that is higher than the optimal temperature of

13 °C. From the from tomatoes are shipped to the distribution center where they can be stored for an average of 72 hours (Hall et al., 2009). From the distribution center, tomatoes are then shipped to stores. During shipping to the stores, the average temperature can range from 2 to 8 °C, depending on the nature of the mixed load. Optimally tomatoes should be handled and shipped closer to 13 °C or higher as lower temperatures will induce chilling injury (Sargent et al., 2005). The objective of the work presented in this chapter was to further understand the impact of chilling (2 °C) and non-chilling temperatures (25 °C) encountered during supply chain on tomato quality. The supply chain scenarios used here were chosen because they had the most negative impact on tomato overall quality (see Chapter 3). Impact cooling at 25 °C had a negative impact on 9 quality attributes (shriveling, CI, decay, overall, L*, a*, acidity, SSC, and carotenoids) of the 21 attributes measured in this study while shipping to the store at 2 °C impacted 6 quality attributes (SCC, acidity, carotene, glucose, fructose, and total sugars) of the 21 attributes. These steps were repeated to evaluate the impact in specific tomato biochemical biomarkers.

Materials and methods

Plant material and supply chain simulation

For the first experiment (first harvest), 50 tomatoes (cv. Beefsteak) were harvested at the light red color stage from Hydro Harvest Farms in Ruskin, Florida on March 27, 2018. For the second experiment (second harvest), 50 tomatoes (cv. Beefsteak) were harvested from Hydro Harvest Farms in Ruskin, Florida on May 4, 2018. Tomatoes were brought to the laboratory within one hour after harvest. Upon arrival to the laboratory, tomato fruit were selected based on color and freedom from defects. Nine of these fruit were used for initial quality evaluations. Nine fruits per treatment (control plus 3 supply chain conditions) were carefully distributed to clamshells and used for non-destructive analysis (i.e., subjective appearance and weight loss). For destructive analysis (color and texture analysis, and chemical analysis), nine fruit each per

treatment (control plus 3 supply chain conditions) were carefully distributed to clamshells. The clamshells containing the tomato for both non-destructive and destructive quality evaluations were then stored for specific periods of time inside temperature and humidity-controlled chambers (Forma Environmental Chambers Model 3940 Series, Thermo Electron Corporation, OH, USA) set at 2.0 ± 0.3 °C, 13.0 ± 0.1 °C, 25.0 ± 0.2 °C and approximately 80 % RH (Figure 23). Since light-red tomatoes were used for this work, the ethylene treatment step was omitted.

Supply chain steps were selected based on the results from previous experiments that determined that impact cooling at 25 °C and shipping to the stores at 2 °C were the most impactful on tomato quality (see Chapter 3). Before and after each time-temperature treatment, within each supply chain step, tomatoes were kept at constant optimum conditions (i.e., 13 °C and 90 % RH) so only the specific segment within each step was different from one step to the other (Figure 23). Tomato optimum storage conditions (13.0 °C and 90 % RH) were selected based on data from from Gross et al. 2004 and Nunes et al., 2008.

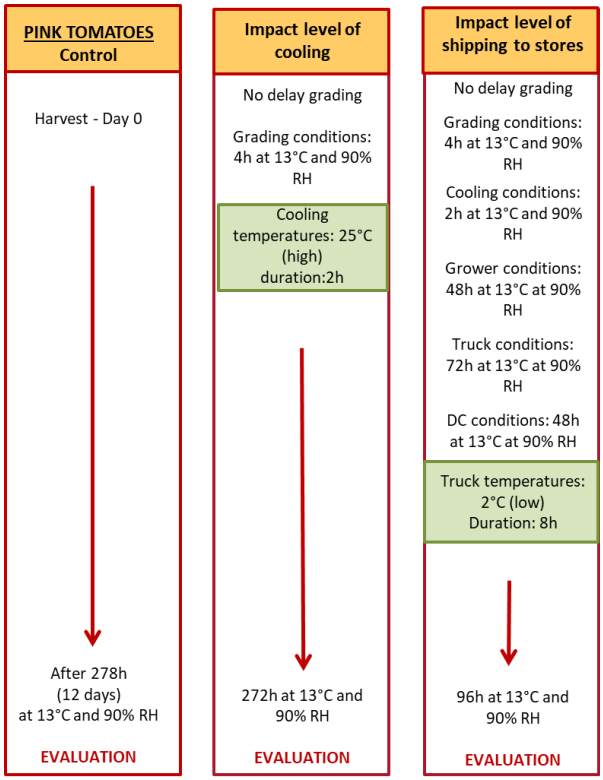

Figure 23. Tomato supply chain simulations of most impactful steps on tomato quality. Each section represents a supply chain step and within each step a best and worst time-temperature scenario were tested.

Temperature and Relative Humidity (RH) Monitoring

The temperature inside temperature and RH-controlled rooms was monitored throughout the study using HOBO® brand U12 data loggers (Onset Computer Corporation, Pocasset, MA, USA), which records within an accuracy of ± 0.35 °C. The RH was monitored using HOBO® brand U12 data loggers (Onset Computer Corporation, Pocasset, MA, USA), which records within an accuracy of ± 2.5 % from 10 to 90 % RH.

Visual Quality

Subjective quality attributes, namely color, shriveling and decay were determined subjectively using a 1 to 5 visual rating scale, and firmness was determined subjectively based on the whole specialty crop resistance to slight applied finger pressure and recorded using a 1

to 5 tactile rating (Table 1). These data were primarily used to determine the end of shelf life due to loss of sensory quality, and to quantify waste. Thus, for each treatment, a limiting quality factor(s) will be established considering the rating value of 3 as the minimum acceptable quality before the specialty crop becomes unmarketable (Nunes, 2015).

Weight Loss

The weight of the tomato was measured using a precision balance with an accuracy of ± 0.01 g (Denver Instruments, Timberline Series Model TP-3102, CO, USA) as described by Proulx et al. (2011).

Total and Individual Sugar Contents

Total sugar analysis was conducted using a Hitachi HPLC system with an RI- refractive index detector and a 300 mm × 8 mm Shodex SP0810 column (Shodex, Colorado Springs, CO) with an SP-G guard column (2 mm x 4 mm). Isocratic solvent delivery of water will be set at 1.0 mL min^{-1}. Standards including sucrose, glucose and fructose were used to identify sample peaks. After comparison of retention time with the standards, the peaks were identified. The amount of total sugar in tomatoes was quantified using calibration curves obtained from different standard concentrations (Chilson and Nunes, 2011).

Total Ascorbic Acid Content

Homogenized tomato (4g) was mixed with 20 ml metaphosphoric acid mixture (6 % HPO_3 containing 2 *N* Acetic acid) and filtered through a 0.22 µm filter prior to HPLC analysis. Ascorbic acid analysis was conducted using a Hitachi LaChromUltra UHPLC system with a diode array detector and a LaChromUltra C18 2 µm column (2 × 50 mm) (Hitachi, Ltd., Tokyo, Japan). The analysis was performed under isocratic mode at a flow rate of 0.5 mL min^{-1} with a detection of 254 nm. Mobile phase was buffered potassium phosphate monobasic (KH_2PO_4, 0.5 %, w/v) at pH 2.5 with metaphosphoric acid (HPO_3, 0.1 %, w/v). After comparison of retention time with the ascorbic acid standard, the peak was identified. The amount of total ascorbic acid content in

tomato was quantified using calibration curves obtained from different concentrations of ascorbic acid standards (Chilson and Nunes, 2011).

Total carotenoid content

Total carotenoids were extracted by mixing 2 g of homogenized tomato tissue with 25 mL of a solution containing acetone: ethanol (1:1) and 200 mg L^{-1} BHT as described by Talcott and Howard (1999). Samples were extracted in the dark, filtered through Whatman No. 4 paper filter, and washed until the residue was colorless. Samples were adjusted to 100 mL, and the absorbance was measured using a microplate reader (Biotek Instruments, Inc., Highland Park, VT, USA). Total carotenoids were calculated according to Gross (1991) using the following equation: µg carotenoid $g^{-1} = (A \times V \times 10^6) / (A^{1\,\%} \times 100 \times G)$, where *A* is the absorbance at 470 nm, *V* is the total volume of extract, *A*1 % is the extinction coefficient for a mixture of solvents arbitrarily set at 2500, and *G* is the sample weight in grams (Gross, 1991).

Statistical Analysis

The Statistical Analysis System computer package (SAS Institute, Inc., 2004) was used for the analysis of the data. Data from the two harvests are shown separately because there was a significant difference between harvests for several of the physicochemical attributes measured. The data was treated by two-way analysis of variance (ANOVA) with harvest and supply chain step as main effects. Significant differences between harvests and between initial, control and supply chain steps were detected using the least significant difference (LSD) at the 5 % level of significance.

Results and Discussion

Sensory Quality

Color. In the first harvest, tomato color was negatively impacted by the temperature scenarios where fruit were exposed to 2 °C and 25 °C, meaning that compared to the control tomato showed a darker red color (Figure 24A). In the first harvest, impact cooling at 25 °C

reduced color quality by 7% and shipping to stores at 2 °C reduced quality by 9%. However, color of tomato exposed to the higher temperature was not significantly different from that of tomato exposed to chilling temperatures (Figure 24A). In the second harvest, color was not perceptively different than the control for either impact cooling at 25 °C or shipping to stores at 2 °C and, there was no difference between the two supply chain steps (Figure 24B). The differences in the color of tomato between the two harvests, was most likely due to the stage of maturity that the tomatoes were harvested. Temperatures higher than 13 °C impact tomato quality more negatively, but it is possible that in the second harvest tomatoes only had minimal to imperceptible color changes (Pek et al., 2010). Chilling temperature had only a slight effect on tomato red color development which may explain why in the second harvest there was no perceivable difference between treatments (Verheul t al., 2015).

Firmness. In the first harvest, there was no perceptible difference in firmness for both impact cooling at 25 °C and shipping to stores at 2 °C (Figure 24C). In the second harvest, however, tomatoes from impact cooling at 25 °C were 6% less firm than the control and tomatoes that underwent exposure to chilling temperature from shipping to stores at 2 °C were 2% firmer than their control counterparts (Figure 24D). Tomato firmness is related to the ripening stage and, in general, the earlier in the ripening stage the firmer the fruit. Temperature fluctuations during handling have been shown to negatively impact tomato fruit firmness (Kader, 1984). For example, previous studies have shown that high temperatures accelerate tomato ripening and cause the fruit to soften prematurely. On the other hand, temperatures below 13 °C delay the ripening process and consequently fruit softening (Tadesse et al., 2015). In the present study, the steps with the largest impact on firmness were the steps where tomato was exposed to high temperature (25 °C). In general, consumer preference is for a tomato that is firm and gives a little to the touch while not being so firm and hard to slice (Oltman et al., 2014). However, high temperature abuses in the supply chain cause fruit to ripen unevenly and cause undesirable softening resulting in objectionable quality at the retail level.

Shriveling. In the first harvest, when compared to the control, shriveling increased in tomato exposed to both supply chain temperature treatments. Impact cooling at 25 °C caused a 6% increase of shriveling and shipping to the stores at 2 °C caused an increase of 7% (Figure 25A) whereas, compared to the control, there was difference in the levels of shriveling in fruit from the second harvest (Figure 25B). Shriveling in tomatoes can be caused by several factors but it is most often attributed to weight loss (Jois et al., 2017). Shriveling is an important contributor to the overall tomato appearance quality as it has a large influence on consumer decision to purchase. While shriveled tomatoes are perfectly edible, their appearance is undesirable. Tomatoes must have a smooth, not shriveled, skin as consumers will not purchase the fruit otherwise (Kader et al., 1978). In this study, fruit shriveling was minimally impacted by any supply chain steps used.

