ABSTRACT

LLOYD, MICHAEL. Carotenoid Processing: The Effects of Microwave-assisted Processing on Carotenoid Ingredients (Under the direction of Dr. Jonathan Allen)

Tomatoes contain carotenoids with 40-hydrocarbon chain structures possessing the ability to indirectly protect cells from damage by absorbing free radicals and directly stimulating metabolism-regulating human biological responses. Tomato processing is a mature domestic market that produces over 30 million tons of carotenoid material annually. While processed tomato production is well characterized in industrialized and standardized convection processes, little work has been done using energy efficient alternative food processing techniques such as volumetric heating which upon exposure to microwave energy generates heat from polar water molecules within tomato fruit homogenate material. The purpose of this dissertation was to use microwave-assisted heating to develop a tomato processing model that can be utilized to investigate knowledge gaps pertaining to processing of frequently consumed carotenoid-containing fruit crops for added value effects and identify industrial applications for energy efficient processing technologies.

In the first study, tomato conditioning and microwave assisted heating were used to develop a mechanical and thermal bioprocessing model system for summer harvested tomato carotenoids with or without processing aids. The impact of microwave-assisted thermal sterilization and bioprocessing with the commercially available processing aids, sunflower oil and sunflower lecithin, on carotenoid isomerization and bioaccessibility were evaluated. Results of *in vitro* digestibility and HPLC analysis indicated that microwave-assisted processing increased beta-carotene and lycopene isomerization and bioaccessibility in the presence of processing aids. Results indicated a significant increase in lutein isomerization occurred in the

presence of processing aid lecithin, however the increase in lutein isomers ($P < 0.05$) did not result in increased lutein micellarization. After treatment of tomato homogenate containing processing aids with microwave heating (90-97 °C, 8-12 minutes), lycopene bioaccessible content was increased 5-fold ($P < 0.01$) and 6.5-fold ($P < 0.01$) for *cis*-lycopene and *trans*-lycopene, respectively. Heated Roma tomato with oil (HRO) treatment showed the largest decrease ($P < 0.05$). in *trans*-lycopene isomer content compared to control. *Cis*-beta carotene bioaccessible content increased 5-fold ($P < 0.01$) in processing aid incorporated microwave heated treatment conditions.

In the second study, the mechanical and thermal bioprocessing model system was used to process and compare fall-harvested tomato carotenoids from red Roma tomatoes and orange tangerine tomatoes to investigate if microwave-assisted thermal processing influenced lycopene bioaccessibility and isomerization. Evidence of isomerization efficiency was observed in microwave processed Roma tomatoes after heating with or without processing aids. The data suggests processing aid incorporation contributed to energizing or oxidation of lycopene double bonds, which effectively altered light absorbance commonly observed for lycopene in crystalline state conformation. Increased susceptibility to isomerization due to bioprocessing conditions was supported by HPLC analysis and UV-spectrophotometer experiments demonstrating a chemical change in lycopene structures and an altered reactivity to light absorbance and refraction in thermally processed treatments. Lycopene bioaccessibility was increased in microwave heated processed Roma tomatoes with or without processing aids, except for lecithin treatment.

In summary, we provide proof of concept that microwave-assisted heating may be used to induce lycopene and beta-carotene isomerization. This system may be used to investigate

some of the emerging questions in fat soluble antioxidant bioaccessibility pertaining to micellarization efficiency, micelle speciation, micelle morphology distribution, process specific carotenoid isomer speciation and electromagnetic energy mediated hydrocarbon energy transfer characterization complemented by mechanism elucidation in cultivar-specific foodstuffs. We provide evidence of translational research and development for post-harvest processing with microwave heating adding value to carotenoid-containing tomato ingredients that may provide functional benefits and industrial applications for the technology. We extrapolate from our findings to suggest that microwave heating after mechanical homogenization may provide an enhanced micellarization opportunity for carotenoids. However, results indicate cultivar and harvest season may impact microwave assisted heating-induced isomerization without processing aids.

Carotenoid Processing: The Effects of Microwave-assisted Processing on Carotenoid Ingredients

by
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BIOGRAPHY

Michael Lloyd is the eldest son of Craig and Janice Lloyd, born and raised in Daytona Beach, Florida. He excelled in scholastics throughout elementary, middle, and high school under the Volusia County Public School System. Michael earned an athletic scholarship to play division 1 college football for South Carolina State University out of high school and ultimately completed his career as a student-athlete at Savannah State University where he earned an undergraduate degree in Biology.

After graduating from the university, he was hired by Diosynth Biotechnology in Durham, North Carolina to work in downstream protein purification vaccine development, and then by Argos Therapeutics, in the same position to work on RNA gene therapy vaccine development. While working full-time, Michael was able to matriculate through graduate school at North Carolina Central University (NCCU) earning a Master of Science degree in Biology/Biomedical Sciences in 2007. He was awarded a National Institutes of Health (NIH) sponsored Bridges-to-the-PhD fellowship for minority STEM students to attend a Historically Black College and University (HBCU) then bridge to a predominately white institution (PWI) such as North Carolina State University (NCSU) for an opportunity to earn a Doctor of Philosophy degree. He constantly split time working in industry and at NCCU's Julius L. Chambers Biomedical/Biomedical Sciences Research Institute within their neuroscience and drug abuse program under the supervision of two-time North Carolina State University (NCSU) graduate Dr. Derek Norford. Michael contributed successfully to the elucidation of cannabinoid mediated signal transduction pathway between cannabinoid receptor 1 (CB1), nitric oxide synthase (NOS) gene regulation and nitric oxide (NO) production in neurons.

At the beginning of 2008, Michael was laid off from his Vaccine Development Scientist position at Argos Therapeutics. This recession engendered event propelled Michael to return to his entrepreneurial roots and launch his product and process development company, Amelioron Corporation. He immediately began working towards commercializing a 4th generation consumer product passed down by his grandfather in 2004. In August of 2008, Michael accepted a Research & Development Scientist position in Vaccine Development at Wyeth Pharmaceuticals. He worked in the Protein Purification Division developing and scaling drug processes for clinical trial material in phase I and II studies. While working on the Prevnar 13 vaccine, Michael commercialized a healthy condiment brand by selling through local specialty retail grocery stores. After roughly 1 year of working full-time while pursing entrepreneurial endeavors in his down time, Michael decided to pursue a PhD in Food Science at NCSU.

Michael is a single father and has 3 wonderful children, Mekhi, Michael and Tianee. Their love, support and inspiration has driven him to improve every day and work tirelessly to complete this work.

Thank you to the company and agencies that have funded and made this project a reality. Thank you to the Amelioron Corporation executive board for approving funding for this project throughout the past 10 years. Thanks to the North Carolina State University Graduate School for providing NIH funding through the Bridges-to-the-PhD program and the Minority Enhancement Education Grant Program. Thanks to my grandfather Archie Faison for emotional and financial support over the years pertaining to this effort.

Special thanks to Jonathan Allen for supporting me throughout my time as a graduate student dating back to my acceptance into the NCSU Nutrition Sciences graduate program in 2009 and the continued support and belief in my abilities. Thanks to MaryAnne Drake for

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Thanks to all my friends, supporters and loved ones that have helped me throughout this journey with advice, encouragement, trials and pure energy to thrust me to the well-rounded diverse Scientist, Entrepreneur, man and father I am today: Archie Lee Faison Jr,, Mekhi Lloyd, Dr. Derek Norford, Richard Abbott, Nelly Faison, Craig M. Lloyd, Merrell C. Lloyd, Janice Lloyd, Mary Speight, Ruby Wilson, Craig A. Lloyd, Debby Alexander, Shayla Lloyd-Jones, Michela Hayes, Vicky Arrington, Julius L. Chambers, Akela Shakir, Dr. Alynn Howlett, Dr. Diedra Guyon, Dr. K.P. Sandeep, Dr. Romanger Worthin, Broderick McGill, Charles Cherry, Katrina Ellis, Mary Stewart Tulak, and to all the supporters of our initial functional condiment brand past and present.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW

CHAPTER 3

Chapter 1

Introduction

Currently, there is a broad societal need to improve the nutrient density of foods to a degree that a functional benefit is achieved through dietary consumption. Today's consumers expect the availability of healthy food options to maintain good health. The tasks of providing nutritionally adequate safe and enjoyable products lies in the hands of professional Food Scientists. Advances in information sharing technologies have created waves of health-conscious consumers that demand transparency, accountability, innovation, and efficiency as it pertains to what is available on the market to maintain healthy lifestyles. Technological advances have also provided Food Scientists with novel methodologies and information required to develop the tools necessary to meet consumer expectations. To this end, investigating carotenoid processing and the impact it has on subsequent value-added products has enormous potential for improving the nutrient density of shelf stable foods and ingredients.

Cardiovascular disease (CVD) is the #1 cause of death in America. Nearly one in three US. adults, or 68 million persons, have hypertension; and nearly 30% of all US adults are pre-hypertensive (Whelton, 2018; CDC, Vital signs hypertension, 2011; Roger, 2012). Hypertension is a major contributor to cardiovascular diseases, which are a leading cause of death, disability, and health-care costs in the United States (Heidenreich, 2011). Cardiovascular disease is often associated with the diagnosis of metabolic related disorders such as diabetes and obesity. Improving the absorption and vascular circulation of carotenoid antioxidants could aid in improving the outcomes of individuals with CVD risk factors and proinflammatory conditions associated with aging. One of the processes associated with aging is a cumulative effect of free

radical damage to lipids, proteins, and nucleic acids. These effects have potential to impact vascular plasticity or flexibility, resulting in hypertension, which then can cause cardiovascular diseases. A lifetime with a higher intake of highly bioavailable carotenoids from colorful plant foods might provide the antioxidant defenses to slow this process.

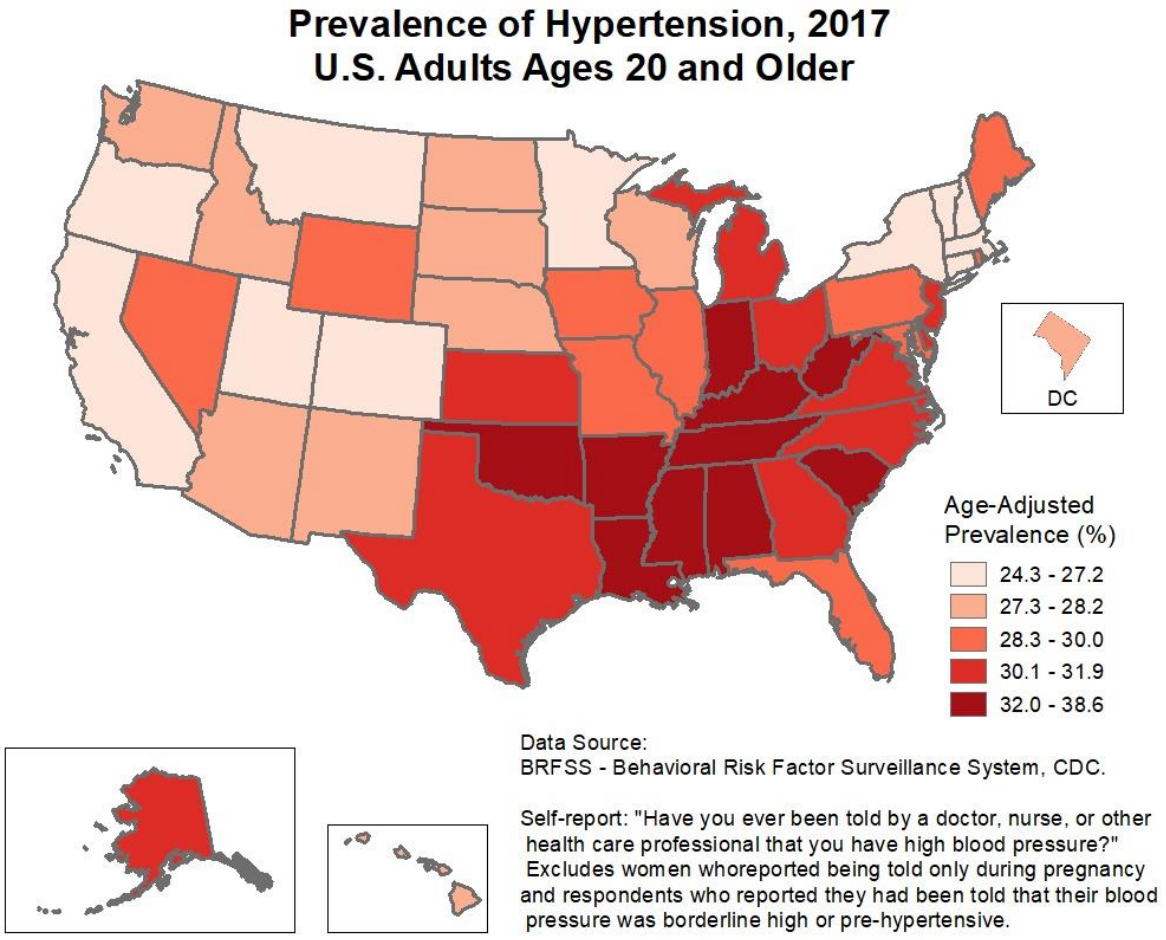

Figure 1.1. CDC Facts about Hypertension in the USA. (Whelton et al. Hypertension. 2018;71(19): e13–115).