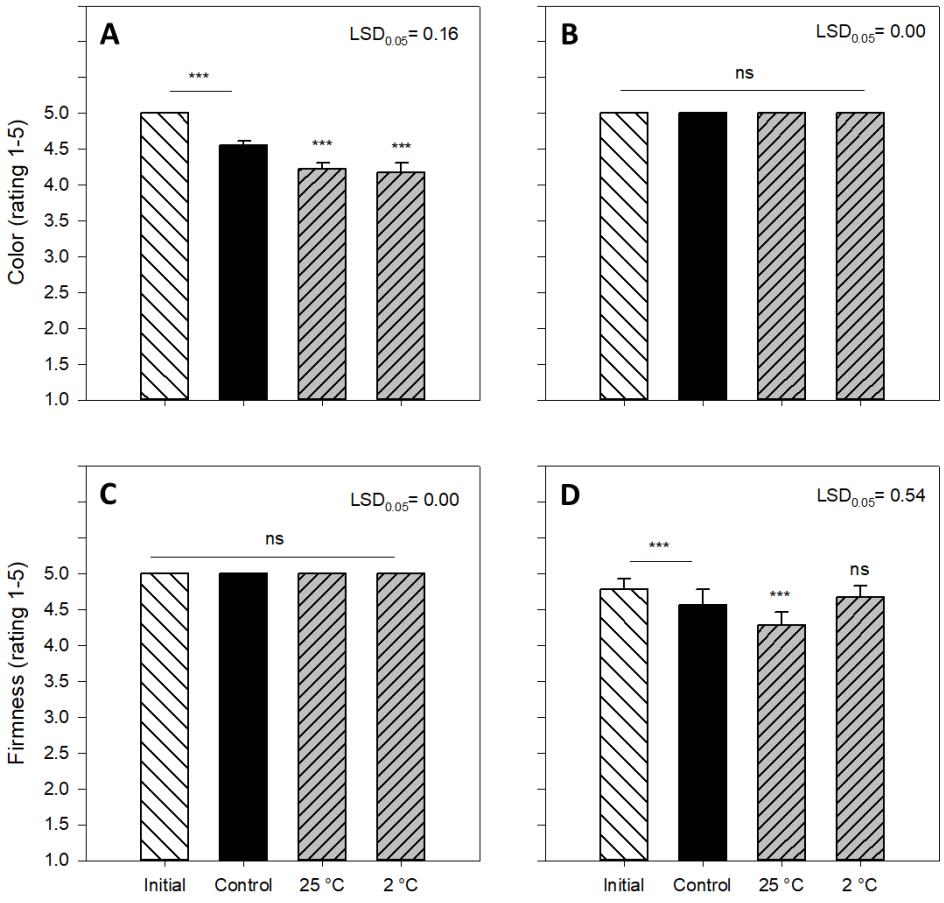

Figure 24: Impact level of impact cooling at 25 °C and shipping to stores at 2 °C on tomato color and firmness. Color (A) first harvest and (B) second harvest. Firmness (C) first harvest and (D) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ***p≤ 0.0001).

Chilling Injury. Chilling injury was apparent in both harvests but was only visible in the low temperature treatment, shipping to the stores at 2 °C (Figure 25C). This is because chilling injury symptoms only occur when a tomato is exposed to temperatures lower than 13 °C. Higher temperatures cause other symptoms such as accelerated ripening, that are not associated with chilling injury. In the first harvest, shipping to stores at 2 °C resulted in a 6% increase in chilling injury symptoms (lower ratings when compared to the control) when compared to the control (Figure 25C) whereas in the second harvest, in tomato exposed to 2 C, chilling injury symptoms

increased by 11% (Figure 25D). Chilling injury symptoms observed in this experiment were mild pitting on the surface of the tomato as well as stripes of uneven ripening. Uneven ripening is also a symptom of high temperature abuse, however the tomatoes that underwent impact cooling at 25 °C showed no signs of uneven ripening so they were not confused as chilling injury symptoms.

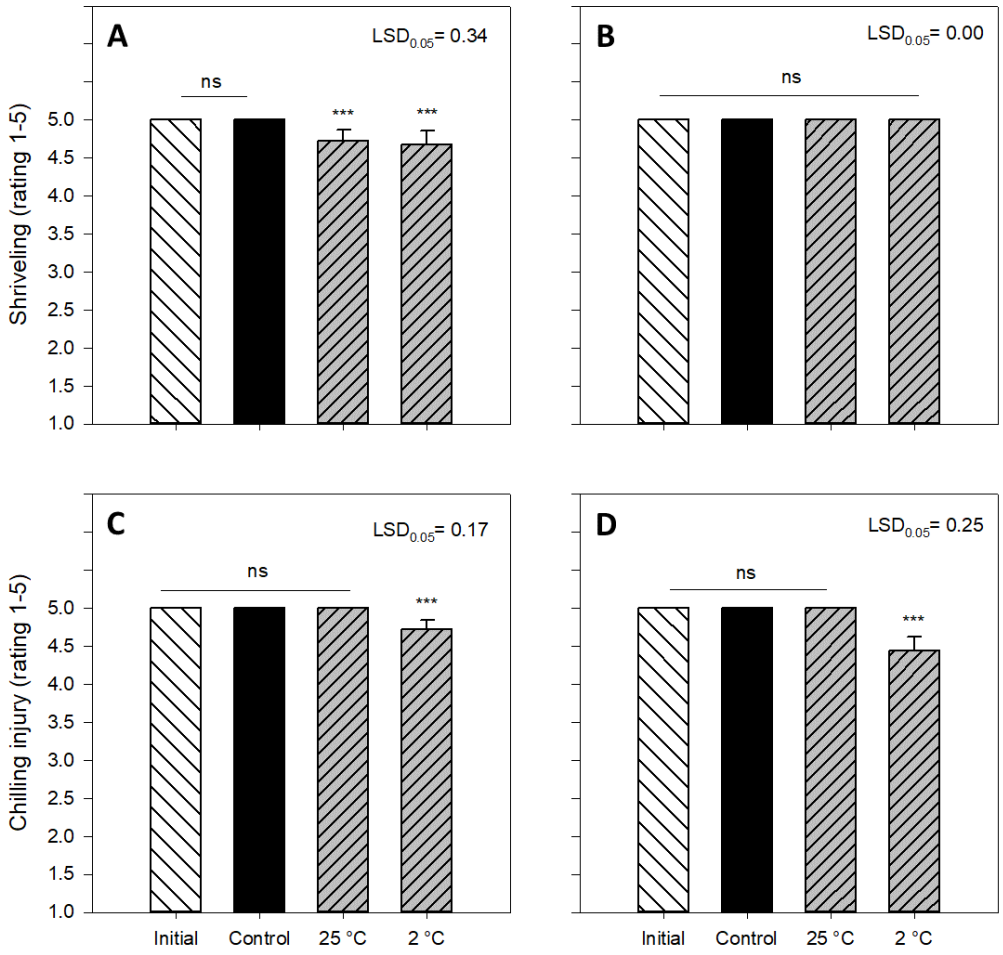

Figure 25: Impact level of impact cooling at 25 °C and shipping to stores at 2 °C on tomato shriveling and chilling injury. Shriveling (A) first harvest and (B) second harvest. Chilling injury (C) first harvest and (D) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ***p≤ 0.0001).

Decay. Development of decay was not impacted by the time temperature treatments for both harvests (Figures 26A and 26B). In the first and second harvest, the amount of perceivable decay found was not significantly different from than that observed in the control for both treatments, impact cooling at 25 °C or shipping to the store at 2 °C. When purchasing tomatoes, consumers look for high quality tomatoes and, decay is the most obvious objectionable defect that a consumer can find. The perception of decay on tomatoes is therefore vitally important to ensuring that tomatoes are sold at market and not wasted (Sargent et al., 2005).

Overall Quality. The overall quality of the tomato fruit was lower after in both supply chain treatments. While there was on overall decline in the quality of tomatoes there was not a significant difference between the supply chain temperature treatments and the control (Figures 26C and 26D).

Weight Loss

Weight loss increased when tomato was exposed to supply chain treatments regardless of the treatment (Figure 27). In the first harvest, there was no significant difference between the weight loss of tomato exposed to supply chain conditions and the control (Figure 27A) whereas in the second harvest, weight loss was lower in fruit exposed to 25 °C compared to the control and there was no significant different in weight loss for fruit exposed to 2 °C (Figure 27B). Although not significant, the low temperature supply chain treatment resulted in higher weight loss when compared to the control. In the first harvest, impact cooling at 25 °C resulted in a weight loss of 1.9% and shipping to stores at 2 °C resulted in a 2 % weight loss (Figure 27A). In the second harvest, impact cooling at 25 °C resulted in a 2 % weight loss and shipping to stores at 2 °C in a 3 % weight low (Figure 27B). The levels of weight loss were not considered objectionable as they never exceeded the threshold of 1% and 2% (Robinson et al., 1975; Hruschka, 1977).

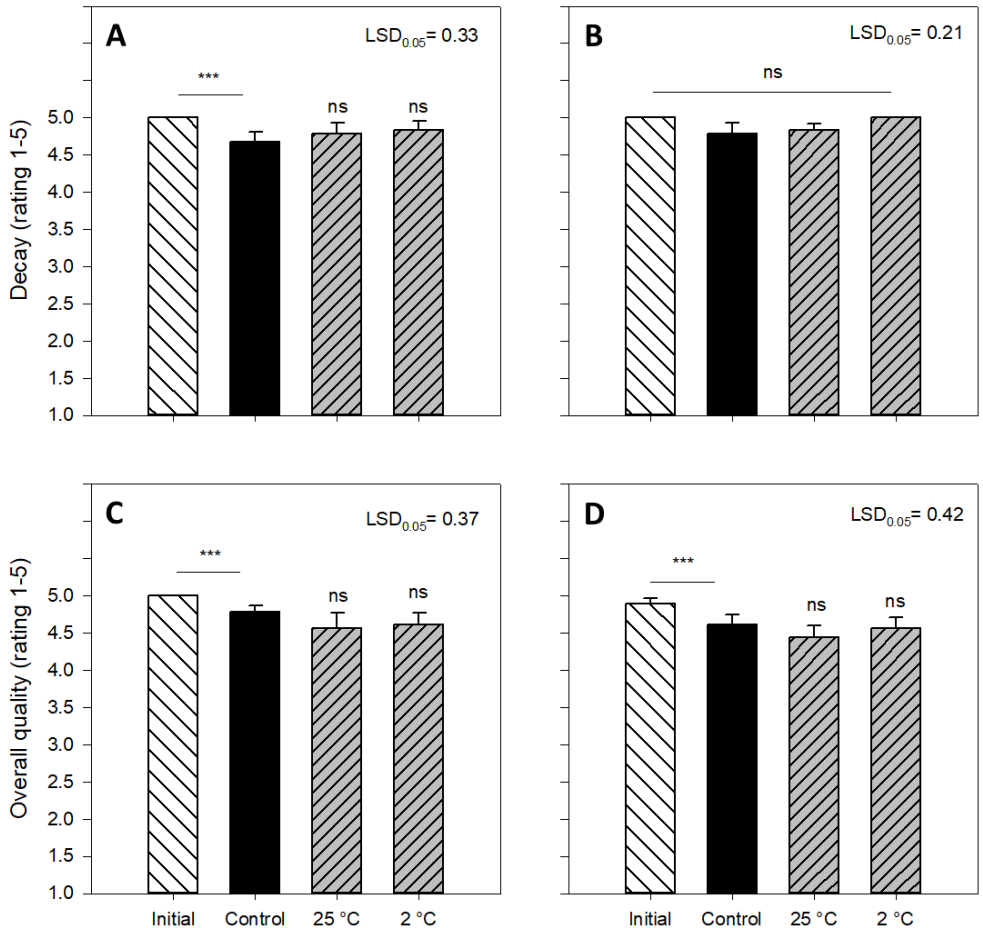

Figure 26: Impact level of impact cooling at 25 °C and shipping to stores at 2 °C on tomato decay and overall quality. Decay (A) first harvest and (B) second harvest. Overall quality (C) first harvest and (D) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ***p≤ 0.0001).