Chapter 2

Literature Review

Carotenoids

Carotenoids have important biological functions, with roles in light capture, antioxidative activity and protection against free radicals, synthesis of plant hormones and as structural components of membranes. They are lipid-soluble compounds that play an important role in human health and nutrition. In nature carotenoids are orange, yellow and red pigments produced by plants, algae, bacteria and fungi. Plants and algae utilize carotenoids to absorb light energy used in photosynthesis and protect chlorophyll from photo damage (Armstrong, 1996). In mammals, carotenoids are obtained from the diet and are stored in fatty tissues. As it relates to the human diet, carotenoid absorption is improved when consumed with fat in a meal (Jayarajan, 1980). Fruits and vegetables provide the majority of the roughly 50 carotenoids found in the human diet with the most common consisting of β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene (Wang, 2014).

Carotenoids are also known as tetraterpenoids, which are all derived from tetraterpenes. Tetraterpenes are terpenes consisting of eight isoprene units and have the molecular formula of $C_{40}H_{64.}$ (Davis, 2000). Epidemiological evidence demonstrates that greater intake of carotenoids offers protection against cardiovascular disease, cancer, macular degeneration and neurodegenerative diseases (Krinsky, 2005). Carotenoid pigments are valuable within the food and pharmaceutical industries due their biological relevance and biocompatible nature. α-Carotene, β-carotene and β-cryptoxanthin are provitamin A carotenoids, meaning they can be

converted by the body to retinol. Lycopene, lutein, and zeaxanthin are non-provitamin A carotenoids because they cannot be converted to retinol.

Beta Carotene

Dietary carotenoids are absorbed intestinally as they are released from the food matrix and incorporated into mixed micelles consisting of bile salts and several types of lipids (Boileau, 1999). Carotenoids have various functions based upon the cell, tissue and vascular system needs. β-Carotene is a primary dietary vitamin A source found in orange flesh sweet potatoes. With vitamin A being an essential micronutrient, its deficiency is an issue of malnourishment that is considered a global threat. Vitamin A deficiency is one of the most prevalent micronutrient deficiency disorders in the world. According to the national surveys vitamin A deficiency prevalence has remained high in pre-school children in much of the less developed world (IVACG, 2003; IVACG, 2004). Vitamin A plays a key role in vision, immunity, cell differentiation, maintenance of cell membrane integrity, embryonic development and growth and reproduction (Garrow, 2000). Vitamin A deficiency is the leading cause for the blindness among approximately 350,000 pre-school children in each year (Garrow, 2000). Also, more than 250 million children suffer from sub-clinical vitamin A deficiency worldwide (IVACG, 2003). Research studies show daily administration of 15 or 45 mg β-carotene significantly increased the plasma β-carotene levels (Dimitrov, 1988). The use of sweet potatoes in the Consumer-Packaged Goods (CPG) food industry often involves processing of the roots into purees that can be subsequently frozen or canned for year-round availability of the produce. Sweet potato purees (SPP) can be used as an ingredient in various products, including baby food, casseroles, puddings, pies, cakes, bread, restructured fries, patties, soups, and beverages (Truong, 1992; Woolfe,1992; Truong, 1995; Walter, 2001).

Lutein and Zeaxanthin

Lutein and zeaxanthin usually referred to as “macula pigments” are known to provide protection against age-related macular degeneration mediated by their ability to scavenge harmful reactive oxygen species formed in the photoreceptors (Landrum, 2001). Lutein and zeaxanthin are found in neural tissue in both the geriatric and pediatric brain, and it is believed that the anti-oxidative properties of lutein may be advantageous at the neural level (Craft, 2004; Lieblein-boff., 2015; Vishwanathan, 2014a; Vishwanathan, 2014b). Reports have shown a relationship between lutein and cognitive and brain health (Johnson, 2014). Lutein, in particular, is known to accumulate across all cortices and brain membranes and has been implicated in the prevention of eye diseases as well as preservation of cognitive abilities in older adults (Erdman, 2015; Ozawa, 2012; Renzi, 2014; Vishwanathan, 2013; Vishwanathan, 2014a; Vishwanathan, 2014b).

Lycopene

Lycopene activates important mechanisms in the human body that protect against pathogenesis of degenerative diseases, especially coronary heart diseases, cancers and an array of other free radical-mediated conditions (Halliwell, 1997; Kritchevsky, 1999). High lycopene and tomato intakes have been found to be associated with a reduced risk of prostate cancer (Giovannucci, 1995). Lycopene derived from tomatoes is the most commonly consumed carotenoid in the US. Lycopene exhibits the highest antioxidative properties among all dietary carotenoids (Miller, 1996; Bohm, 2002). Tomatoes and tomato-based products are also the dietary sources of quercetin and kaempferol, polyphenols that are present almost exclusively as conjugates (Vinson, 1998; Stewart, 2000). According to Paganga (1999) tomatoes also contain naringenin (flavanone), and chlorogenic acid. Of 22 vegetables analyzed by Vinson and colleagues, tomatoes

were 17th in the ranking of total phenols (368 mg/kg), but 1st in phenol consumption (42 mg/day) in the US diet (Vinson, 1998).

In vivo studies report that tomato products improved the antioxidative activity of the plasma and reduced LDL oxidation in healthy men (Lee, 2000; Bub, 2000). Tomato value-added products such as ketchup, barbeque sauce, salsa, and pizza sauce represent the majority of carotenoid-based foods typically consumed by Americans. Tomato farmers in California grow roughly 300,000 acres for tomato processors producing 12 million tons (23 billion lbs.) of product annually, worth an estimated $8 billion (Hartz, 2008; Carter, 2008). The state of California accounts for over 90% of U.S. production and 35% of world production (Hartz, 2008). Tomato processing is a high value market in the US, yet the market's full potential related to carotenoid heart health benefits is not represented in many commonly consumed processed food products.

Tomato Processing and Manufacturing

Tomatoes are currently processed using a series of standardized methods dating back to the processing expansion era in California. These processes involve hot break (HB) methods to produce the most prevalent form of process tomatoes, tomato paste and tomato puree. Paste is the preferred form throughout the market of manufacturers, co-packers and ingredients distributors/brokers. Tomato value-added product manufacturers prefer paste because many of their recipes are based upon paste form as they have the flexibility of reconstituting with water to increase yields and control quality.

Tomato paste is a dispersion of solid particles (pulp) in an aqueous medium serum resulting from the concentration of tomato pulp after the removal of skin and seeds. Tomato paste is manufactured by an industrial process that includes unit operations such as heat-

treatment and pulping-finishing, which have different objectives: inactivation of the pectolytic enzymes, softening of tissues and removal of skin and seeds (Marsh, 1979). The rheological behavior of tomato paste is dependent on these processing variables (Sanchez, 2002). Tomato paste may be an ingredient in other derivatives, for example tomato ketchup. Tomato ketchup has unique viscosity and physical behavior properties which are particularly important from both engineering and consumer acceptance viewpoints (Charm, 1971; Mackley, 1994; Rao, 1977).

In 2006, Heinz controlled 50% of the ketchup market and was a major beneficiary of the research and development that took place in California (Hartz, 2008). Tomato paste worked optimally with their production and branded Heinz tomato seeds and cultivars (Hartz, 2008). Tomato ketchup is made by combining 97 lbs. of 31% NTSS, HB (Hot Break) tomato pastes with 153 lbs. of water to produce 250 lbs. of tomato sauce with viscosity and flow like Heinz ketchup. The formulated product is heated to 180^{o}F for 3 seconds and hot filled above 160^{o}F. This technology and the products produced by the systems have been supplying the markets for 70 years and not much innovation has occurred in the field during this time. Research and developers in the private sector are looking to innovate the stale tomato processing category, for example, by adding pungency to tomatoes through introducing expression of capsaicinoid genes with gene editing techniques or developing more flavorful cultivars. In keeping with the current trends and indicators in the market, consumers are looking for evolving change towards product innovation or superior quality differentiation in traditional categories. An indicator of change in consumer demand was evident in 2014, when the article “Ketchup Isn’t King” was published showing mayonnaise had overtaken ketchup’s #1 ranking of American condiments with sales at $2 billion annually compared to $770 million for ketchup. Previously, over the past 50 years tomato ketchup was the predominant condiment consumed in America. This consumer demand

change indicator is indicative of the sentiment that consumers are looking for perhaps superior, heathier, better-for-you, clean-label, uniquely flavored, novel, innovative and/or sustainably produced products.

Innovative Carotenoid Processing

Novel carotenoid processing may be a viable solution for innovating the tomato processing and condiment categories. Thermal processing is one of the main methods to preserve food products with increased shelf life and has been used for some time. Such methods rely on heating through conduction and convection creating the issue of the heating apparatus's surface becoming much hotter than the product itself. The problem with those methods (including but not limited to retort) is that heat is typically transferred to the food from an outside source resulting in a significant amount of heat loss, lower efficiency of heat transfer and low product quality due to overheating (Fasina, 2003; Lopez, 1987). A new thermal processing method involving microwave-assisted heating has been established and commercialized in North Carolina. This represents one of the emerging technologies in food processing, offering fast and efficient heating for the purpose of reaching sterilization levels without degrading nutrients and reducing poor product quality in processed foods, particularly lipophilic in nature, such as carotenoid-containing foods or dairy products (Coronel, 2003; Coronel, 2005). Yamco of Snow Hill, NC, has worked with NC State University researchers to develop and commercialize processing methods for #2 sweet potato crops (culls) into sterile aseptic sweet potato puree food ingredients. The β-carotene biofortified ingredients are used as starting material for baby food formulations. Thermal processes with the capacity to improve the quality of carotenoid ingredients and value-added end products may be applicable to the tomato processing industries.

Previous studies at NC State University's Thermal Processing Lab have reported processed tomato products have suitable properties for sterilization using a microwave-assisted heating method (Kumnar, 2008). This method uses high heat, low duration heating strategies to reach sterilization temperatures in seconds as opposed to minutes, thereby avoiding prolonged cook time as observed with conventional thermal sterilization. Microwave-assisted processing heats products by energizing polar water molecules and ionic salt compounds through friction within a food matrix, resulting in efficient and uniform heating throughout the volume of the food product. It has been hypothesized that microwaved-thermal processing can influence carotenoid isomerization and perhaps bioaccessibility. In 2006, a patent was granted to BASF by the U.S. Patent and Trademark office for thermal isomerization of lycopene using conventional thermal processing, however the technology has yet to be commercialized and marketed (Wegner, 2006). Inefficient energy transfer resulting in prolonged processing time may be preventing commercialization as the patent indicates processing time to achieve lycopene isomerization is between 16 and 40 hrs. (Wegner, 2006).

Microwave processing may represent a path towards innovation, sustainability and product improvements consumers expect today from the food industry and producers. Lower processing time requirements for carotenoid homogenate material may improve product quality by maintaining desirable organoleptic flavor profiles and perhaps improve antioxidant bioavailability. Previous studies have shown that a combination of homogenization and heat treatment enhances the bioavailability of carotenoids from vegetables (Rock, 1998; Van Zeben, 1948). Carotenoid bioavailability is influenced by numerous factors such as food matrix, quantity and composition of carotenoids and food preparation. In tomato tissue, lycopene is primarily located in the chromoplasts, in the form of crystals associated with membrane

structures (Nguyen, 2001). Stable crystal structures may prevent *trans*-lycopene from being oxidized or isomerized to a more bioavailable isomer. Nguyen and colleagues (2001) report thermal processing influenced isomerization in lutein and β-carotene, but not lycopene. On the contrary, studies by Van het Hof et al. (1999) reported severe homogenization (200 bar) and thermal treatment increased lycopene bioavailability. Knockaert et al. (2012) reported an increase in lycopene isomerization and in vitro bioaccessibility after high pressure and thermal processing of homogenized tomato puree containing oil. A possible conflict between carotenoid isomerization outcomes may be because lycopene, but not lutein and beta carotene; is conformed and confided to its stable crystalline structure and compartmentalized within chromoplasts and cell walls throughout the tomato pericarp. Novel processing conditions and methods applied to tomato material that effectively destabilize or denature inherent food matrix constructs may ameliorate lycopene post process quality correlated with optimal bioaccessibility.

Consumer Trends

The literature is inconsistent relating to the effects of processing on improving carotenoid overall quality or abundance of bioaccessible isoforms, however consumers have been loud and clear with their demands for higher quality food products. The Specialty Food Association and Mintel International reports the specialty food market grew 9.8% between 2016 and 2018 to $148.7 billion. At retail, specialty food sales outpaced the growth of all food, up 10.3% compared with 3.1% during the two-year period. According to a Specialty Foods Consumer report, nearly three in four consumers purchase specialty food products which may be defined as premium, produced in small batches, or featuring authentic recipes and high-quality ingredients. Product innovation and broader availability of specialty foods appear to be propelling the industry forward. According to the Specialty Food Association president, “Diverse consumer

lifestyles are taking specialty foods mainstream and to reach these consumers and increase their own sales, food merchants (like Whole Foods Market) have embraced the vast assortment of specialty products." This is consistent with research conducted by the Food Marketing Institute (FMI) 2019 Power of Health and Well-Being in Food Retail report indicating consumers broadly eye food as "medicine" to boost health, consumers believe in the health and social benefits of eating meals at home with family, shoppers exhibit strong opinions about food labels, health and transparency, and consumers have new wellness expectations from food retailers.

Recently the highest growth has been seen in plant-based alternative meats and alternative dairy products. Beyond Meat has reported revenue of $ 32 million in 2017 and recently has issued an Initial Public Offering (IPO), raising $250 million in order to increase their customer base, supplier network and co-manufacturing partners, expand marketing channels, invest in distribution and manufacturing facilities, hire additional employees and enhance technology and production. According to their prospectus, the strategy is to boost the plant-based meat category to the same proportion as alternative milk brands have cut into the US dairy industry market. The meat category is worth roughly $270 billion and Beyond Meat has targets of $35 billion in U.S. sales. Alternative milk brands now represent 13% the US dairy industry at sales of $2 billion in 2017. Plant -based alternative market demands are rising, but supply must keep up with demand. Ingredients supply issues with pea protein production have plagued Beyond Meat's growth efficiency. Perhaps this market has room for a carotenoid based alternative condiment containing higher quality carotenoid ingredients to complement plant-based meats.

In the U.S., milk sales are expected to drop by 11% between 2015 and 2020. This trend is expected to continue as milk alternatives grow in popularity along with the plant based-diet

trend. Sales of alternative milk such as almond, soy, and flax have spiked by more than 61 percent in the past five years leading some CPG milk advocates to express concerns about the use of the term "milk" compared to the current "standards of identity". Throughout the past half century, traditional milk has served as a vitamin fortification delivery vehicle for fat soluble vitamins D and later vitamin A. These hydrophobic essential nutrients have been added during the production process of milk for several decades to lower the risk factors for diseases associated with vitamin D deficiency. Today, due to vitamin D fortification programs diseases associated with vitamin D deficiency such as rickets are rare, however vitamin D deficiency remains a health concern issue for specific demographics and the general U.S. population. In the U.S., other than dairy milk, there is no common dietary access to vitamin D. Like milk, dietary carotenoid-based ingredients such as tomatoes are fat soluble. Due to the fat-soluble nature of carotenoids along with high volume consumption in America, processed tomatoes could potentially in the future serve as a reliable alternative shelf stable non-dairy fortification delivery vehicle for vitamins D and A.

Fortification

Fortification is a practice used across the world to prevent or reduce onset of disease (Kuczora, 2012). According to the World Health Organization (WHO) & Food and Agriculture (FAO), the process of fortification is defined as the addition of a nutrient to a food to improve its quality for people who consume it, generally for the purpose of reducing or controlling a specific nutrient deficiency (WHO, 2006). The deficiencies tend to be dependent on age, gender and ethnicity (WHO, 2006). Fortification strategies have been replicated with various micronutrients (e.g., Vitamin D, Iodine, Vitamin A) and has proven to be the most effective way to meet community health needs safely in a population-based approach. However, deficiencies in some

groups of people at risk require supplementation (Dary, 2002; Tulchinsky, 2010). Supplements are provided by healthcare professionals as an extreme public health measure in order to combat malnutrition (Feng, 2015). Despite the benefits, dietary supplementation has limitations.

Determinant factors such as low income, education level, community infrastructure, age and household size conditions impact micronutrient supplementation and accessibility (Feng, 2015; Marini, 2003). Other independent factors such as migration, inadequate program support, and inadequate delivery system could negatively impact treatment compliance and thus, the intended effect of supplementation (Feng, 2015; Galloway, 1994). Iron-deficiency anemia patients tend not to consume iron supplements because its delivery vehicle tends to cause abdominal discomfort and hardness of their stools (Feng, 2015; Galloway, 1994). Carotenoid based functional ingredients could serve as a viable food matrix delivery vehicle designed to alleviate both reported negative effects caused by iron supplementation. Utilizing carotenoids such as lycopene or beta-carotene that are already accepted by consumers in the form of value-added food products. Therefore, carotenoids would be ideal iron supplement delivery vehicles because carotenoids are consumed daily or weekly and they don't have side effects associated with intake. The incorporation or fortification of iron supplements to carotenoid-based foods result in carotenoid products being transformed into functional foods with health benefit advantages minus negative side effects. Market data indicates the supplements industry is expected to lose market share to functional foods in the near and distant future.

Personalized Nutrition

Ready-to-Use Therapeutic Food (RUTF) has been shown to be an effective malnutrition intervention approach for humanitarian programs. Malnutrition is a condition in which the body

does not absorb enough nutrients for its adequate development (Tulchinsky, 2010). RUTFs have simple ingredient compositions that include non-costly vegetable protein or grains, milk powder or whey protein isolate, vegetable oil, micronutrient fortifications and sugar (Annan, 2014). Examples of RUTFs available are pastes or drinks that can be consumed straight out of the package. Other fortified blended foods are provided as powdered products (intended to be cooked as porridge) and other dietary supplements (Annan, 2014). Ingredients processed to render carotenoid micronutrients and antioxidants more bioaccessible can be utilized as a base product for RUTF. Such ingredients can also be used as a base material for Functional Foods or Medical Foods.

Functional Foods

Foods provide nutrients or other substances that furnish energy to sustain growth or maintain/repair vital physiological processes. Functional foods move beyond necessity to provide additional health benefits that may reduce disease risk and/or promote optimal health (Hasler, 2004). The functional foods market in the USA is estimated to be worth $11 billion, while the global market is valued at $28 billion. The US functional foods market is driven by the high popularity of dietary supplements amongst sections of the population. Subsequently there is a high level of awareness regarding certain botanicals with anti-cancer properties, for example. Another major market driver has been the use of permitted FDA structure-function claims associated with certain ingredients and medical conditions, particularly within the heart health sector. This has been evidenced by the frequent use of health claims relating to the cholesterol-reducing properties of oatmeal, for example. Lycopene is well established in the scientific literature for its antioxidant benefits to heart health, but there is currently no commonly used tomato value-added food product or brand available on the market for consumers use, as is the case with oatmeal. Currently

traditional ketchup, BBQ sauces, or salsa do not qualify as heart health beneficial in part because of the high sodium content necessary to maintain an adequate desirable flavor profile and the lack of enhanced bioaccessible carotenoid content. There are currently no viable functional condiments available in the US market.

Medical Foods

Medical Foods are often confused with dietary supplements and functional foods that are neither targeted for specific disease conditions nor require medical supervision for product administration. To penetrate the medical foods market, participants must have a robust research and development (R&D) set-up, invest in regional product differentiation, and focus on product innovation. With life expectancy along with disease incidence rates going up, the cohort of people receiving medical attention is expanding and boosting the volume base of the medical foods market. Interest in clinical nutrition to treat diseases is gaining pace, fueling the demand for medical foods. The global medical foods space is well-consolidated, with the top few participants holding majority of the market share partly due to the prevalence of regional participation. The leading medical food manufacturers have been engaging in R&D, which is driving advances in food technologies and increasing the number of disease targets for medical foods. Immediate disease targets for carotenoid based medical foods are individuals at risk for cardiovascular disease, prostate cancer, skin cancer, dementia and neonatal abnormalities (Agarwal, 2000; Rao, 2002; Kim, 2011; Burri, 2009; Wang, 2015; Cooperstone, 2017, Feart, 2016; Hanson 2018). Once manufacturers start focusing on building physician and end-user awareness on medical foods, the market will inch towards its true value.

Carotenoid Production in North Carolina

Carotenoid production is a big part of agribusiness in the state of North Carolina. In the United States, North Carolina ranks first in the production of sweet potatoes, supplying nearly 50 percent of the market (North Carolina Sweet Potato Commission, 2016). More than 78,000 acres are grown in the state annually (Schultheis, 2018). Majority of the crops are sold as fresh to market whole produce. The sweet potatoes are scaled into sizes with a grading for specific retail markets. The roots that do not adequately meet specifications, known as culls, are generally regarded as waste, and often left behind in the fields. However, culls can also be processed into juices, purees and powders that can be added into other value-added product applications such as energy bars, breads, or sauces (Truong, 2010). The utilization of sweet potatoes previously discarded as waste streams has been successfully commercialized and is profitable in the state of North Carolina. Yamco, LLC of Snow Hill, NC produces sweet potato puree from culls homogenized and thermally processed using microwave-assisted methods to produce ingredients for baby foods produced by national brands. Additional research on value-added products and nutritional qualities derived from processed sweet potatoes could contribute to expanded uses as ingredients, as well as align with current consumer trends in healthy eating, novel functional ingredients and exotic flavors.

North Carolina orange fleshed varieties of sweet potatoes (*Ipomoea batatas L.*) are biofortified with β-carotene, which is a precursor form to vitamin A and retinoid. Vitamin A deficiencies are associated to xerophthalmia, blindness and premature death, mostly in developing countries (Yanggen, 2006). In developed countries like the U.S. micronutrient deficiencies are less prevalent due to national food fortification and enrichment programs (Hermann, 2014). For example: salt is fortified with iodine, sugar and milk are both fortified

with vitamins A (Arroyave, 2010), milk and dairy products with vitamin D, while cereals and flours are enriched with thiamin, niacin, riboflavin, folic acid, and iron (Feng 2015). However, in US, there are smaller demographics that have increased incidence in vitamin and mineral deficiencies such as Native Americans, African Americans and recently those maintaining strictly vegan, and vegetarian diets. The sweet potato matrix can provide a naturally fortified vitamin A source for non-dairy consumers to ensure adequate daily intake.

The micronutrient malnutrition that affects people worldwide can be responsible for many chronic diseases, such as osteoporosis and osteomalacia, thyroid deficiency, colorectal cancer, and cardiovascular diseases (Tulchinsky, 2010). This form of malnutrition in the Native American and African American communities is largely due to food insecurity and the prevalence of Food Desserts often associated with residing in poverty-stricken environments. Food insecurity is defined by the United States Department of Agriculture using different parameters as the lack of access to enough food for an active, healthy life for all household members with limited or uncertain availability of nutritionally adequate foods (USDA, 2017). As of 2016, according to the USDA about 31.1 million U.S. households were identified as food insecure (USDA, 2017). According to the USDA food deserts are defined as parts of the country void of accessible fresh fruit, vegetables, and other healthful whole foods, usually occurring in impoverished areas. Access to more fresh vegetables, fruits, greens, roots, beans and protein can help to minimize food insecurity and food deserts. Additionally, consumption of biofortified foods that contain provitamin A and subsequent value-added processed foods such as biofortified blend homogenate mixes may help improve health outcomes and minimize health disparities in minority communities. According to the CDC, minority populations have the highest rates of

cardiovascular disease in the US (CDC, 2011). In 2010, African Americans were 30 percent more likely to die from heart disease than non-Hispanic whites (CDC, 2014).

Sweet potato is known to be rich in nutrients that include carbohydrates, protein, fiber, β-carotene, vitamin C, E, phenolics, anthocyanins and contain significant iron and zinc (Truong, 2010; Wang, 2016). It ranked highest in nutritional value among vegetables available in the United States (Wang, 2016). Retinoids including retinol, retinal, retinoic acid, retinyl esters and certain carotenoids are forms of fat-soluble vitamin A (Arroyave, 2010). Vitamin A can be consumed in the diet in two forms: as preformed vitamin A or provitamin A carotenoids (Feng 2015). Provitamin A in the form of alpha-carotene, beta-carotene and beta-cryptoxanthin is found in green, orange, and yellow vegetables, and fruits such as apricots, broccoli, cantaloupe, carrots, pumpkin, dark leafy greens, squash, sweet potatoes, and tomatoes (Feng 2015). The human body converts these plant pigments into vitamin A (Feng 2015).