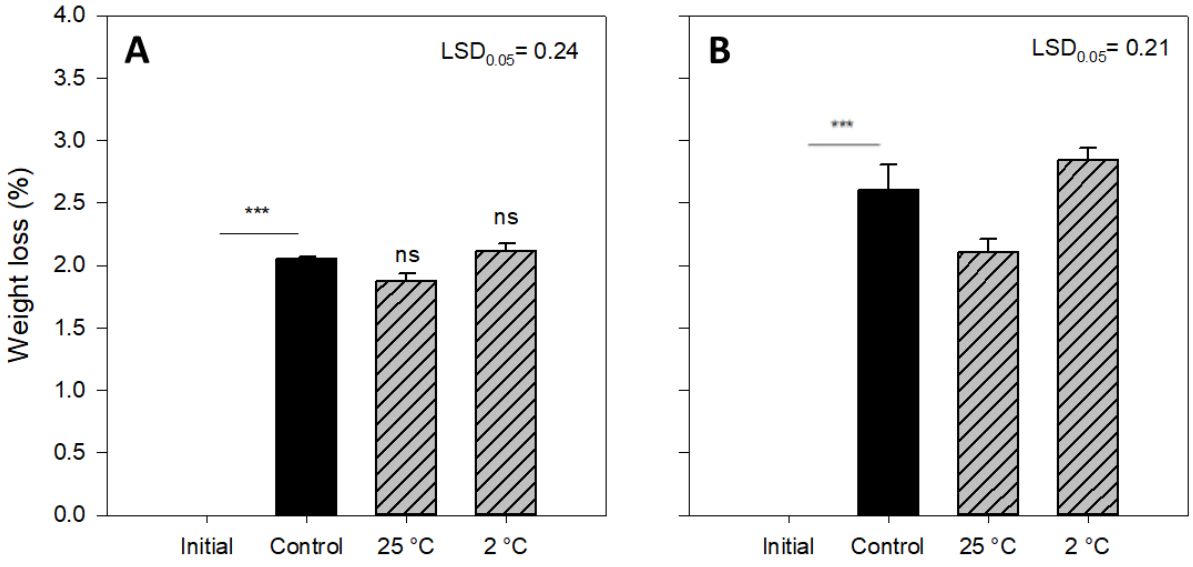

Figure 27: Impact level of each step along the supply chain on tomato weight loss. (A) first harvest and (B) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ***p≤ 0.0001).

Total and Individual Sugar Contents

In both harvest, fructose content of tomato fruit decreased significantly from initial levels at harvest, regardless of the temperature treatment (Figures 28A and 28B). However, for both harvests, there was no significant difference between fructose content of tomato exposed to supply chain conditions and the control. Similar results were found for glucose and total sugars contents (Figures 28D, 28E and 28F) with the exception of the first harvest, where glucose levels were significantly lower in tomato exposed to supply chain treatments compared to the control (Figure 28C). In the first harvest, when compared to the control, there was a decrease of 13% in glucose levels after impact cooling at 25 °C and a decrease of 18% after shipping to stores at 2 °C (Figure 28C). Exposure to low temperatures also result in decreased overall tomato quality and are notable for having a large negative impact on glucose levels (Kader et al., 1978).

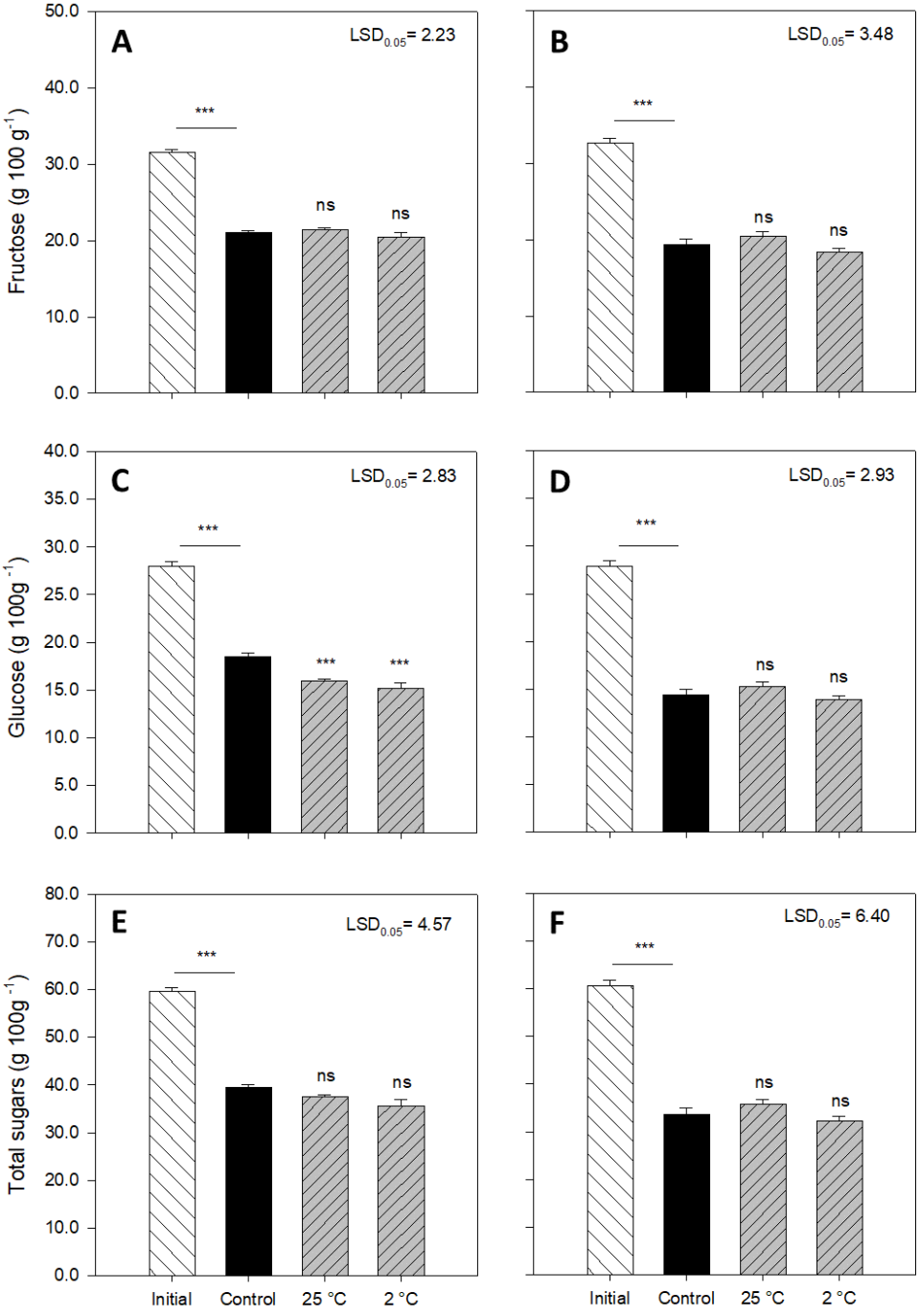

Figure 28: Impact level of impact cooling at 25 °C and shipping to stores at 2 °C on tomato fructose, sucrose, and total sugar content. Fructose (A) first harvest and (B) second harvest. Glucose (C) first harvest and (D) second harvest. Total sugars (E) first harvest and (F) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ***p≤ 0.0001).

Total Carotenoid Content

Overall, carotenoid content of the tomatoes was negatively impacted by the supply chain temperatures used in this study (Figure 29). In the first harvest, when compared to the control, there was a decrease in carotenoid content of 10% after shipping to stores at 2 °C but no significant difference between the control and the supply chain temperature treatment at 25 °C was observed (Figure 29A). In the second harvest, there was a decrease in carotenoid content of 11% and 15% for impact cooling at 25 °C and shipping to stores at 2 °C, respectively (Figure 29B). These results are in agreement with previous published studies which showed that there is a large negative impact of temperature on carotenoid biosynthesis (Farneti et al., 2012; Fattore et al., 2016; Zhang et al., 2016). These studies have found that as temperature fluctuates from 13 °C that the quality of carotenoid content diminishes, specifically finding that tomatoes stored at 4 °C resulted in a loss of lycopene content and a substantial loss of red color as well.

Total Ascorbic Acid Content

Compared to initial values at harvest, ascorbic acid (AA) content in the tomato decreased, regardless of the temperature treatment (Figures 29C and 29D). Disruption of the ripening process by exposing the tomatoes to either high or low temperature abuses is detrimental to AA content. However, other studies have shown that AA levels remain constant throughout the ripening process and do not fluctuate when exposed to temperature abuses (Toor and Savage, 2005; Stevens et al., 2008; Mellidou et al., 2012). In this study, although there was a decrease in AA content during storage at constant temperature and after supply chain simulations, compared to the control, there was no significant difference in the AA content of tomato exposed to a high and low temperature supply chain treatment (Figures 29C and 29D).

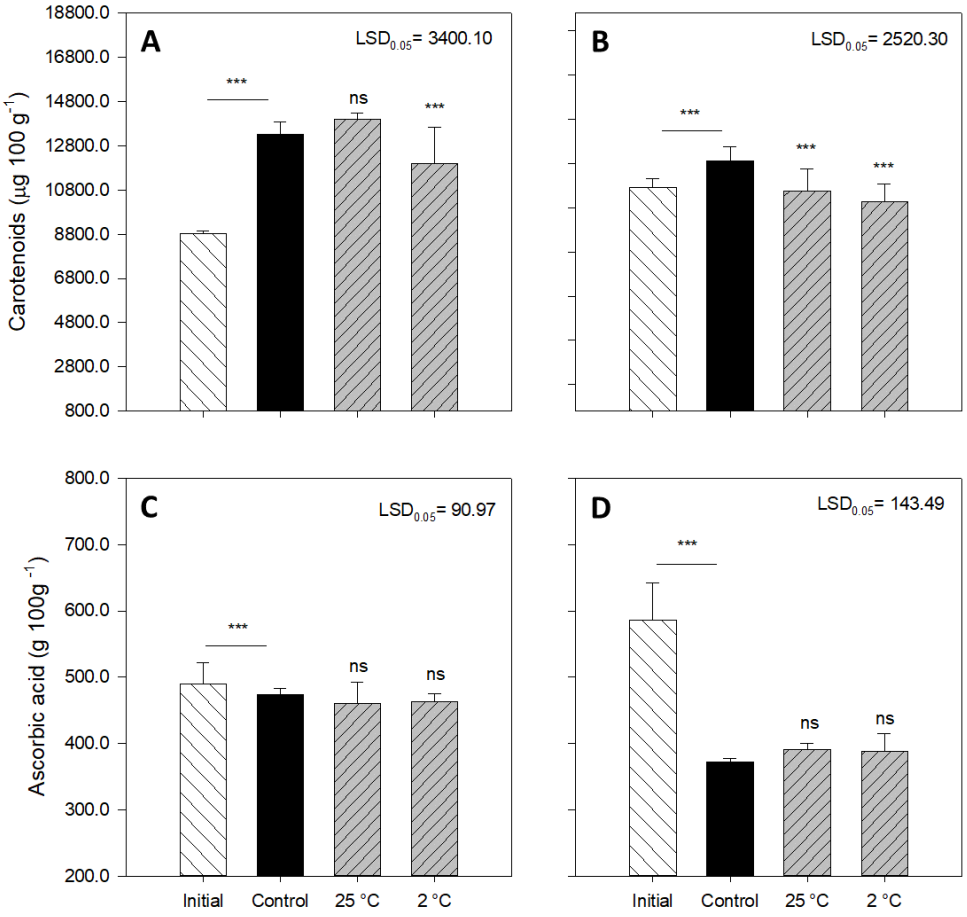

Figure 29: Impact level of impact cooling at 25 °C and shipping to stores at 2 °C on tomato carotenoid and ascorbic acid content. Carotenoid (A) first harvest and (B) second harvest. Ascorbic acid (C) first harvest and (D) second harvest. Bars are means ± SE of 9 biological replicates. Asterisks indicate significant differences between initial quality at harvest and control (constant temperature at 13 °C) and between the control and the three critical steps along the supply chain with the highest decline in appearance (ns: non-significant; ***p≤ 0.0001).