North Carolina Tomato Crop Production

Tomatoes crop production is the other major source of carotenoid derived Agribusiness generated by the state of North Carolina. Tomatoes are rich in vitamins A, C, B_3 and minerals potassium, magnesium, phosphorus, and small amounts of calcium and Vitamin E and other B vitamins. In 2017, 3,057 acres of tomatoes were harvested in North Carolina (USDA NC Census, 2017). Approximately 97% was harvested for fresh market while less than 3% was utilized for processing. In 2016, a total of 961,000 cwt. of tomatoes were produced in North Carolina for a value of $51,894,000 (USDA, 2016).

North Carolina Tomato Producers

In 2017, tomato production in NC was spread out over 1,317 farms (USDA NC Census, 2017). Patterson Farm in Mt. Ulla is one of the largest tomato producers in North Carolina. They produce grape, Roma, mature green, and vine-ripened tomatoes. Their operation includes the complete tomato cycle from start to finish including plant, grow, harvest, grade, package and ship. They only sell whole tomatoes destined for fresh markets. The top five tomato-producing counties in NC are Brunswick, Henderson, Buncombe, Rowan, and Haywood. This pattern has been consistent for the past five years, which indicates that their tomato markets are stable and their crop growing efficiencies are adequate should demand increase in the future. In 2017, Henderson County produced 545 acres of tomatoes and has maintained this average tomato production since 2015 (Hamer 2017).

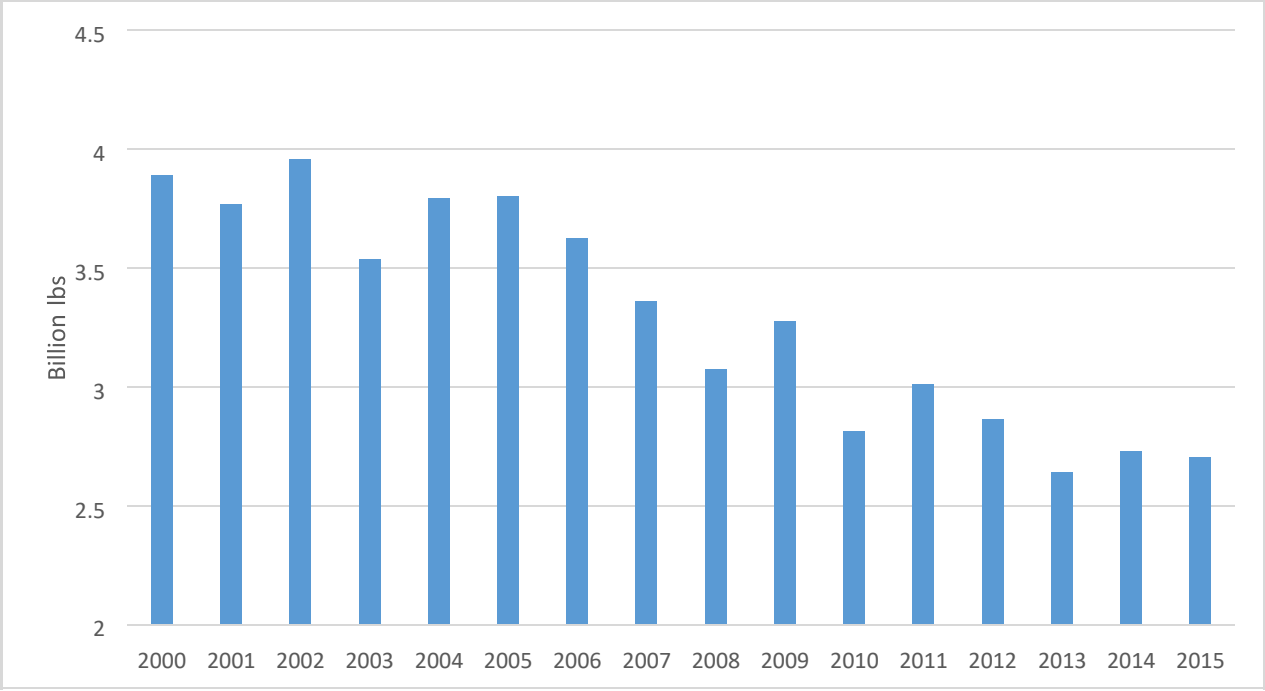

Figure 2.4.1: U.S. fresh tomato production, 2000 to 2015 Source: USDA, NASS .

Targeting these markets would be ideal for a North Carolina based processing company to capitalize upon because there are no tomato processors in NC. A lack of competition in the state would grant a company complete control over the new niche in the tomato processing market. Comparatively, the tomato processing industry is extensive in California, with a portion of the tomatoes that NC produces being shipped there to be processed into other usable forms. Eliminating the need for interstate shipping for farmers in rural areas in NC would certainly assist in building a base of customers for local tomato processing operations and perhaps increase farmer acreage production for processing tomatoes.

Fresh Market Competition

The United States is one of the world's leaders in fresh tomato production. In 2015, 2.7 billion pounds of fresh tomatoes were produced in the United States (Guan, 2017). Domestic production accounts for about 40% of the total domestic demand for fresh-market tomatoes (Guan, 2017).

The rest of the demand is met by imports, mostly from Mexico and Canada. Florida and California account for about two-thirds of the national fresh tomato production, while North Carolina ranks 3rd at the national level (Wu, 2017, Zhu, 2013). Since 2000, fresh tomato production in the United States has exhibited a steady declining trend. Total US fresh tomato production dropped from 3.9 billion pounds in 2000 to 2.7 billion pounds in 2015 (Figure 2.4.1). One major reason is the increased competition from Mexico (Guan, 2017). While NAFTA eliminated trade barriers and encouraged year-round imports from Mexico, Mexican government subsidy support and lower cost of production make Mexican tomatoes more competitive in the global market than US tomatoes (Diario, 2010; Victoria, 2011, Wu, 2017). Increasing imports coupled with production issues at home such as labor shortages and the phase-out of methyl

bromide have significantly affected the market share and profitability of US growers (Guan, 2017).

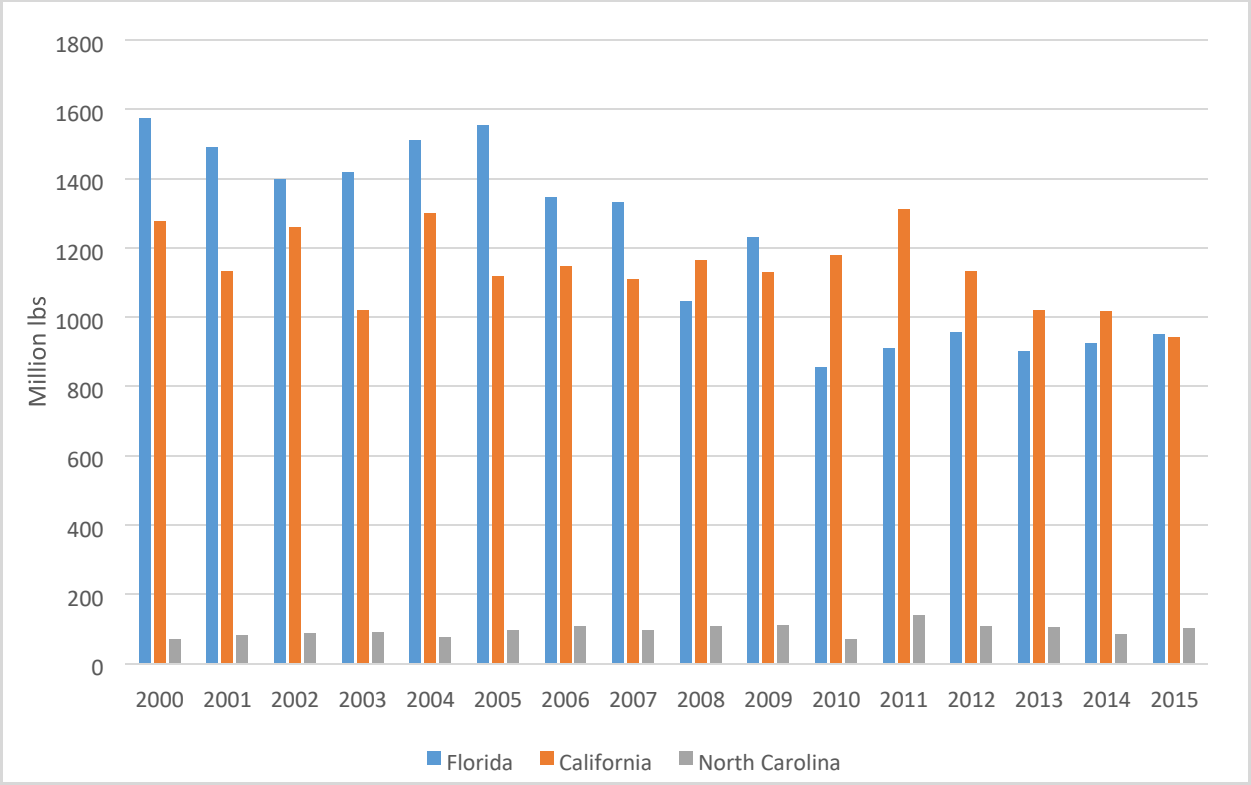

Figure 2.4.2: Fresh tomato production in the top three states FL, CA, NC, 2000 to 2015 Source: USDA.

United States Tomato Processing

East Coast Tomato Processing

In the early 20th century, processing tomatoes were mainly produced in the eastern U.S. with the leading states being New Jersey, Maryland, and Indiana. At that time, shipping costs from these states to the major markets in the East and Midwest were lower than those produced in California (Valdes, 1994). Despite this disadvantage, production was considerably expanded in California from the 1940s to 1960s. Some of the factors contributing to the increase in production

were higher productivity on irrigated land, progress due to research at the University of California on mechanical harvesting and development of suitable tomato varieties and the availability of cheap labor from Mexico (Rasmussen, 1968; Valdes, 1994). Technological innovation in harvest machinery catalyzed the geographic shift in production of tomatoes.

Today's processing east of the Mississippi river consists of canning operations supporting CPG and food service industries. A primary example is Furmano's, a Northeastern regional family-owned grower and packer of tomatoes out of Northumberland, Pennsylvania. Furmano's uses a network of over 30 trusted family-owned and operated farms in PA, MD, DE, and NJ. Today, they have over 600 acres of land farmed annually. According to the company's web site, at the end of their growing season, they had 5 harvesters in a field at one time to bring the crop in before the frost. They had over 1,300 tons of tomatoes brought from one farm in one day and harvested over 5,000 tons in 3 days from the family farm, resulting in 8.9 million cans of tomatoes from one field in just 3 days. At an average of 30 tons per acre, 600 farmed acres produces 18,000 tons or 36,000,000 lbs. of tomatoes. If this volume of material were grown and processed in North Carolina, at $0.22- $0.23 per/lb. farmers would generate roughly $8,000,000 in revenue, averaging $13K-$14 per acre. The tomato processor would also be profitable by selling 55 gallon and 300-gallon sizes for $0.45 - $0.60 per lb, at a competitive rate compared to processed tomatoes from California (including shipping costs). Processing 600 acres of tomatoes could have an economic impact for the state of NC that exceeds $18,000,000 in sales and creates much needed jobs in the food processing sector. Recently, the state of North Carolina has developed a Food Processing and Manufacturing Initiative to leverage its agricultural resources, industrial capacity, and research innovation assets to catalyze the economic growth of the value-added food manufacturing industry (NC Food, 2015). This initiative is designed to fill the void left by the economic decline of

traditional industrial strengths including textiles, furniture, and tobacco (NC Food, 2015). In 2017, tomatoes were produced from 3,057 acres, but only 2.7% of the acreage grown was utilized for processed tomatoes (USDA, 2017). Therefore, establishing a robust tomato processing industry in North Carolina represents an opportunity to capitalize on economic growth potential for farmers and the value-added food manufacturing industry.

West Coast Tomato Processing

California alone accounts for roughly 95% of the U.S. processed tomato production (Guan, 2017). Tomato paste is the main product of processed tomatoes, which is used as a raw material to make ketchup, sauces and other tomato-related products. Processors of tomato paste usually sell to re-packaging companies known as manufacturers which add different additives to make specialized retail products such as ketchup, sauces, and juices. Tomato paste is also used as tomato substitute in restaurants, hotels, and individual households.

Detailed Production Process

The production process will involve the following steps:

Washing

Fresh tomatoes arriving at the plant in trucks are unloaded into a collection channel (also known as flume), a stainless steel or cement duct into which a quantity of water 3 to 5 times higher than the amount of unloaded tomato is continuously pumped. For example, a 10 tons/hour rate requires at least 30 m^3/hour of water.

Sorting

This water flow carries the tomatoes onto the roller elevator, which then conveys them to the grading station. The delivery trucks park-up alongside the flume and, while the trailers containing the tomatoes are being tilted towards it, an operator, using a special tube, pipes a vast quantity of water inside the truck, so that the tomatoes can flow out from the special 50 x 50cm opening. In this way the tomatoes and the water will be gradually feed into the flume without getting damaged.

The tomatoes then arrive at the grading station, after having been rinsed under a clean water spraying system (preferably drinking water). Here the staff removes the green, damaged and excessively small tomatoes which are placed on a reject conveyor (or an auger) and then collected in a large box or directly inside a truck to be taken away.

Chopping/ Crushing

The tomatoes suitable for processing are transported to the chopping station (a hammer mill or a special mono-pump provided with pre-feeding screw) where they are chopped (broken and pulped).

Pre-Heating

The pulp is pre-heated to 65-75°C for Cold Break processing or to 85-98°C for Hot Break processing. The main control panel on the evaporator regulates the pre-heating temperature.

Pulping & Refining

The heated tomato pulp (fiber, juice, skin and seeds) is then conveyed via a special pump to an extraction unit composed of two operating stations: a pulper and a refiner, equipped with two sieves having different sized meshes. The first sieve processes solid pieces up to 1 mm,

while the refiner processes solid pieces up to 0.6 mm, depending on the type of sieve fitted on the machine (the manufacturer can supply sieves with different sized holes if necessary). Two products come out of the extraction unit: refined juice for concentration and waste for disposal. The average extractor yield varies according to different factors: the pulp's temperature (a higher temperature will mean an increased juice yield), the variety of tomatoes treated, and the type of sieve fitted, the rotation speed and the shape of the rotor on each de-juicing body unit. On average, however, the yield is about 95%. For example, if the extractor is fed with 100 kg of hot pulp, it will produce 95 kg of juice and 5 kg of waste. In addition, there is also a hypothetical product waste of about 1-3% from the grading stations. Therefore, 100 kg of tomatoes unloaded from the trucks will produce about 93-94 kg of juice to be concentrated. At this point the refined juice is collected in a large tank with an agitator which constantly feeds the evaporator. This tank is equipped with maximum and minimum level indicators-adjusters which control the pump supplying juice to the evaporator.

Evaporation/ Concentration

The juice in this storage tank is fed to the evaporator which automatically regulates juice intake and finished concentrate output; the operator only has to set the Brix value on the evaporator's control panel; during normal working conditions, the evaporator does not require any further regulations. The juice inside the evaporator passes through different stages where its concentration level will gradually increase until the required density is obtained in the final stage or "finisher". Here the tomato paste is automatically extracted via a pump controlled by an electronic refractometer.

The entire concentration process (evaporation) takes place under vacuum conditions and at low temperatures, significantly below 100 °C. Product circulation inside the various

concentric tubular exchangers is carried out by special stainless-steel pumps which are designed to ensure that the product is conveyed inside the exchanger tubes at a speed of over 1.2 m/sec to avoid “flash evaporation” thus avoiding getting burnt. This means it is possible to process for extensive periods without having to shut down the machine.

Evaporator output is measured in liters of evaporated water per hour while concentrating tomato juice with an initial 5° Brix concentration and producing tomato paste double concentrate at 30° Brix. All the tomato juice evaporators are designed according to these parameters. The evaporative capacity of tomato juice concentrators is greatly influenced by the viscosity level. If the tomato paste has a low Bostwick value, then the concentrator’s output level will also be low; on the other hand, a higher Bostwick value means an increased output level. It is therefore fundamental to know if the productivity data supplied by a manufacturer refers to Hot Break (HB) or Cold Break (CB) finished products.

Aseptic Filing & Packaging

The concentrate is sent from the evaporator directly inside the aseptic system tank. From here it is pumped at high pressure inside the aseptic sterilizer-cooler and then to the aseptic filler, where it is filled into pre-sterilized aseptic bags housed in metal drums. The sterilization temperature and the holding time vary according to the product’s pH value. Generally speaking, a product with a pH value equal to or less than 4.2 could have a sterilization temperature of 115°C measured at the end of the holding section, and a holding time of at least 60-90 seconds. On the other hand, if the pH value is greater than 4.2, it is advisable to acidify the product in order to bring it to about 4.1, improving taste and final product quality.

The sterilized tomato paste is cooled down to about 35-38°C before being piped into pre-sterilized aluminum bags housed in special metal or plastic bins via a special aseptic filler. The packaged concentrate can be kept up to 24 months depending on its pH value and ambient conditions.

Storing

When storing for over 12 months, it is however advisable to conserve it in refrigerated cells, more to reduce oxidization, which could cause darkening than to protect the product's aseptic quality.

PROCESS FLOW CHART:

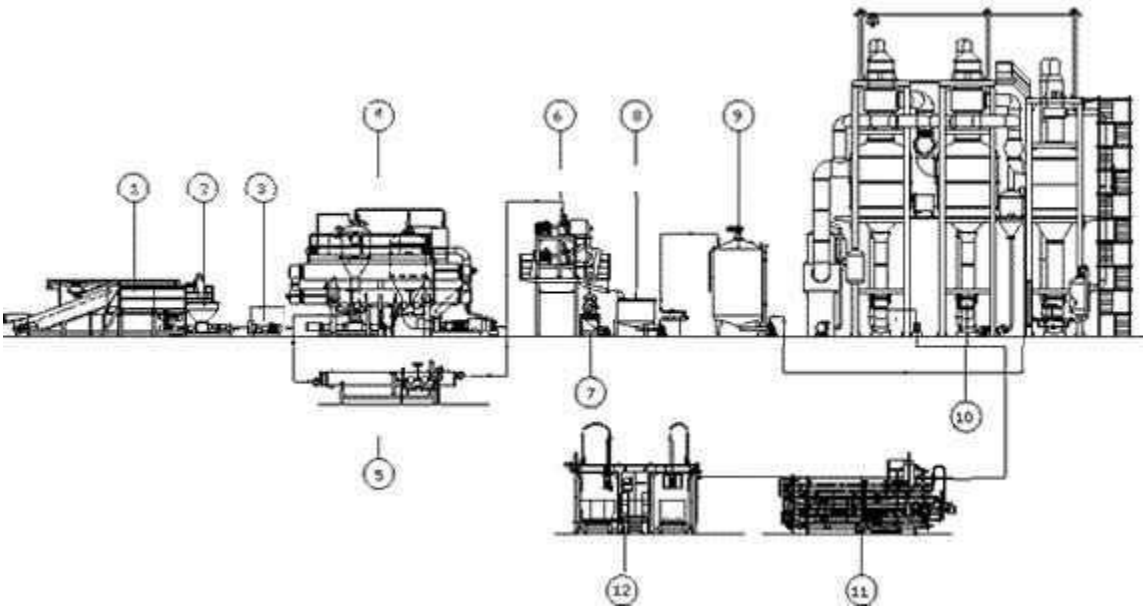

Figure 2.5.2 Industrial scale processing tomato flow chart diagram.

Production Flow is summarized below:

- The tomatoes, discharged from trucks, are hydraulically transferred to the sorting tables, where they are washed, and quality checked (1). Water recycling and filtering provide optimal operation of the system.

- After sorting, the whole tomatoes are collected in one tank, then pumped (2) to the chopping system (3).
- Then, depending on customers' requirements, they are fed either to the Hot-Break enzyme inactivation unit (4) or to the Cold-Break heater (5).
- After this treatment, the product is fed to the refining groups (6) for separation of the liquid from the solids, producing the refined juice and waste (peels/seeds/stalks). The refined juice is collected in a tank (8) while the waste is treated to recover the remaining part of the juice still present (7).
- Both the product collection (8) and the storage tanks (9) feed the evaporator continuously by means of centrifugal pumps.
- The concentration phase allows, during the evaporating process (10), production of concentrated products with different Brix characteristics.
- UHT sterilizing treatment (ultra-high temperature) and cooling (11).
- Aseptic filling into flexible bags, with a capacity of up to 1,500 lbs. (12).

Hot Break is a method where in tomatoes/selected fruits are heated by passing through a steam heated tubular heater. This method gives better yield of pulp having higher viscosity without being separated into juice & pulp. The heat intensity applied deactivates or denatures enzymes that degrade pectin vital to achieving desired viscosity and texture of finished ingredient product. This process also kills microorganisms.

Cold Break is a method where tomatoes are not heated to get the pulp. The pulp obtained through Cold Break process is of lower quality when compared to pulp obtained by Hot Break that rely on viscosity to define standards of identity for high volume value-added condiment end products such as ketchup. Cold Break process tomato material is of higher value for markets

targeting soups and beverages that do not have consumer end product mouthfeel sensory standards facilitated by viscosity.

The finished ingredient product could be stored either in tin packing or in aseptic bags of 200 kg. As regards the end use, there is no hard and fast rule for hot process or cold process. Generally, hot process is preferred as it gives a better aroma, better yield and is in use for production of processed product by the industry.

Condiment History

The American culture is inundated with the use of condiments. This practice can be linked to the historical popularity of barbeque style cooking, which was originally adopted by colonists from Native Americans and African slaves (Early, 2006). Barbeque remained an east coast and southern tradition until it was spread across the U.S as “Black culture” Americans, knowledgeable of cooking the less desirable and meaty cuts of meat, migrated to the northern and western states (Early, 2006). As a result, barbeque became prevalent in cattle and rail towns throughout the country (Early, 2006). The tremendous popularity of barbequing led to the use of sauces as a complementary food flavor enhancer. Tomatoes are considered the first ingredient for most national barbeque sauce brands. A value added biproduct of tomatoes that has become a staple in the American culture is ketchup. The popularity of ketchup grew exponentially as the fast-food revolution took hold of the American culture during the 1960s. Tomatoes naturally produce volatile and non-volatile flavor compounds including glutamate complexes that interact with umami receptors of the tongue. Although a plethora of desirable flavor molecules exist within a tomato homogenate matrix, today it is generally accepted that any health benefits of consuming lycopene from ketchup or tomato-based BBQ sauces are off-set by their high sodium levels.

Sodium Reduction

High sodium content in foods is an issue of concern for Americans. Salt and other ingredients containing sodium are added to food by manufacturers for many reasons. For example, salt can play multiple roles in flavoring, food processing, and safety at relatively low costs (Institute of Medicine. Strategies to reduce sodium intake in the US). In many cases sodium content exceeds the concentrations needed for food safety or essential food processing (IOM, 2010). Excessive concentrations of sodium function to enhance flavor directly at the taste bud level, and indirectly at the olfactory level. Additionally, salt can induce the volatilization of nonpolar flavor compounds in fluid food matrices such as soup, sauces, or ketchup. This effect increases the likelihood of flavor compounds interacting with odor receptors. The association of volatile compounds binding to specific clusters of odor receptors can influence or dictate the perception of a desirable flavor profile associated with a particular food product. Food recognition based on characteristic flavor compounds become key identifiers of corresponding foods by the brain. Over time, flavor recognition or detection can result in varying degrees of desirability. In some cases, achieving desirable flavor responses in foods with elevated sodium content may lead to passive addiction to foods that are not healthy for the steadily growing at risk populations. In the past, ketchup manufacturers have attempted to decrease sodium levels in their products, but the aroma flavor profile was altered to a degree that consumer acceptance is negatively impacted. Low sodium ketchups simply do not sell well in the marketplace. The reason is that the low-sodium ketchup is not recognized as ketchup by the consumer, compared to the “standard of identity” for ketchup containing salt concentrations of 160 mg/Tbs (15 ml). Therefore, opportunities exist in the market to improve the availability of ingredients with desirable flavor profiles leading to the development of consumer products not requiring excess sodium to achieve acceptance.

The US government-funded school lunch program is a target market for the homogenate ingredient and end products derived from the proposed work. According to the CDC Vital Signs report (2012), more than 90 percent of US children, aged 6-18 years, eat more sodium than recommended, putting them at risk for developing high blood pressure and heart disease later in life. US children aged 6 to 18 years eat an average of about 3,300 mg of sodium a day before salt is added at the table (CDC, 2012). The 2010 Dietary Guidelines for Americans recommend that children eat less than 2,300 mg per day total. Most sodium is already in food before it is purchased or ordered (CDC, 2012). To help reduce the amount of sodium children are consuming daily, parents and caregivers, as well as schools, communities, and places that sell, make or serve food, are all encouraged to take steps to ensure more low-sodium options.