Overall, results showed that there was a significant decline in quality for both chilling and non-chilling temperature treatments when compared to the control for most of the biochemical and sensory markers. In most cases it was found that there was not a significant difference between harvests suggesting that the two temperature treatments had a similar impact on the overall quality of the fruit. Although the mechanisms for loss of quality might be different, the results are similar suggesting that

both heat stress and chilling injury can result in tomato quality loss to the consumers perception.

CHAPTER 5:

USING A PROTEOMIC APPROACH TO DETERMINE THE IMPACT OF CHILLING AND NON-CHILLING TEMPERATURES ENCOUNTERED DURING SUPPLY CHAIN ON TOMATO QUALITY

Introduction

The use of proteomics allows for the examination of the global genes that are produced in the cell in any condition or state. The advancements of genomic sequencing and protein mapping have allowed proteomics to become one of the largest fields of study (Park, 2004). Proteomics has seen such large success do to its ability to study protein-protein interactions, mapping of organelles, concurrent descriptions of genomes and proteomes, and the ability to identify and quantify complex biological samples (Aebersold and Mann, 2003). Plant proteomic research has mostly been focused on food security to ensure that climate change does not lower food output for an increasing global demand (Wheeler and Braun, 2013). However, there is a growing significance of using proteomics to study plant protein complexities and how stress conditions impact the modifications of proteins and the impact on plant growth and health.

Tomato fruit has been a preferred candidate for proteomic studies because it has been the focus of substantial developmental, physiological and genetic research. Therefore, tomato fruit is a well-studied crop with established genomic resources and has been well characterized with the whole genome being sequenced in 2012 (Barone et al., 2008; Tomato Genome Consortium, 2012). The genome of the most studied cultivar, *Heinz*, was found to be 950MB divided between 12 chromosomes, with a total of 35,000 unique genes (Tomato Genome Consortium,

2012). The proteomic database that contain protein sequences are derived from genomic predictions and gene transcripts (Sant'ana and Lefsrud, 2018).

Understanding the importance of stress tolerance in plants is an important step in the context of food security and the investigation of the plant response to stresses found in the food supply chain is essential to preventing tomato quality degradation that leads to food waste. Previous studies have found that high temperature stress results in reduced biosynthesis and photosynthesis in tomato plants (Zhang et al., 2014 and Shaheen et al., 2015). Low temperature chilling injury has shown changes in proteins associated with carbon metabolism, photosynthesis, protein degradation, and protein processing in tomatoes (Vega-García et al., 2010).

The largest hurdle in tomato proteomics is that sample preparation is not standardized (Alvarez and Naldrett, 2016) with the plant cell wall being the largest challenge to protein extraction due to the cellulose interfering with protein readouts. Plant cells also contain large vacuoles that carry phenolic compounds and proteases that impair protein quality making standardization of plant proteomics difficult (Laing and Christeller, 2004). For example, Rubisco, a ubiquitous enzyme, interferes with protein extraction causing precipitation and thus requiring the use of phenol or acetone inactivate the enzyme (Alvarez and Naldrett, 2016). The use of phenol helps plant proteomics as it also dissolves other cellular debris not found in mammalian cell lines in addition to inactivating interfering enzymes. The objective of the work presented in this chapter was to determine the impact of chilling and non-chilling temperatures encountered during supply chain on tomato proteome and, to identify which proteins are upregulated and downregulated with a particular focus on the impact of temperature stress on major metabolic pathways.

Materials and Methods

Plant material and supply chain simulation

Tomatoes (cv. Beefsteak) were harvested at the light red color stage from Hydro Harvest Farms in Ruskin, Florida on March 27 and May 4, 2018. A total of 50 tomatoes each harvest were brought to the laboratory within one hour after harvest. Upon arrival to the laboratory, tomato fruit were selected based on uniformity of color and freedom from defects. Nine of these selected fruit were used for initial evaluations (i.e., protein extraction and analysis). In addition, nine fruits per treatment (i.e., one control at 13 °C plus two supply chain conditions at chilling and non-chilling temperatures) were carefully distributed to clamshells and stored for specific periods of time inside temperature and humidity-controlled chambers (Forma Environmental Chambers Model 3940 Series, Thermo Electron Corporation, OH, USA) set at 2.0 ± 0.3 °C, 13.0 ± 0.1 °C, 25.0 ± 0.2 °C and approximately 80 % RH. Supply chain steps were selected based on the results from previous experiments that determined that impact cooling at 25 °C and shipping to the stores at 2 °C were the most impactful on tomato quality (see Chapter 3). Before and after each time-temperature treatment, within each supply chain step, tomatoes were kept at constant optimum conditions (i.e., 13 °C and 90 % RH) so only the specific segment within each step was different from one step to the other (Figure 23). Tomato optimum storage conditions (13.0 °C and 90 % RH) were selected based on data from from Gross et al. 2004 and Nunes et al., 2008.

Temperature and Relative Humidity (RH) Monitoring

The temperature inside temperature and RH-controlled rooms was monitored throughout the study using HOBO® brand U12 data loggers (Onset Computer Corporation, Pocasset, MA, USA), which records within an accuracy of ± 0.35 °C. The RH was monitored using HOBO® brand U12 data loggers (Onset Computer Corporation, Pocasset, MA, USA), which records within an accuracy of ± 2.5 % from 10 to 90 % RH.

Protein Extraction

Tomatoes were diced into small cubes of roughly 5 mm in size. Diced tomato cubes were immediately frozen in liquid nitrogen and ground into a fine powder to obtain a total of 10 g of powder. The finely ground powder was suspended in 20 mL of extraction buffer (0.7 M sucrose, 0.1 M KCl, 0.5 M Tris–HCl, pH 7.5, 50 mM EDTA, 2% w/v β-mercaptoethanol, 1 mM PMSF) with 20 mL of tris-phenol. The solution was then shaken on ice for 30 minutes and then centrifuged at 4,700 x g for 45 minutes at 4 °C. The supernatant was collected and added to a new tube, the extraction process was repeated twice for a total of three cycles. Once the supernatant was collected for the final time, 15 mL of chilled precipitation solution (0.1M ammonium acetate in methanol) was added and allowed to sit overnight. The precipitated proteins were centrifuged for 20 min at 4700 g at 4 °C to yield pellet (protein). The pellet was washed once with 10 mL ice cold methanol and twice with 10 mL ice cold acetone being centrifuged for 20 min at 4700 g at 4 °C between each wash. The pellets were then transferred to a 1.5 mL centrifuge tube using chilled acetone and centrifuged for 15 min at 19000 x g 4 °C. After centrifuging the pellets were dried on ice for 30 mins and stored at -80 °C (Hurkman and Tanaka, 1986; Bianoc et al., 2009).

Sample preparation and LC-MS/MS analysis

Protein extracts were separated by SDS-PAGE and Coomassie-stained for visualization. The gel was divided into 3 fractions, and each gel section was minced and de-stained before being reduced with dithiothreitol (DTT), alkylated with iodoacetamide (IAA), and finally digested with Trypsin/Lys-C overnight at 37˚C. Peptides were extracted using 50/50 acetonitrile (ACN)/H2O/0.1% formic acid and dried in a vacuum concentrator (Labconco). Peptides were resuspended in 98%H2O/2%ACN/0.1% formic acid for LC-MS/MS analysis. Experiments were performed in triplicate.

Peptides were separated using a 50cm C18 reversed-phase HPLC column (Thermo) on an Ultimate3000 UHPLC (Thermo) with a 180-minute gradient (2-32% acetonitrile with 0.1% formic acid) and analyzed on a hybrid quadrupole-Orbitrap mass spectrometer (Q Exactive Plus, Thermo Fisher Scientific) using data-dependent acquisition in which the top 10 most abundant ions are selected for MS/MS analysis.

Raw data files were processed in MaxQuant (www.maxquant.org) and searched against the current UniprotKB Solanum lycopersicum (Tomato) protein sequence database. Search parameters included constant modification of cysteine by carbamidomethylation and the variable modification, methionine oxidation. Proteins were identified using the filtering criteria of 1% protein and peptide false discovery rate. The MaxQuant protein groups file were then analyzed using Perseus, software developed for the analysis of omics data (Cox and Mann, 2016). Protein intensities were Log2-transformed, and then filtered to include proteins containing at least 60% valid values (reported intensities) in at least one experimental group. Finally, the missing values in the filtered dataset were replaced using the imputation function in Perseus with default parameters (Tyanova et al., 2016). Statistical analyses were carried out using the filtered and imputed protein groups file.

Two approaches were used for assessing statistical significance. For bioinformatic analysis, statistical significance was established using the Student's t test with a threshold of $p<0.05$. For bioinformatic analysis, FDR correction were not applied for improved depth of coverage and enhanced bioinformatic output (Stevens and Bickford, 2016). Statistically significant proteins were uploaded to Ingenuity Pathway Analysis (IPA), which provides information about localization, molecular function, protein interaction pathways, as well as upstream regulator analysis. Additional dataset filtering using a more stringent approach included a significance criteria of both a $|z\text{-score}|>1$ and Welch's t test $p<0.05$ (Stevens and Bickford, 2016). Welch's t test was performed on LFQ intensity values. The z-score was

calculated (t test difference of protein)-(median t test difference of dataset)/(standard deviation of t test difference of dataset).

Results

Impact of exposure to non-chilling temperature (25 °C) during supply chain on tomato proteome

To determine the effect of impact temperature abuse on the tomato proteome, LC-MS/MS analysis was performed on protein extracted from tomatoes that were exposed to high temperature conditions (25 °C) for 2 hours during a simulated supply chain scenario (Figure 23). Results from protein analysis showed over 3500 proteins with significant value. Of these proteins, 19 were expressed differently between tomato fruit that was kept at its ideal temperature of 13 °C for 12 days and tomato exposed to 25 °C. However, of the 19 proteins, only 6 have been well characterized in terms of function and localization namely, the enzymes Mitogen-Activated Protein (MAP) kinase, RNA cytidine acetyltransferase, two different lipoxygenases, Remorin 1 and ferredoxin (Table 2). In both tomato harvest, the enzymes MAP kinase and the RNA cytidine acetyltransferase were downregulated in fruit exposed to 25 °C compared to the control (13 °C) whereas the lipoxygenases, Remorin 1, and a ferredoxin were upregulated in tomato fruit exposed to 25 °C compared to the control (13 °C) (Figures 30-37). As discussed in the previous chapter, tomatoes exposed at 25 °C showed no abnormal signs of ripening and were considered marketable yet high temperature abuse have been shown to have a high impact on the function of proteins that control thylakoid membrane carbon metabolism and stroma photochemical pathways (Shaheen et al., 2015; Zhang et al., 2014). Most significantly, rubisco activity is suppressed via the downregulation of rubisco activase and S-adenosyl-L-homocysteine hydrolase (Yamamoto et al., 1981). In our study, these proteins were not found to be significantly impacted most likely due to the fact that in the study conducted by Yamamoto et al. (1981) tomato were exposed to 30 °C for 16 days, whereas in our study

temperature stress at 25 °C was applied for only 2 hours. However, when compared to the control (i.e., tomato fruit kept continuously at 13 °C) there was a significant decrease in the activity of enzymes MAP kinase and the RNA cytidine acetyltransferase.

Mitogen-activated protein kinase (MAPK8) is a member of the MAP kinase subfamily that is responsible for the regulation of MAP enzyme activity and ATP binding in the plant cell (Jonak et al., 1996; Kong et al., 2012) (Table 2). These proteins are believed to take part in the important role of heat stress response in the plant cell and help regulate abiotic stress (Link et al., 2002). The reaction cofactor is Mg^{2+}, however, in heat stress it has been shown that there is a calcium component needed as well (Link et al., 2002, Vega et al., 2018). The expression of MAPK8 was lower when exposed to 25 °C for 8 hours suggesting that MAPK8 is activated negatively impacted by high temperatures (Figure 30A). It is possible that MAPK8 is a repressor and by downregulating the number of MAPK8 tomato cells attempt to upregulate heat shock proteins (HSP).

RNA cytidine acetyltransferase is part of the RNA cytidine acetyltransferase family and the NAT10 subfamily that plays a role to catalyze the formation of the N_4-acetylcytidine in 28S rRNA in order to assist with the formation of the ribosomal subunits (Table 2). RNA cytidine acetyltransferase is required for early nuclear cleavages and is a precursor to rRNA synthesis. The reaction requires ATP and acetyl-CoA to proceed (Sinclair et al., 2017). The exact reason is not well understood, but it is known that RNA cytidine acetyltransferase is helpful for codon stability and helps translation by safeguarding cognate codon-anticodon interactions (Dominissini and Rechavi, 2018). Results from this study showed that there was a downregulation of RNA cytidine acetyltransferase in tomatoes that were exposed to heat stress (Figure 30B). It is possible that heat stress impedes the function RNA cytidine acetyltransferase leading to inability of cells to maintain microtubules, but further studies are needed to prove this theory.

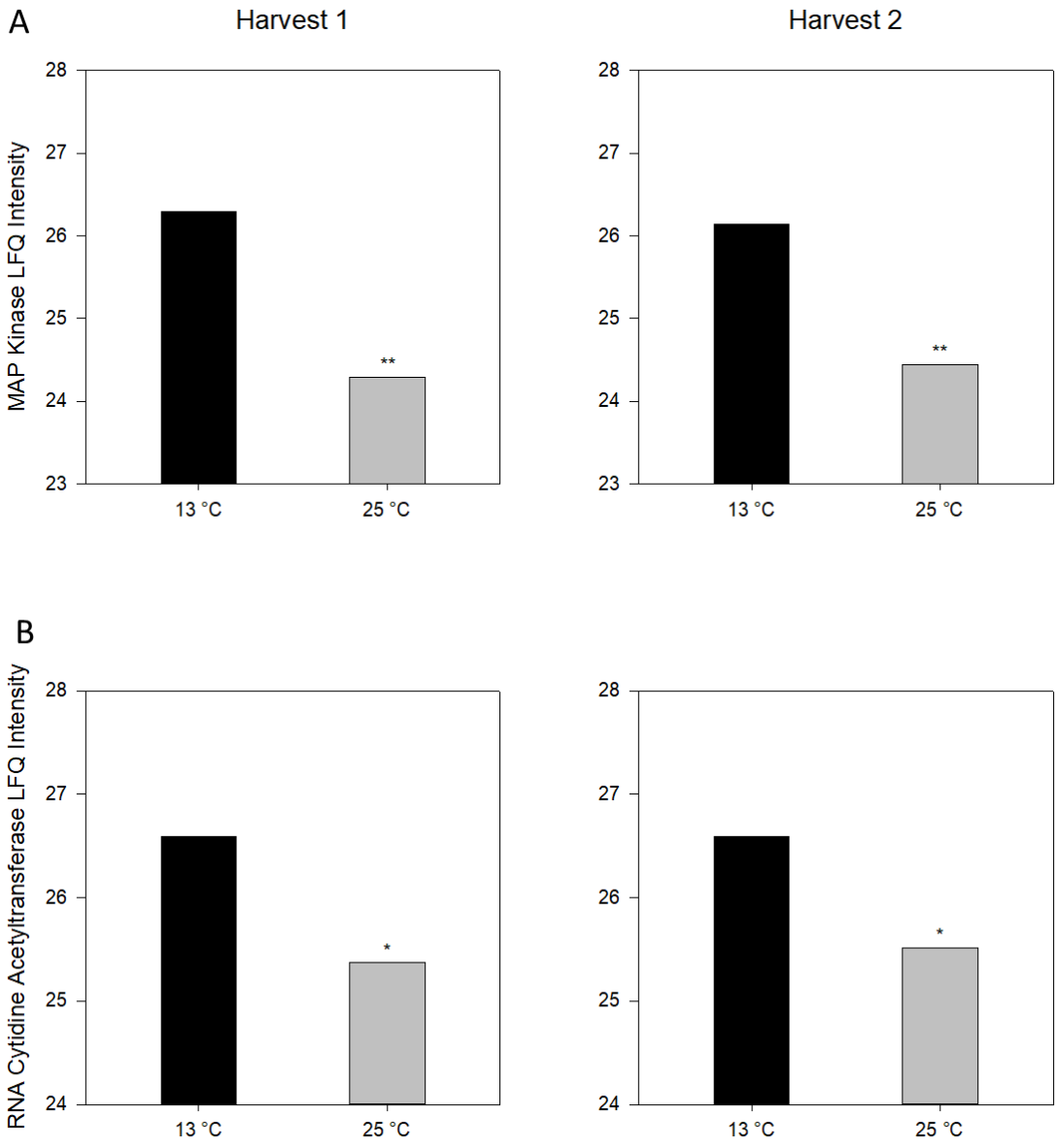

Figure 30: Protein expression after tomato exposure to a constant optimum temperature (13 °C; control) and to non-chilling temperature (25 °C). (A) MAP kinase and (B) RNA cytidine acetyltransferase. Asterisks indicate significant differences between control and non-chilling temperature.

Lipoxygenase belongs to a family of proteins with a large diversity of roles in the tomato plant including growth, development, senescence, or responses to damage (Shen et al., 2014). Lipoxygenase is involved with the oxylipin biosynthesis pathway and is activated by an iron cation cofactor (Table 2). The activation of lipoxygenase results in increased lipid metabolism and the amount of lipids being generated by tomato cells. Lipoxygenase are well studied in

tomatoes as they can impact fatty acid synthesis which impacts fruit flavor volatiles (Chen et al., 2004). A study has shown that tomato cells will rapidly metabolize exogenous linoleic acid into fatty acid oxidation products in a pathway that is heavily regulated by lipoxygenase (Todd et al., 1990). At a temperature of 22 °C there was a rapid increase in fatty acid metabolism but as the temperature increased to 30 °C a dramatic decline in fatty acid metabolism was observed suggesting that heat negatively impacts lipoxygenase activity. The results from this study showed that there was an upregulation of lipoxygenase in tomato fruit as a response to heat stress which could explain the decline in fatty acid metabolism observed the previously mentioned study (Todd et al., 1990) (Figure 31A). Potentially, the cell could be reacting to lipoxygenases being degraded by heat stress and attempting to synthesize more protein (i.e., more lipoxygenases) in order to compensate for its degradation and reduced function.

Remorin proteins (Table 2) are thought to play a role in signal transduction processes in human cell lines (Lefebvre et al., 2010). In tomato plants, there is a lack of studies performed on this protein's activity. Nonetheless, one study found that remorin 1 was closely associated to membrane rafts in potato plants cells assisting with cellular communication and restricting viral movement between cells (Perraki et al., 2012). In this study it was found that remorin 1 was upregulated when the tomato underwent heat stress (Figure 31B). Tomatoes exposed to heat conditions will ripen more quickly than those stored in a cooler environment, so it is possible that in our study, the upregulation of remorin 1 was a result of cells communicating to each other the need to mature and ripen.

Finally, ferredoxin belongs to the 2Fe2S plant-type ferredoxin family of proteins. Their role in the cell is to regulate the flow of electrons in the electron transport chain in photosystem I (Fukuyama, 2004). The protein's regulation helps the flow of electrons from photosystem I in the thylakoid membranes to ATP synthase, and generates ATP (Golbeck, 1987). In order to function, ferredoxin needs to be attached to a sulphur-iron box which is as such considered the enzyme cofactor (Hanke and Mulo, 2013). The results of our study showed that ferredoxin is

upregulated in tomato plant cells that have been exposed to high temperatures (Table 2 and Figure 32).

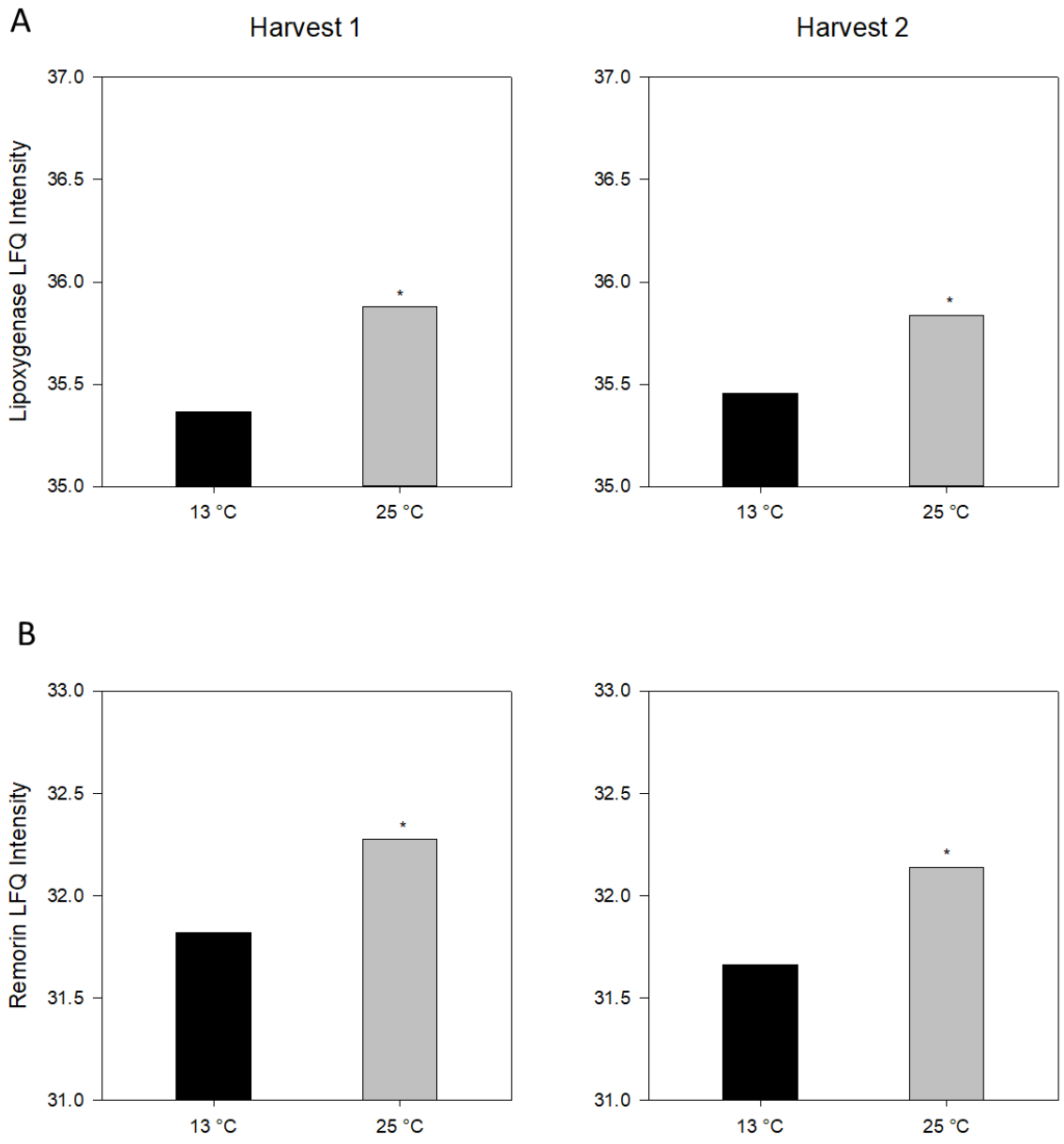

Figure 31: Protein expression after tomato exposure to a constant optimum temperature (13 °C; control) and to non-chilling temperature (25 °C). (A) Lipoxygenase and (B) Remorin. Asterisks indicate significant differences between control and non-chilling temperature.