The USDA Nutrition Standards for foods served during the school day has been set, and functional ingredients biofortified with desirable flavor compounds and profiles may be utilized as ingredients in food preparations or as flavor enhancing condiments to be applied to vegetables and protein preparations to discourage food waste amongst students. In keeping with a healthy lifestyle based on proper nutrition promoted by the USDA, the www.choosemyplate.gov website has been created to improve healthy eating habits.

This website indicates tomatoes and sweet potatoes are classified in the red and orange vegetable group and lists the recommended amount of red/orange vegetables per week to consume for healthy eating (Table 2.5.4). Additionally, according to a Food and Agriculture Organization report on human vitamin and mineral requirements the needs of preschool-aged children are 500 micrograms or 1,650 IU retinol/day (FAO, 2001). Carotenoid-based condiments can help consumers meet healthy eating goals each day.

USDA ChooseMyPlate.gov

United States Department of Agriculture

		Dark green vegetables	Red and orange vegetables	Beans and peas	Starchy vegetables	Other vegetables
		AMOUNT PER WEEK**				
Children	2–3 yrs old	½ cup	2½ cups	½ cup	2 cups	1½ cups
	4–8 yrs old	1 cup	3 cups	½ cup	3½ cups	2½ cups
Girls	9–13 yrs old	1½ cups	4 cups	1 cup	4 cups	3½ cups
	14–18 yrs old	1½ cups	5½ cups	1½ cups	5 cups	4 cups
Boys	9–13 yrs old	1½ cups	5½ cups	1½ cups	5 cups	4 cups
	14–18 yrs old	2 cups	6 cups	2 cups	6 cups	5 cups
Women	19–30 yrs old	1½ cups	5½ cups	1½ cups	5 cups	4 cups
	31–50 yrs old	1½ cups	5½ cups	1½ cups	5 cups	4 cups
	51+ yrs old	1½ cups	4 cups	1 cup	4 cups	3½ cups
Men	19–30 yrs old	2 cups	6 cups	2 cups	6 cups	5 cups
	31–50 yrs old	2 cups	6 cups	2 cups	6 cups	5 cups
	51+ yrs old	1½ cups	5½ cups	1½ cups	5 cups	4 cups

Table 2.5.4 ChooseMyPlate.gov United States Department of Agriculture.

Tomato Antioxidants

Tomatoes are an abundant source for the antioxidant lycopene. This branched chain molecule is largely responsible for the health benefits associated with consuming tomatoes. Research studies show high dietary lycopene consumption is inversely correlated with the incidence of prostate cancer, breast cancer, cardiovascular disease (Agarwal, 2000; Clinton, 1998; Giovannucci, 1999). Tomatoes possess many beneficial phytonutrients but the most well studied is lycopene. Lycopene exists predominately in either the *trans* or *cis* isoforms. *Trans*-lycopene isoform is abundant in the native unprocessed tomato. As tomatoes are processed and/or cooked, the *trans*-lycopene is converted to the more bioavailable *cis*-lycopene isomer form, which has been shown to increase absorption by 2 to 3-fold. (Gartner,1997; Stahl, 1992; Rao, 2002). Studies by Burri and Ishida (2009) sponsored by Agricultural Research Services showed the tangerine

tomato's tetra-*cis*-lycopene is more efficiently absorbed by human bodies than is the *trans*-lycopene of red tomatoes (Burri, 2009). Scientists also determined that oxidative damage decreased with both treatments, but the decreases were greater following consumption of the products made with the tangerine-tomato (Burri, 2009). Studies in the Schwartz lab at Ohio State investigating the bioavailability of *cis*-lycopene containing tomato products showed 8.5 times increase in absorption in human studies and it was concluded that tomato-based food products could be manipulated by temperature processing to favor the formation of specific isomer patterns to improve lycopene bioavailability (Cooperstone 2015, Cooperstone 2016, Unlu, 2007). Collectively, the findings in these studies allude to the significance of establishing a proficient heat induced *cis*-lycopene conversion bioprocessing system for the readily available and less expensive red tomatoes in the production of post-harvest sterile value-added tomato ingredients and end products.

Lycopene is the most potent antioxidant of the carotenoid family. It contains 11 conjugated double bonds arranged linearly in the center portion of the molecule and 2 unconjugated double bonds at each end (Nguyen, 1999). This highly unsaturated hydrocarbon carotenoid has a molecular formula of $C_{40}H_{56}$. Lycopene is the longest carotenoid in the family, and its configuration provides a singlet-oxygen free radical quenching ability twice as high as that of β-carotene and 10 times higher than that of α-tocopherol (Nguyen, 1999; Weisburger, 2002). The radical scavenging activities of lycopene are related to its ability to trap peroxyl radicals (ROO^-) and diffuse oxygen radicals (O_2^-) (Foot, 1968; Burton, 1984). Lycopene has been reported to deactivate an array of free radicals, such as hydrogen peroxide, nitrogen dioxide, thyl, and sulphonyl (Bohm, 1995; Lu, 1995; Mortenson, 1997). Additionally, multiple investigations

demonstrate that lycopene is a more potent ROS scavenger than many other dietary carotenoids and other antioxidants (Di Mascio, 1989; Di Mascio, 1991; Devasagayam, 1992; Miller, 1996).

Lycopene Biological Responses

The major sources of lycopene in the typical American diet come from tomato products such as spaghetti sauce, tomato juice, pizza sauce, and ketchup. These foods provide over 80 percent of the lycopene consumed in the U.S. The half-life of lycopene is 7 to 14 days in human blood, and a diet absent of lycopene for 1 week significantly lowers plasma concentrations (Allen, 2002; Hadley, 2003; Rao, 2002; Schwedhelm, 2003). Interestingly, investigations by Hadley and colleagues (2003) suggest that 1 week of consuming processed tomato products low in lycopene resulted in increased levels of the circulating *cis*-lycopene concentration. The results from this study indicate that low dose lycopene consumption from perhaps condiment sources may be enough to improve circulating lycopene levels. Research investigating low dose lycopene intake by Rao and Shen (2002) report that lycopene intake of 5-20 mg produced a significant increase in serum lycopene levels for both ketchup and lycopene capsules. Moreover, these studies suggested lycopene mediates a protective effect by significantly reducing lipid and protein oxidations in the body (Rao, 2002). Commonly used ketchup contains 5.1 mg of lycopene per 2 tablespoons, according to the USDA/NCC Carotenoid Database for U.S. Foods (USDA, 1998).

Compared to *trans*-lycopene, the absorption potential of *cis*-lycopene is enhanced due to the lower tendency to self-aggregate and form crystals (Britton, 1995). The solubility of *cis*-isomers is high in the lipophilic phase; therefore, they are more efficiently incorporated into bile acid micelles, pass across the intestinal barrier via passive diffusion, and are preferentially incorporated into chylomicrons (Bohm, 1999; Boileau, 2002, Cartner, 1997). Lycopene, as well as

other carotenoids are absorbed through enterocyte uptake as well (Johnson, 1997; Tyssandier, 2002). In fact, studies suggest that serum response of lycopene is improved in the presence of other carotenoids such as β-carotene (Tyssandier, 2002; Tyssandier, 2003 Johnson, 1997; White, 1993).

After gaining access to the body via lymphatic system or portal circulation, carotenoids accumulate in the liver, where they are packaged and released into circulation with lipoprotein particles. In blood plasma, lycopene (and other carotenoids) is mainly associated with low density lipoproteins (LDL) and very low-density lipoproteins (VLDL). The lycopene-lipoprotein interaction truly represents a symbiotic relationship. While the lipoproteins provide lycopene a transport mechanism to specific tissues, the lycopene protects the lipoprotein from oxidative damage (Agarwal, 2001). Carotenoids are transported to various tissue sites that have many low-density lipoprotein receptors and/or a high rate of lipoprotein uptake such as adrenal, prostate, lung, kidney, pancreas, and ovary (Gerster, 1997; Schmitz, 1993; Erdman, 1993). Lycopene is deposited in the liver, lungs, prostate gland, colon, and skin in the human body, and its concentration in body tissues tends to be higher than those of all other carotenoids (Rao, 1998; Rao, 2002; Shi, 2002; Shi, 2003). The protective role of lycopene to LDL in circulation is critical to decreasing the risk of coronary heart disease. Oxidized LDLs are directly involved in the formation of foam cells and arterial plaques, which increase the risk of cardiovascular disease (Jialal, 1996; Parthasarathy, 1998; Ames, 1995). Oxidation of LDL is responsible for the increased presence of activated macrophages and is believed to contribute to the development of atherosclerosis (Santanam, 2002). This is plausible because macrophage receptors preferably recognize the oxidized LDL form and could, therefore, be taken up by vascular tissue macrophages to induce the fatty-streak lesion of atherosclerosis (Lavy, 1993). The development of

atherosclerotic plaques along arterial walls often leads to a decrease in vascular lumen size, which can result in elevated blood pressure and cardiovascular disease.

Studies suggest lycopene protects the body from lipid peroxidation (Bhuvaneswari, 2001; Velmurugan, 2002; Yeh, 2002). This is significant because several lipid peroxidation products were identified as cytotoxic and genotoxic and were shown to play an important role in the etiology of several chronic diseases, including acute coronary syndromes (Tsimikas, 2003; Boyd, 1991). Lipid peroxidation refers to the oxidative degradation of lipids. During this process free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. Fortunately, lipophilic antioxidants can provide protection as they are embedded entirely within the nonpolar inner environment of the membrane and exhibit limited mobility. In the case of obese individuals, due to their excess stored energy, lipid cellular components would be likely in abundance and thus prone to oxidative damage as chronic low-level inflammation is a hallmark of obesity. Due to the characteristic lipophilic properties of lycopene, it appears that the localization of carotenoids in the lipophilic component of the cell provides a greater resistance for lipid and lipid proteins to oxidative damage (Clevidence, 1993; Ribaya-Mercado, 1995). The availability and accessibility of bioavailable lycopene in systemic circulation throughout the vasculature may provide an additional layer of protection from oxidative damage.

Lycopene's lipid peroxidation protective effects may be accompanied by a lycopene induced activation of the cellular endogenous antioxidant defense system. This innate cellular defense mechanism that protects the body from oxidative damage consists of enzymes such as glutathione peroxidase, catalase, and superoxide dismutase. Velmurugan and colleagues (2002) conducted an animal study to examine lycopene antiperoxidation effect by using a potent carcinogen that produced toxic and highly diffusible reactive oxygen species. They reported a

significant reduction in lipid peroxidation in both plasma and erythrocytes was observed in the animal group that was administered lycopene; and accompanied by enhanced levels of glutathione (GSH) and biotransformation enzymes, such as glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR) (Velmurugan, 2002). This is consistent with previous work by this group that shows the ingestion of lycopene significantly decreased the lipid peroxides and enhanced the activities of hepatic biotransformation enzymes in a hamster carcinogenesis model (Bhuvaneswari, 2001; Bhuvaneswari, 2002).

Although there could be a question of whether the toxic chemicals present actually combined with lycopene to synergistically activate glutathione enzymes in the tested *in vivo* systems, the data does suggest lycopene may possess the capacity to activate endogenous defense mechanisms. With obesity being a chronically toxic condition that produces chronic medium to low doses of various inflammatory reactive oxygen species, the presence of circulating lycopene could be an important antioxidant defense system with protective effects as observed in studies by Velmurugan *et al* (2002) and Bhuvaneswari *et al* (2002). In clinical studies, lycopene has been shown to increase superoxide dismutase (SOD) activity. In studies by Kim *et al* (2011), patients received a daily 6-mg or 15-mg lycopene supplement for 8 weeks, and results showed that SOD activity increased by 1.73 units per milliliter and 2.37 units per milliliter, respectively. The results were correlated with a significant improvement in endothelial function and a 57% reduction in C-reactive protein (CRP) levels in the high dose group (Kim, 2011). The beneficial effects of lycopene displayed in this study are significant and relevant to obesity and cardiovascular disease, as high CRP levels are positively correlated with obesity and the progression of atherosclerosis (Visser, 1999; Cook, 2000; Rohde, 1999). Moreover, the ability of low to moderate levels of

dietary lycopene to upregulate SOD activity further substantiates the suggestion that dietary lycopene may provide a protective effect beyond its traditional free radical quenching activities.