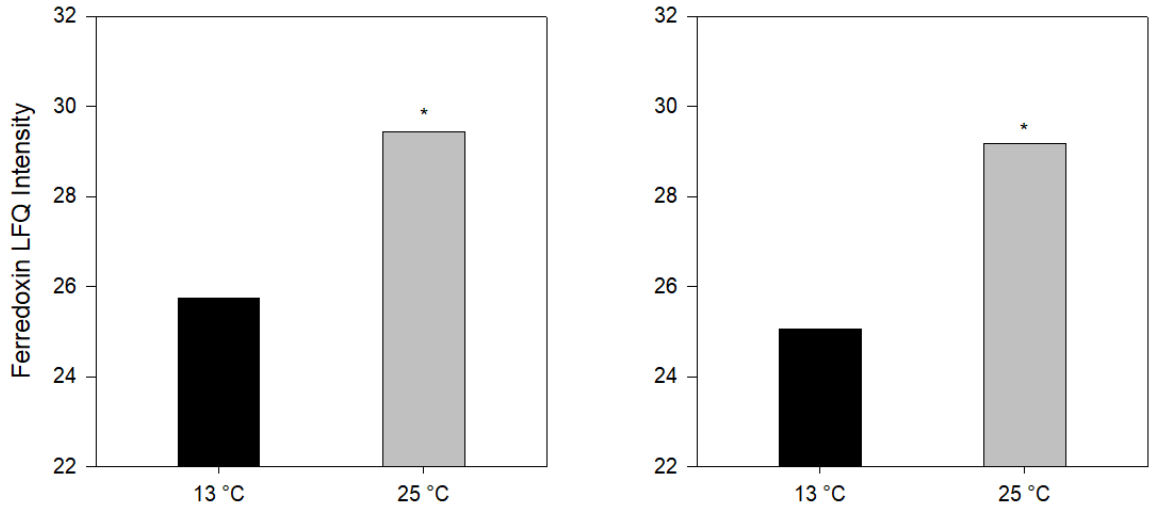

Figure 32: Expression of protein ferredoxin after tomato exposure to a constant optimum temperature (13 °C; control) and to non-chilling temperature (25 °C). Asterisks indicate significant differences between control and non-chilling temperature.

Impact of exposure to chilling temperature (2 °C) during supply chain on tomato proteome

To determine the impact of exposure to chilling temperature during supply chain (i.e., shipping to the stores at 2 °C) on the tomato proteome, LC-MS/MS analysis was performed on fruit exposed to chilling temperature for 8 hours during a simulated food supply chain scenario (Figure 25). Proteomic analysis resulted in over 3500 proteins being significantly expressed. Of these proteins there were 37 that were expressed differently between tomato fruit that was kept continuously at optimum temperature of 13 °C and tomato exposed to 2 °C. Of the 37 proteins identified, only 8 have been well characterized in terms of function and localization within the cell (Table 3). The up downregulated proteins in fruit exposed to chilling temperature were a vacuolar protein sorting-associated protein, UBC4, proteasome subunit alpha, phosphomannomutase, glycosyltransferase, prefoldin subunit 4, and heme oxygenase 1 (Table 3 and Figure A-D). The upregulated proteins were cleavage and polyadenylation specificity factor subunit 2 and ferredoxin (Table 3 and Figure E). As discussed in the previous chapter, tomatoes showed no abnormal signs of ripening and were considered marketable after either exposure to optimum temperatures of after a short exposure to 2 °C. Results from our study

agree with results from previous studies which examined the impact of chilling injury over a longer period of time (25 days) at 2 °C compared to the 8 hours exposure at 2 °C used in our study.

Vacuolar protein sorting-associated protein is part of the SNF8 family of proteins that are responsible for the regulation of the endosomal sorting complex (Table 3). Vacuolar protein sorting-associated protein is required to the formation of multivesicular bodies (MVB) and is essential to endosomal cargo proteins being loaded into MVBs (Cullen et al., 2008). In other eukaryotic cells it has been observed that vacuolar protein sorting-associated protein are important for autophagy and play a role in the membrane of vacuoles. Their proximity to the surface means that they are important for the communication of vacuole pathways and assist in vacuole delivery mechanism (Saftig and Klumperman, 2009). The results of our study showed that vacuolar protein sorting-associated proteins are downregulated in tomatoes exposed to chilling temperature (Figure 33A). Although the role of vacuolar protein sorting-associated protein during chilling injury is poorly understood, the downregulated expression of the protein could be an indicator of a decrease in the cellular internal communication system resulting in an erroneously vacuole binding.

UBC4 is a protein that belongs to the ubiquitin-conjugating enzyme family of proteins that are responsible for the attachment of ubiquitin to proteins that are tagged for a proteasome (Table 3). In yeast cells, UBC4 is involved in the destruction of short lived and abnormal proteins, and the absence of UBC4 results in irregular or arrested cell cycle (Seufert and Jentsch, 1990). In tomato fruit, studies have shown that the attachment of ubiquitin to a protein is a signal for a proteasome to destroy and recycle the target. This cellular behavior has been associated with uneven ripening in the tomato fruit (Zhou et al., 2016). In our study, we found that there was a lower amount of UBC4 in tomato fruit exposed at 2 °C compared to 13 °C (Figure 33B). Tomato uneven ripening is one of the symptoms caused by chilling injury and thus it is possible that the disruption of UBC4 could be responsible for this type of abnormal

discoloration (e.g., disruption of normal chlorophyll breakdown and/or lycopene synthesis that occurs during normal tomato ripening).

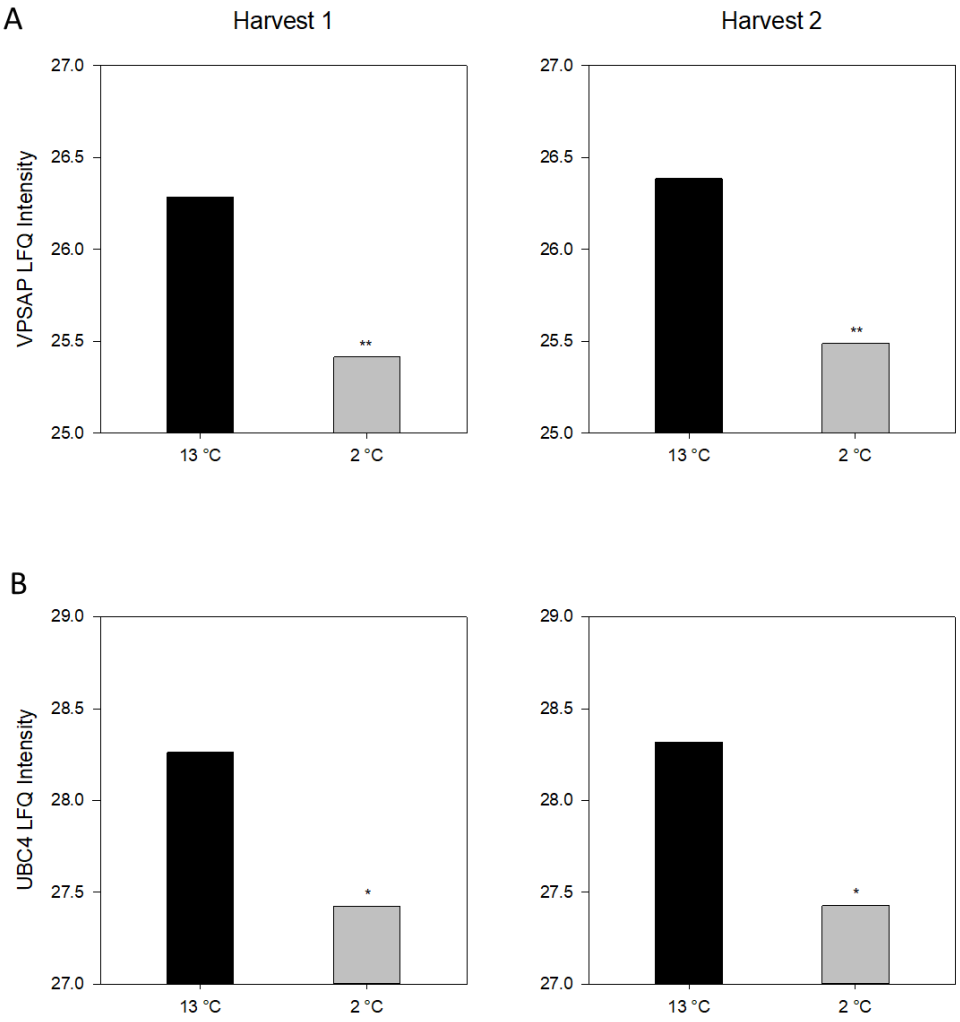

Figure 33
: Protein expression after tomato exposure to a constant optimum temperature (13 °C; control) and to a chilling temperature (2 °C). (A) VPSAP and (B) UBC4. Asterisks indicate significant differences between control and chilling temperature.

Proteasome subunit alpha belongs to the peptidase T1A family of proteins that are responsible for forming proteasomes (Table 3). Proteasome subunit alpha is only a small piece of the proteasome but plays a vital role in the cleavage of a broad range of peptides that are Arg, Phe, Tyr, Leu, and Glu residues. This subunit is significant because it is the active site of the enzyme and its inclusion is important to the normal function of the proteasome. A previous

study has reported that when a tomato plant is exposed to periods of salinity stress there is an alteration of the catalytic site in the proteasome (Kovacs et al., 2017). In this study there was a downregulation of proteasome subunit alpha indicating that there is a possible loss of cell viability (Figure 34A). It is possible that chilling injury can induce this same or similar reaction in the proteasome resulting in a reduced functionality of the enzyme. This reduced functionality is correlated to loss of cell viability (Kovacs et al., 2017).

Phosphomannomutase belongs to the eukaryotic PMM family of proteins that are responsible for the synthesis of nucleotide-sugars (Table 3). In tomato, the enzyme phosphomannomutase is pivotal to the formation of ascorbic acid because it catalyzes the synthesis of alpha-D-mannose 1- phosphate into D-mannose 6-phosphate (Ruggieri et al., 2016). D-mannose 6-phosphate is a precursor to many important tomato metabolites besides ascorbic acid, and it is also used to synthesize sugars that the cell uses in primary metabolism. Mainly found in the cytoplasm, this enzyme is very important to the tomato's overall health (Ruggieri et al., 2016). In this study, it was found that tomatoes exposed to chilling injury had downregulated levels of phosphomannomutase (Figure 34B). There was not a significant difference between the levels ascorbic acid of tomato exposed at 2 °C compared to 13 °C (Figure 31), however the downregulation of this one enzyme might not be enough to impact the AA content of the tomato. The AA pathway is complex and might have other means of circumnavigating this downregulation.

Glycosyltransferase is a member of the glycosyltransferase family of proteins that are responsible for glycosidic linkages and sterols (Williams et al., 2008) (Table 3). These proteins have been shown to play an important role in plants for the development and maintenance of the cell membrane integrity (Ramirez-Estrada et al., 2017). Membrane fluidity and permeability have been shown to deteriorate due to chilling injury stress which results in cellular death due to leakage of cytosol. The tomato plant regulates membrane integrity by controlling the overall amount of membrane linked sterols which are regulated by glycosyltransferase (Larsson et al.,

1990). In this study, the expression of glycosyltransferase was lower in tomato fruits that were exposed to chilling injury when compared to fruit stored at ideal temperatures suggesting that membrane integrity might have been compromised in fruit exposed to 2 °C (Figure 35A).

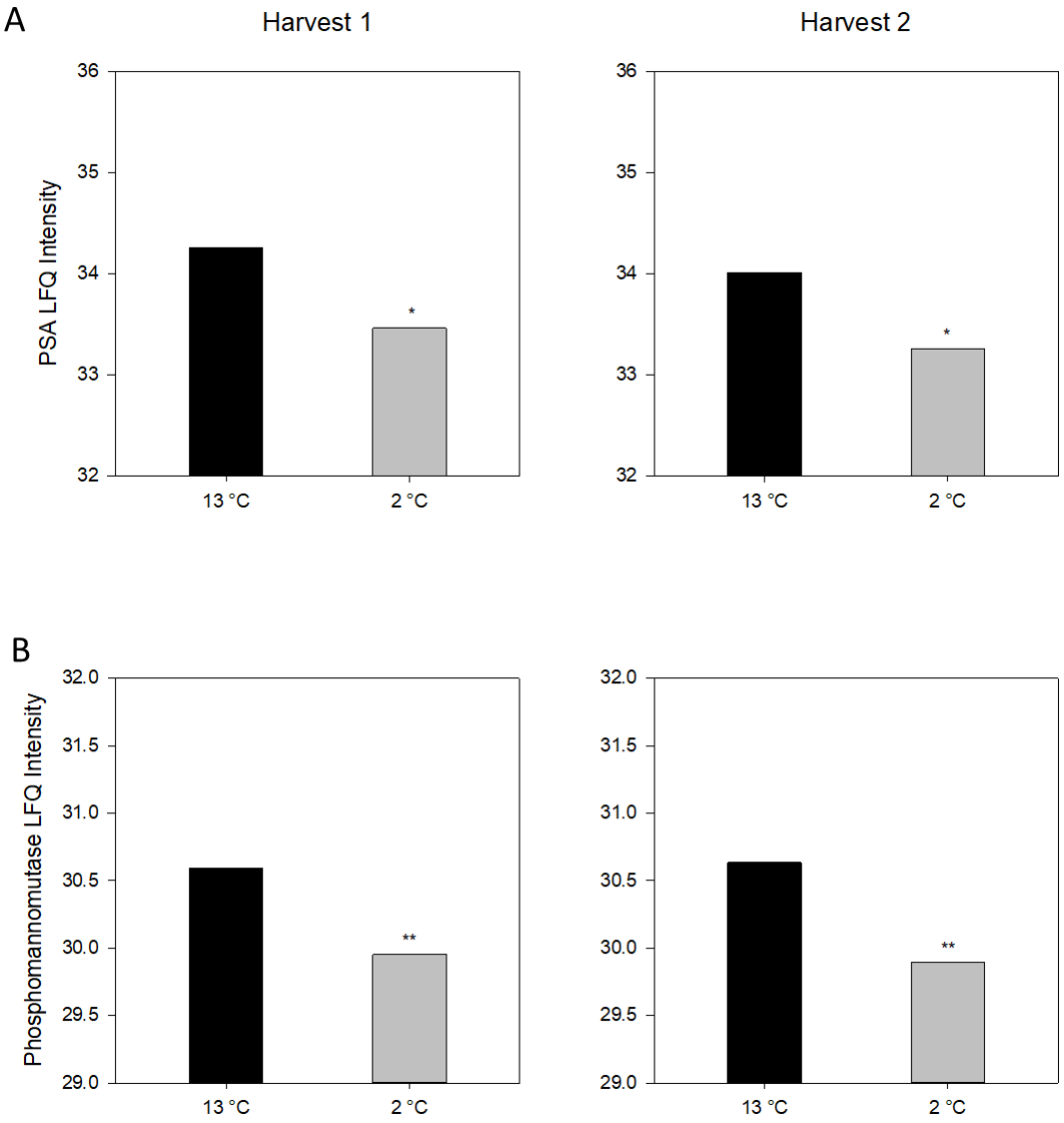

Figure 34: Protein expression after tomato exposure to a constant optimum temperature (13 °C; control) and to a chilling temperature (2 °C). (A) PSA and (B) Phosphomannomutase. Asterisks indicate significant differences between control and chilling temperature.

Prefoldin subunit 4 is a member of the prefoldin subunit beta family of proteins that promotes the folding of polypeptide chains, especially in unfavorable environments such as tomatoes stored at abuse temperatures. The full molecule of prefoldin is a hexameric molecule

present in all eukaryotic cells. However, there is little information on the protein's evolution in plant cells (Cao, 2016). In maize, the gene controlling the expression of two prefoldin genes is significantly downregulated in stress conditions including low temperatures, while two other prefoldin genes are upregulated (Cao, 2016). Low temperatures have been associated with poor microtubule stability, including changed orientations, root elongation, and depolymerization (Liu et al., 2014; Bartolo and Carter, 1991). The data from our study shows that in tomato exposed to 2 °C, there was a downregulation of the prefoldin subunit 4, but not the whole protein (Figure 35B). It is possible that the cell response to chilling injury results in reduction of the production of one subunit of the entire hexamer in response to the cold damage in order to maintain microtubule integrity.

Heme oxygenase 1 is a member of the HSP family and is responsible for heme oxygenation or decyclizing (Table 3). Although in the tomato plant this enzyme is not well studied, it has been associated with root growth (Xu et al., 2011). Another study has found that heme oxygenase's play an important role in regulating carotenoid development and impact fruit development (Auldridge et al., 2006). In our study, there was a downregulation of the enzyme heme oxygenase 1 in tomato fruits that were exposed to chilling temperatures compared to those kept continuously at 13 °C, suggesting that heme oxygenase was repressed by cold temperatures (Figure 36). It is well known that chilling injury causes an uneven and/or delayed ripening in tomato fruit due to abnormalities in carotenoid synthesis. Consequently, delayed ripening may result in lower carotenoid synthesis and thus downregulation of proteins such as heme oxygenase 1.

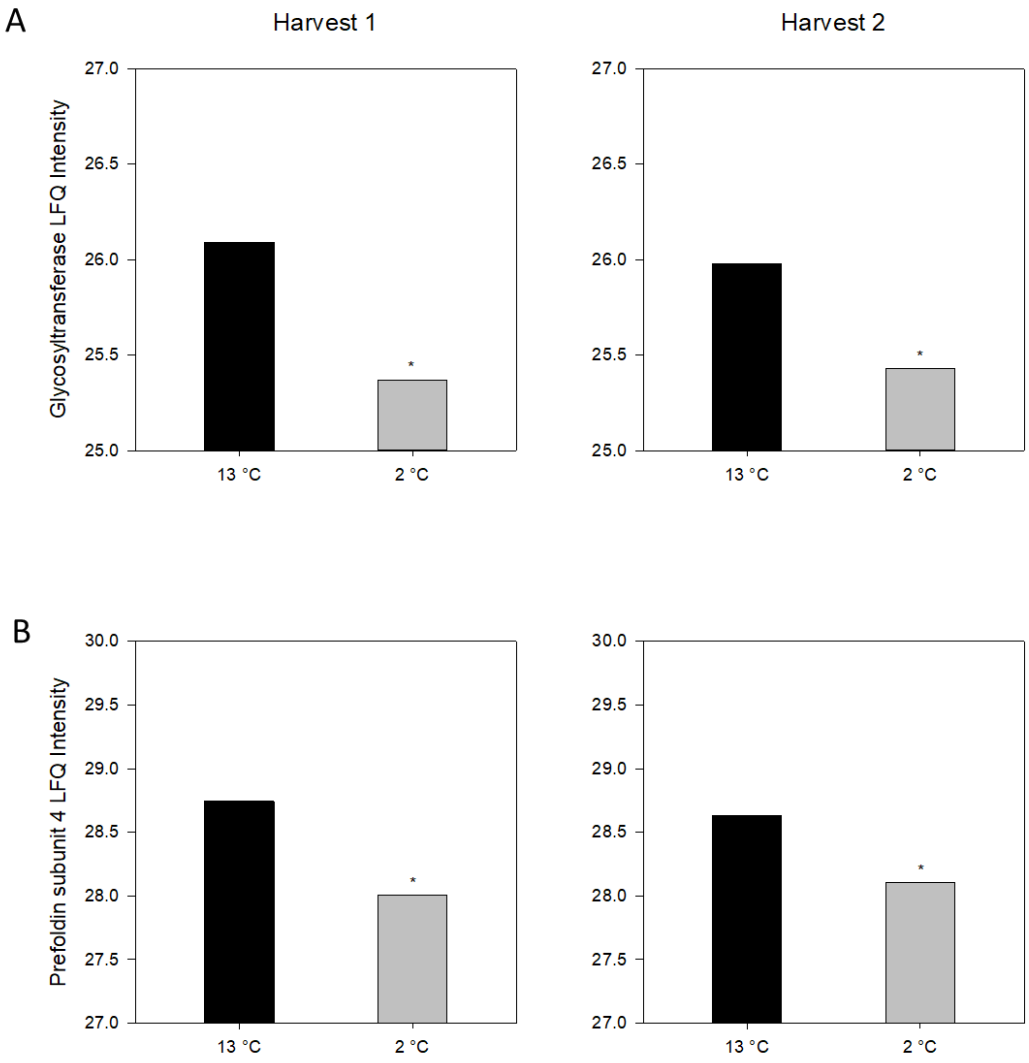

Figure 35: Protein expression after tomato exposure to a constant optimum temperature (13 °C; control) and to a chilling temperature (2 °C). (A) Glycosyltransferase and (B) Prefoldin subunit 4. Asterisks indicate significant differences between control and chilling temperature.

Cleavage and polyadenylation specificity factor subunit 2 (CPSF 2) perform a role in mRNA post transcription modifications (Table 3). In plant cells, CPSF 2 is not well studied, however it has been described well in yeast cell lines. In yeast, CPSF 2 is a cleavage factor that is vital to the mechanism of mRNA 3'-end formation (Zhao et al., 1997). In our study, CPSF 2 was upregulated in tomatoes that were exposed to chilling temperatures compared to those maintained at 13 °C (Figure 37A). Tomatoes exposed to cold temperatures tend to have lower metabolic rates thus CPSF 2 being present in higher amounts in chilled tomatoes, may suggest

that the fruit was trying to overcompensate for the lower metabolic activity (Rugkong et al., 2010).

Ferredoxin belongs to the 2Fe2S plant-type ferredoxin family of proteins (Table 3). Their role in the cell is to regulate the flow of electrons in the electron transport chain in photosystem I (Fukuyama, 2004). The protein's regulation helps the flow of electrons from photosystem I in the thylakoid membranes to the ATP synthase in order to create ATP (Golbeck, 1987). Because ferredoxin needs to be attached to a sulphur-iron box in order to function, iron can be considered the enzyme's cofactor (Hanke, 2013). The results from our study showed that ferredoxin is upregulated in tomato plant cells that have been exposed to chilling temperatures compared to fruit maintained at 13 °C (Figure 37B). Ferredoxin is upregulated in both high and low temperature abuses indicating that it acts either as a regulator for temperature stress or is highly prone to temperature changes and that tomato cells need to produce more to compensate.