Thermal Processing of Tomato and Carotenoid Products

Recent advances in thermal processing technology have resulted in the establishment of methods possessing the potential to rival standard bioprocessing techniques used in today's food industry (Richardson, 2001). A significant factor in producing a more nutrient rich tomato-based condiment is selecting the way the product is processed post formulation. Tomatoes are the key ingredient, therefore a processing technique that facilitates the conversion and protection of lycopene, is paramount. Within the pericarp tissue of the ripe tomato fruit, lycopene is localized in the chloroplasts, in the form of fine crystals that are associated with the membrane structure (Bouvier, 1998). While in the crystalline state, lycopene is configured in its most thermodynamically stable *trans*-lycopene form. This structurally "grounded state" accounts for 90-96% of total lycopene in red tomato fruit, and is a predominant factor influencing the accessibility of lycopene to the human body. During mechanical and thermal processing, it is possible that the stable lycopene isoform is freed from the fleshy membrane-bound matrix and isomerized to a more energy-rich state known as *cis*-lycopene isomers. Under the influence of heat, light, or certain chemical reactions, seven bonds comprised within the natural *trans*-lycopene molecule can perhaps be isomerized to the mono- or poly – *cis* form. The *cis* isomers of lycopene have physical characteristics and chemical behaviors distinct from the all-*trans* structure, and in human serum and tissue *cis*-lycopene isomers contribute more than 50% of total lycopene (Shi, 2000; Schierle, 1996). Data from research studies show that the human body absorbs *cis* isomers of lycopene better than the *all-trans* form (Boileau, 2002; Cooperstone, 2015). The mechanism elucidating how lycopene consumed from tomato sources containing over 94% *all trans* lycopene

changes to over 50% *cis*-lycopene of total lycopene in blood serum is poorly understood. Possibilities include greater absorption rates from the diet for the *cis* form, greater excretion or degradation of the *trans* form, or transformation of the *trans* to *cis* form in the body.

During the processing of tomatoes to value-added products (such as condiments), heat intensity exposure and time duration are the most critical factors influencing isomerization or degradation. A study by Shi et al. (2003) involving dissolved extracted lycopene in canola oil and heated to 25 °C, 100 °C, or 180 °C produced results that suggest degradation of lycopene was the main mechanism of lycopene loss when heated above 100 °C. Conversely, at lower heating temperature of 70 °C, Schierle et al. (1996) reported data that shows *cis* isomerization levels increased proportionally to heating time. The presence of certain macromolecules in tomatoes may provide additional protection for lycopene during heat treatment. It was observed that lycopene loss was less while heating tomato pulp in comparison to heating lycopene in organic solution (Cole, 1957; Cole, 1957). In a separate study by Shi et al (2002), tomato puree was subjected to heat treatments of 90 °C, 110 °C, 120 °C and 150 °C for 1-6 hours to characterize isomerization and degradation of lycopene. The results indicate that the concentration of total lycopene steadily decreased with treatment (the higher the temperature, the faster the degradation), while the *cis* isomer levels increased but only during the first 2 hours of heating. Under the influence of heat energy, light, or certain chemical reactions, seven bonds comprised within the natural *trans*-lycopene molecule can isomerize, which involved the relocation of the single or double bond of one form of carotenoid into another (Kuki, 1991; ESA, 2009). The authors suggested that oxidation of lycopene was the main mechanism of lycopene loss when heated above 100 °C, and that an optimum heating condition could be found to promote *cis* isomerization in tomato-based foods (Shi, 2002; Shi, 2003). Data from Schwartz's laboratory

indicates that the *trans*-lycopene isomer is relatively stable to isomerization at temperatures between 50 and 100 ˚C (Nguyen, 1998; Nguyen, 1999). Conversely, studies from this same lab reported industrial level thermal processing influenced isomerization of lutein and beta-carotene, but not lycopene (Nguyen, 2001).

Microwave Thermal Processing

Microwave assisted thermal processing offers similar benefits to conventional methods, but with improved product quality and reduced time of exposure to energy (Canumir, 2002). Several studies have successfully been conducted on the microwave pasteurization of fruit juices as it preserves the natural organoleptic characteristics of the juice and reduces the time of exposure to energy, with the subsequently lower risk of losing essential thermolabile nutrients (Igual, 2010). It is generally accepted with microwave-assisted heating the come-up time for sterilization temperature range is shorter compared to conventional heating, thus limiting heat exposure time. This occurs because the product is being heated both from the inside and outside using polar and charged molecules within the fruit puree matrix. Microwaves are electromagnetic radiation waves with frequencies that lie between infrared and radio and TV waves. The principal mechanism of microwave radiation in fruit puree sterilization is the water present within food matrices act as an electric dipole, which contains both positively and negatively charged molecules. When an electromagnetic radiation is passed through the food, heat energy is produced due to intermolecular frictions resulting from the movement of electrical charges produced by forces of attraction and repulson (Datta, 2013). Another mechanism of heating is due to ionic conduction. The application of electromagnetic field causes migration of the ions towards oppositely charged regions. This results in release of heat due to multiple billiard ball-like collisions and disruption of the H-bonds in water (Venkatesh, 2004). The amount of heat produced in food during microwave heating is

proportional to food matrix's dielectric properties, including dielectric constant and dielectric loss factor (Tang, 2005).

Studies performed by Kumnar et al. (2008) at NC State University showed that thermophysical and dielectric properties of tomato value-added food materials and ingredients are suitable for microwave heating at sterilization temperatures. Unlike conventional thermal sterilization, microwave heating causes uniform heating of the entire volume of the food, and provided water content is adequate, the energy is absorbed extremely fast, which causes rapid heating (Venkatesh, 2004). The ability of microwave heating to transfer energy uniformly and rapidly allows for an opportunity to identify ideal conditions for standardized lycopene isomerization conditions that stop short of severely oxidizing or degrading bioactive antioxidants and desirable organoleptic characteristics. Additionally, studies have previously shown that applying thermal processing to tomato juice can enhance lycopene *in vitro* bioaccessibility (Jayathunge, 2017).

Carotenoid Processing & Bioaccessibility Research

Carotenoid bioaccessibility is generally defined as the fraction of carotenoids transferred from the food matrix to mixed micelles and, thereby, made available for subsequent uptake by the intestinal mucosa (Brown 2004, Reboul 2006). The fraction of bioaccessible carotenoids that are absorbed and available for utilization and storage in the body is referred to as the bioavailability of carotenoids. Studies reported by Dhuique-Mayer et al., (2018) showed industrial processes to manufacture orange flesh sweet potato (OFSP) into baby food improved bioaccessibility and carotenoid isomerization compared to homemade preparation. Industrial processing required various treatments including but not limited to sweet potato peeling,

grinding, blanching (3 min 90 ^{o}C), packaging, pasteurizing (15 min, 100 ^{o}C), sterilizing (30 min, 123 ^{o}C), adding oil and/or adding oil + emulsifier (Dhuique-Mayer, 2018). Estimations of the vitamin A activity expressed in percentage of RDA in OFSP-based baby food purees were calculated using a classical estimate from food (Dhuique-Mayer, 2018). Considering bioaccessibility and applying a 50%, conversion to retinol shows that a 115 g baby portion of the OFSP-puree with 2% of added oil or emulsifier could provide 31.4 or 85.5% (respectively) of the daily vitamin A requirement (RDA) for children under 6 years (Ekesa 2012, Dhuique-Mayer 2018). Comparatively, 115 g of OFSP puree homemade would provide only 3.5% of the RDA, while the industrial sample and the laboratory sterilized puree provided similar percentages, 19.7 and 21.8% (respectively) of RDA (Dhuique-Mayer, 2018). These data correlate with information highlighted by Bengtsson et al., (2009) and Tyssandier et al., (2003) that indicates a more efficient micelle incorporation of isomer 13-cis-β-carotene was observed in relation to corresponding all-trans-form during digestion. Based on these results, the authors recommend the development of functional baby food from OFSP puree to reduce the risk of vitamin A deficiency and improve public health (Dhuique-Mayer, 2018).

Recently, it has been reported that microwave-assisted heating of tomatoes improves lycopene bioaccessibility (Page, 2012). The study by Page et al. (2012) was designed to determine whether hot break (HB) or cold break (CB) treatment also impacted the carotenoid content, composition, and bioaccessibility of tomato purees. They found that HB purees were much more viscous than CB tomato material. Hot break conditions also led to a significant decrease in lycopene content, while increasing the extractability of lutein (Page et al., 2012). It was interesting to observe within the data that the crystalline chromoplast and cell wall membrane structures containing lycopene-rich particles of HB samples remained intact, while

the CB specimen displayed free lycopene-rich particles separated from the cell wall membrane and chromoplast matrix (Figure 2.7.2). The HB treatment produced viscous purees by thermally inhibiting the endogenous cell-wall lytic enzymes, primarily pectin methyl-esterase (PME) and endo-polygalacturonase (PG), exceedingly early in the process (Anthon, 2010; Page et al, 2012). It appears that CB processing enabled an enzymatic breakdown of cell wall material (Page Et al, 2012). The capacity of the food matrix to retain or liberate compounds during processing or digestion influences carotenoid bioaccessibility. Some within the scientific community believe the intact crystalline chromoplast structure prevents thermally induced isomerization of lycopene. This highlights the complexity of factors involved with carotenoid bioaccessibility.

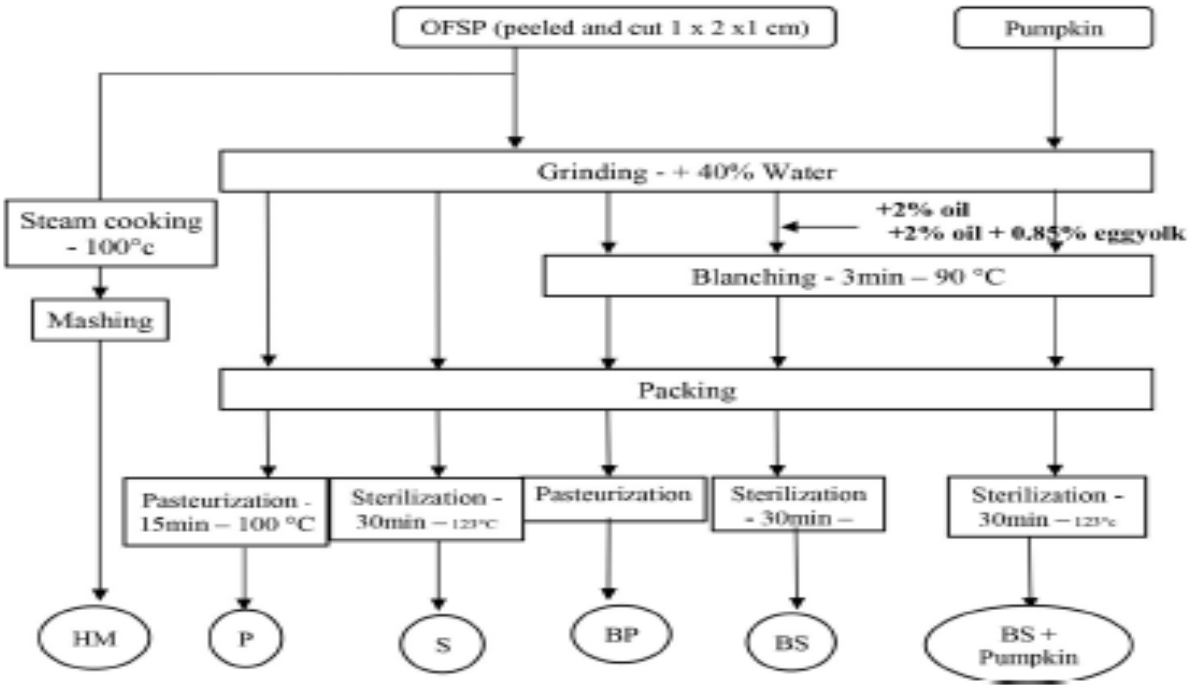

Figure 2.7.2 | Schematic overview of the experimental process. HM, Home-made Process; BP, Blanching and Pasteurized or BS, Blanching and Sterilized; P, pasteurized, or S, Sterilized. Dhuique-Mayer et al 2018.