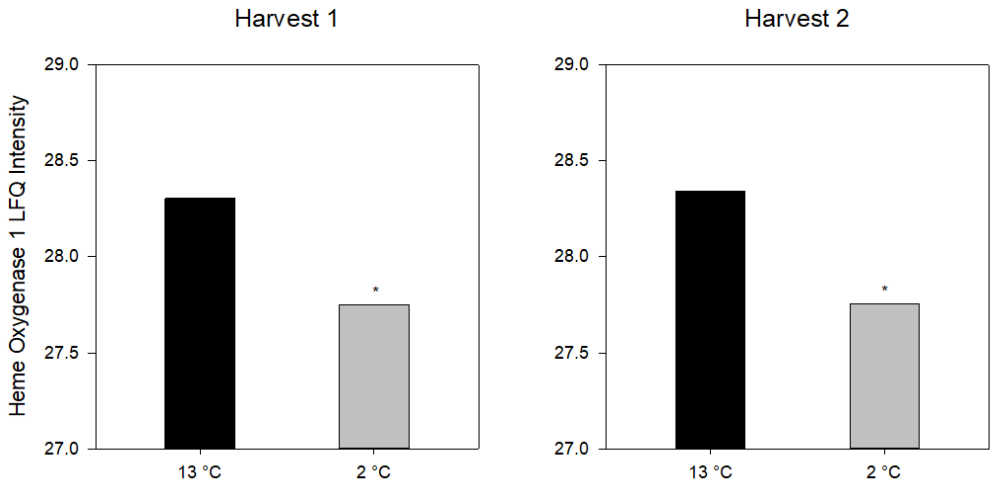

Figure 36: Expression of protein heme oxygenase after tomato exposure to a constant optimum temperature (13 °C; control) and to a chilling temperature (2 °C). Asterisks indicate significant differences between control and chilling temperature.

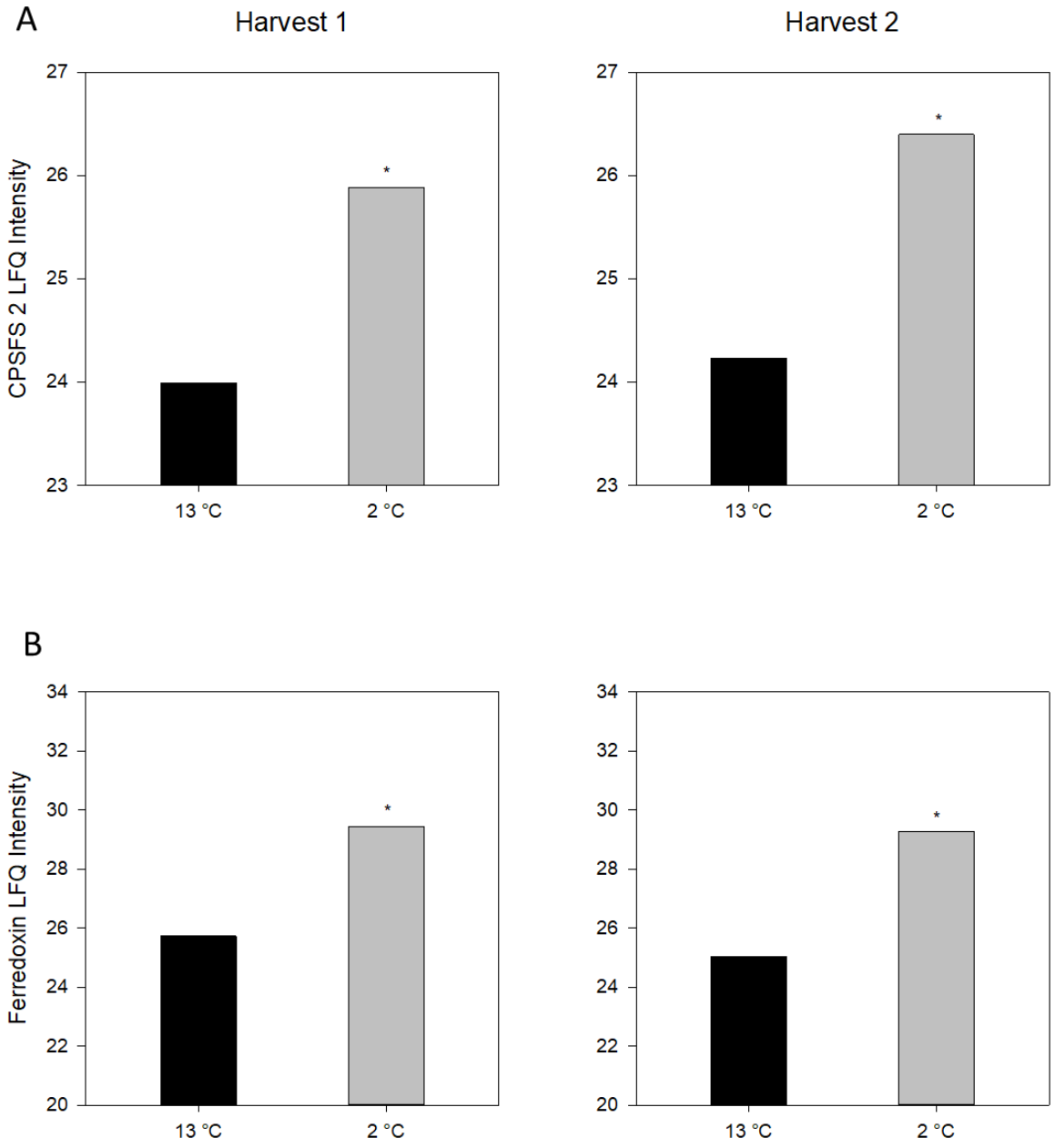

Figure 37: Protein expression after tomato exposure to a constant optimum temperature (13 °C; control) and to a chilling temperature (2 °C). (A) CPSFS 2 and (B) Ferredoxin subunit 4. Asterisks indicate significant differences between control and chilling temperature.

Proteome analysis of tomato fruit exposed to chilling and non-chilling temperatures showed that depending on the temperature, specific enzymes can be up- or downregulated. Overall, the heat stress proteins that were differentially expressed were HSP regulating proteins

and metabolic proteins. High temperature environments result in accelerated tomato ripening and so these proteins might have been stimulated by the warm environmental conditions used in this study (25 °C versus 13 °C). It is possible, that while the HSP regulating protein might be activated to prevent or reduce cell damage caused by heating, the metabolic proteins increased the rate of tomato ripening. Tomato response to cold stress resulted in an upregulation of mRNA modifier proteins and in a photosynthetic protein. Proteins that were downregulated in response to cold temperature (2 °C) were proteins that control ascorbic acid synthesis, metabolism, cell wall integrity, and intracellular communication. Chilling injury is known to damage metabolic processes, impacting the levels of several important tomato chemical components. All proteins that were downregulated as a result of exposure to chilling temperature are vital to tomato normal metabolism thus chilling injury may result from the reduced activity of these proteins. Finally, cell wall and membrane leakages associated with chilling injury might be attributed to the downregulation of proteins responsible for maintaining the integrity of the cell wall when tomato fruit is exposed to chilling temperatures.

Table 2. Proteins differentially regulated during exposure of tomato fruit to 25 °C.

Number	Protein ID	Protein Name	Function	Localization	Effect
1	D9IV43	MAPK8	Regulation of gene expression via ATP binding	Nucleus	Downregulated
2	K4BSC6	RNA cytidine acetyltransferase	Required for nucleolar cleavages of rRNA	Nucleus	Downregulated
3	C0KKU8	Lipoxygenase	Required for the biosynthesis of lipids	ER	Upregulated
4	Q9XEX8	Remorin 1	Plant specific protein responible for cyclin-dependent protein serine/threonine kinase activity	ER	Upregulated
5	K4B1W7	Ferredoxin	Transfer of electrons to ATP synthase in ETC	Chloroplast	Upregulated

Table 3. Proteins differentially regulated during exposure of tomato fruit to 2 °C.

Number	Protein ID	Protein Name	Function	Localization	Effect
1	K4CXG3	Vacuolar protein sorting-associated protein	Regulates MVB formation and organization of vacuoles	Endosome	Downregulated
2	K4CYK1	UBC4	Binds ubiquitin to proteins	Cytoplasm	Downregulated
3	K4DC02	Proteasome subunit alpha	Multicatalytic proteinase resposible for the destruction of misfolded proteins	Nucleus	Downregulated
4	K4C108	Phosphomannomutase	Synthesis of the GDP-mannose and dolichol-phosphate-mannose	Cytoplasm	Downregulated
5	K4BV87	Glycosyltransferase	Ttransferring glycosyl groups and cell wall organization	Golgi Apparatus	Downregulated
6	K4B0B7	Prefoldin subunit 4	Promotes protein environment, especially when there are many competing protein confermations	Cytoplasm	Downregulated
7	Q94FW7	Heme oxygenase 1	Helps break cyclical bonds	Chloroplast	Downregulated
8	K4BF27	Cleavage and polyadenylation specificity factor subunit 2	Posttranscriptional gene silencing	Nucleus	Upregualed
9	K4B1W7	Ferredoxin	Transfer of electrons to ATP synthase in ETC	Chloroplast	Upregualed

Chapter Six:

General Conclusions

The results from the work presented in this thesis show that tomato physical and chemical quality are differentially impacted by each step along the supply chain. Overall, the two steps that showed the most significant impact on tomato quality were impact cooling at 25 °C and shipping to the supermarket at 2 °C. Cold storage at 2 °C, contributed significantly to a decline (19 to 45%, depending on the attribute measured) in tomato chemical and nutritional quality specifically a decline in the levels of acidity, soluble solids content, carotenoids, fructose, glucose, and total sugars. During impact cooling at 25 °C there was also a decline in tomato sensory, chemical and nutritional quality (5 to 23%, depending on the attribute measured) with fruit showing increased shriveling and decay, decline in overall appearance quality, increased softening and decreased acidity, fructose, glucose, carotenoid contents. Both temperature abuses (2 °C and 25 °C; chilling and non-chilling temperatures) resulted in a lower tomato salability because of eventual lower consumer perceived quality.

In this study, it was also found that there were distinct trends in protein regulation between chilling and non-chilling temperature exposure. Shipping to the stores at 2 °C resulted in an upregulation of mRNA proteins and photosynthetic proteins and a downregulation of control ascorbic acid synthesis, metabolism, cell wall integrity, and intracellular communication proteins. Impact cooling at 25 °C resulted in differential expression of proteins that are responsible for HSP regulation and proteins responsible for metabolism.

Further studies on tomato quality and proteome should focus of how the entire supply chain impacts the proteome with a more thorough investigation into the proteins that regulate

important chemical markers such as ascorbic acid and carotenoids. Phosphomannomutase is a pivotal part of the ascorbic acid metabolic pathway and is downregulated when tomato fruit is exposed to chilling temperatures due to the repressed ripening associated with exposure to cold temperatures. Because the ascorbic acid pathway is a large and interconnected pathway with great influence on tomato health and quality, there are possibly more proteins that are impacted by exposure to temperature abuses and need further research.

Based on the results presented in this thesis, future studies should also investigate how different tomato cultivars are impacted by the supply chain. Results from this and further, more in-depth studies, on the impact of temperature on tomato proteome will provide new understandings into possible regulation mechanisms. Such findings not only will provide proteomics information on the regulation of ascorbic acid and carotenoid biosynthesis on tomato fruit but will also lead the way to further proteomic studies on other fruit models. Finally, finding biomarkers of pre- and postharvest abiotic stress in tomato fruit, which can with additional work be applied to other fruits, will constitute a useful tool for the food industry in helping to identify mishandling.

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