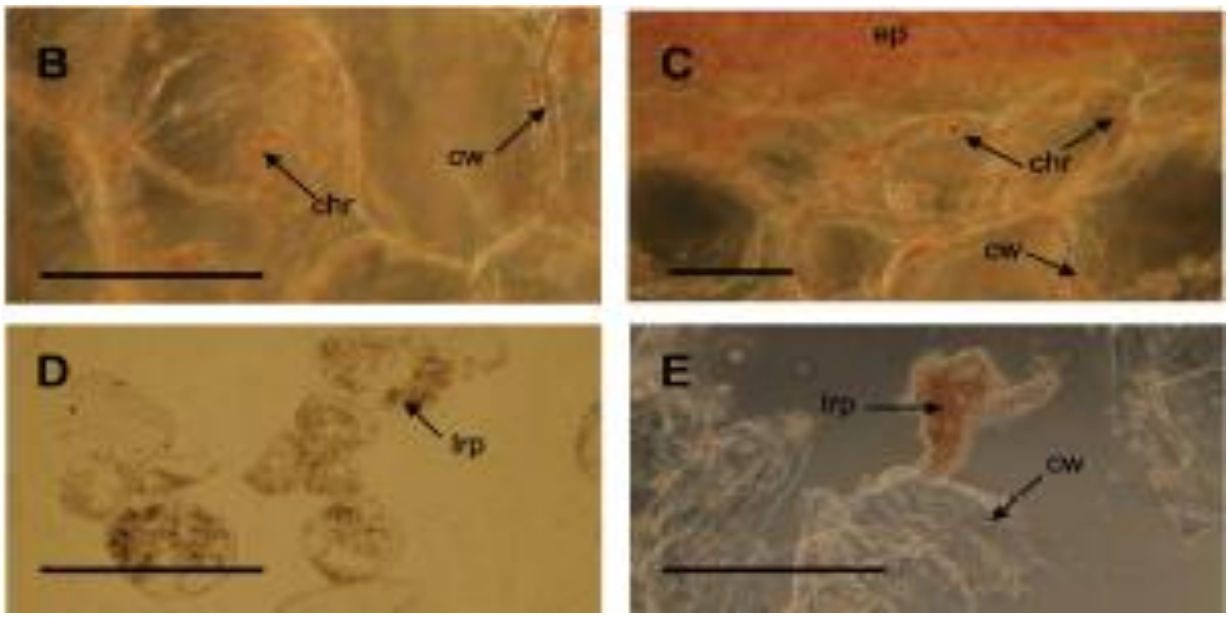

Figure 2.7.3 Particles characterization in M82 purees: (B and C) Photonic microscope observation of fresh tomato pericarp. (D) Particles from hot break puree, € particles from cold break puree (chr, chromoplasts; cw, cell wall; ep, epidermis; lrp, lycopene-rich particles): bars represent 100 µm. Page et al., 2012.

Microwave Processing, Ohmic Heating & Tomato Homogenate Hydrocolloid

When tomatoes and food materials are heated changes in the food matrix dielectric properties may occur. Therefore, the dielectric properties of a food material, its dependence on temperature and frequency must be understood. Food materials such as tomatoes belong in a group of dielectric materials that are neither conductors nor insulators.

Electromagnetic waves propagate into dielectric materials, but the amplitude of the waves decreases, and the energy that is lost by the waves is converted into heat inside the food material matrix (Englender, 1991). This phenomenon is characterized by the dielectric constant and dielectric loss factor. The dielectric constant relates to the amount of energy that is reflected or transmitted by the material and to the ability of the material to store electromagnetic energy. The loss factor relates to the ability of the material to lose the energy transmitted. The conversion of

the energy into heat, or ohmic heating is usually described as a function of frequency, electric field intensity and loss factor (Metaxas, 1983; Buffler, 1995). As it relates to microwave processing of homogenized tomatoes, the questions needing additional research are: what is the influence of the material's stored electromagnetic energy, and what is the overall impact of the lost energy if the food material is neither a conductor nor an insulator? The answer to these questions may be derived from investigating the qualities and components of the food material matrix.

Processed tomatoes can be considered a colloidal dispersion that consists largely of hydrophobic polymer hydrocarbon chains comprised as the colloidal continuous phase and water as the discontinuous phase. It is generally accepted that water and ions (polar molecules) are responsible for the ohmic loss of microwave energy within a food (Nelson, 1994; Venkatesh, 2004). The mobility of ions provided by the discontinuous water phase enables transmission of microwave energy through the dielectric food material matrix. Hydrocolloids are polymers and have two characteristics that affect dielectric properties including changing during heating and binding water. Heating leading to temperature rises affects molecules by increasing the internal vibrations of their bonds, elongating such bonds, increasing the mobility of molecules and the internal energy in them (Van Wylen, 1994). Due to the changes in mobility of polar molecules dielectric properties must be affected by temperature. By binding water, the mobility of ions is restricted, therefore loss factor is minimized. Subsequently the availability of free water throughout a tomato homogenate matrix could optimize or maximize microwave energy transmission. Available data of dielectric properties of food materials shows that most liquid products follow the behavior observed in water dielectrics (Kent, 1987; Nelson, 1973; Tinga, 1973; Venkatesh, 2004). Dielectric properties are a macroscopic effect of molecular interactions with the electromagnetic field. Being that dielectric properties are the result of polarization of

molecules, temperature affects the mobility of the molecules, changes molecules, as well as some macro characteristics of the food materials, such as gelation, protein denaturation, carotenoid degradation, or carotenoid isomerization, which all have an important effect on the dielectric properties. Kumnar reported tomato salsa possessed dielectric properties suitable for microwave sterilization (Kumnar, 2008). Nevertheless, due to the complexity of food systems, predictive equations associated with microwave heating fail to describe products they target; therefore, the effects of microwave heating must be observed and measured experimentally for each material (Funebo, 1999). To this end, the effects of a potential industrially scalable microwave processing energy transfer system on post-harvest tomato carotenoid antioxidants is a major focus of this work.

Consumer Demand in the U.S.A.

Data from the proposed studies may contribute to elucidating the mechanisms involved with improving the bioaccessibility or nutrient density of processed carotenoid rich ingredients and value-added end products using novel thermal sterilization methods. Consumers appear to be ready to make healthier changes to their diets, therefore it would be ideal to generate research data that may lead to improving the availability of functional ingredients and end products such as homogenates/condiments that may be used to combat malnutrition in a personalized disease specific manner for at risk populations in the United States.

Consumer demand to personalized nutrition as a means of preventative maintenance disease avoidance has led to a boom of better-for-you products and niche markets such as gluten free diets, vegan diets, resulting in demands for meat alternative products, dairy alternative products, and a host of other wellness products and healthy lifestyle brands. In keeping with the

new market demands research looking to improve carotenoid bioavailability of commonly consumed products is on par with current consumer demands. Therefore, with the proposed studies we look to identify if and how novel processing methods can improve the nutrient density and effectiveness of condiments in delivering antioxidants and micronutrients through convenient dietary consumption.

Homogenate Potential Usage Compared to Dietary Supplements or Functional Beverages

Currently available fat-soluble antioxidant delivery systems for protective phytonutrients include pill supplementation, beverage consumption, or traditional fruit and vegetable consumption. A limitation of pill supplements is that the active compounds are often not absorbed effectively, as is the case with lycopene pill supplements. Beverage consumption has a less than ideal effect on increasing total antioxidant absorption due to the effectiveness of clarification processes that remove many protective hydrophobic phytonutrients, while leaving only water, sugar, and hydrophilic antioxidants in the clarified fruit-based beverage. Subsequently, the delivery of health promoting antioxidants is minimal throughout the $100 billion-dollar US non-alcoholic beverage industry. Limitations associated with fruit and vegetable consumption pertaining to phytonutrient antioxidant delivery are a lack of consistent access to fresh fruits and vegetables in certain demographics; an inability of poor and middle-class populations to consistently afford fresh fruit and vegetables; population segments in need being culturally unaccustomed to consuming fresh produce; or consumer populations that are ignorant of nutrient and health benefits of fresh produce. Increasing antioxidant intake by consuming fruits and vegetables can also be limited by the preservation or processing methods used to extend the product's stability and shelf-life (Lopez, 1987).

Conversely, the use of homogenates to deliver hydrophilic and hydrophobic phytonutrient antioxidants can improve the availability of products in the marketplace designed to deliver health promoting phytonutrients to consumers through common food consumption. This trend is projected to increase in popularity. Recently a regenerative medicine company, Healthy Cell, has closed on eight-figure financing to produce products targeting healthy aging demographics by producing bioactive multi-gel packs that deliver essential nutrients to help consumers meet or exceed the 100% daily value for micronutrients with one slurp of the fruit-flavored gel pack. The use of a tomato-based condiments as an antioxidant delivery vehicle can effectively deliver high levels of both hydrophilic and hydrophobic antioxidants in a safe, efficient, and complementary manner to daily meal consumption. The use of tomatoes as the primary base for the condiment is ideal because selectively processing tomatoes can produce more bioavailable carotenoids (Cooperstone 2016, Cooperstone 2015, Unlu, 2007). The notion of antioxidant delivery systems in the form of condiments is novel, yet conducive to the American culture. The per capita consumption of processing tomatoes is over 73 lb. (farm weigh equivalent) (Carter, 2008). This is high by international standards, as it is almost double the per capita consumption level in Europe (Carter, 2008). Due to the historic relationship with condiments and the prevalence of a thriving condiment market, the use of condiments to deliver dietary carotenoid antioxidants (lycopene and β-carotene) provides an opportunity for immediate acceptance of ingredients and subsequent value-added end products by the American population.

Conclusion

The proposed studies to evaluate the effects processing has on carotenoid bioaccessibility and isomerization are relevant to assessing the potential of microwave processing on carotenoid cash crops in North Carolina. The final phase of this project will include the design of a prototype

microwave thermal processing method for processing whole tomato into tomato puree as a base for an antioxidant-rich homogenate ingredient for prospective downstream functional condiments. Based on the physical and chemical properties of tomatoes, measuring the effects of processing tomatoes using microwave heating offers the best opportunity to generate data while producing sterile high quality ingredient materials that can be scaled for industry.

The use of microwave heating systems in processing of food materials is a promising technology application. However, more knowledge is required on the interaction between foods and microwave energy in customized bioprocessing systems. The following studies attempt to provide some knowledge in these areas by focusing on practical matters such as application and translation.

Chapter 3 presents a bioprocessing system that identifies mechanical and thermal processing parameters suitable for using batch microwave heating for post-harvest carotenoid produce destined for value added consumer product applications, and the results of applying such methods to carotenoid quality and micellarization efficiency. Chapter 4 presents data on post-harvest tomato processing conditions and effects microwave processing with or without processing aids have on lycopene quality, isomerization, and bioaccessibility.

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Chapter 3

The Effects of Processing on Bioaccessibility of Tomato Carotenoids

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ABSTRACT

The inclusion of bioavailable antioxidant ingredients from regionally grown North Carolina tomatoes into traditional popularized American condiments is a potentially sustainable strategy to conveniently provide key shortfall anti-inflammatory phytonutrients and micronutrients including lycopene and beta-carotene to at risk populations. However, only limited information is available on long-term health effects of these materials, including their potential to modify vascular inflammatory cascades associated with diseases positively correlated to the American diet, or the impact novel thermal energy transfer methods achieved with post-harvest sterilization processes has on absorption of fat-soluble tomato carotenoid antioxidants. The present study investigated the impact of repeated exposure of fresh Roma tomatoes to various processing treatments to assess changes in carotenoid isomerization and subsequent carotenoid micellarization from sterilized ingredient material. To study antioxidant isoform energy state conformations from bioprocessing exposure, Roma tomatoes were locally purchased in whole form, mechanically homogenized with or without biocompatible non-synthetic processing aids sunflower oil and/or sunflower lecithin and sterilized for 6-12 minutes using microwave-assisted volumetric heating at a range of 90-98° Celsius. Tomato bioprocessing was followed by an *in vitro* digestion model simulating the human oral, gastric and small intestine digestion. Carotenoid assessment of isomerization and micellarization was conducted using liquid chromatography with photodiode array detector (LC-DAD). Alterations in select tomato carotenoid isomers were observed by LC from microwave-assisted thermal treatment conditions with and without processing aids. Specifically, *cis*-lycopene and beta-carotene content were observed to significantly increase with exposure to processing treatments with processing

aids of red Roma tomatoes. Significant improvement ($p < 0.05$) in carotenoid micellarization and absolute bioaccessibility was observed with different processing methods incorporating oil.

Introduction

Carotenoids protect human skin against UV light-induced photo-oxidative stress, preventing erythema formation, premature aging, and cancer, as well as other diseases associated with oxidative stress in this organ (Johnson, 2000). Carotenoids are one of the most consumed phytonutrients in the United States of America. The majority of consumption is in the form of processed tomatoes. The per capita consumption for processed tomatoes is 73 lb. Carotenoids are health promoting antioxidants that are hydrophobic in nature and often do not have high absorption characteristics. Advances in bioprocessing technologies have created an opportunity to improve the quality of hydrophobic health promoting ingredients which may lead to improving the nutrient absorption of commonly consumed value-added processed carotenoid-based foods.

Carotenoid bioavailability represents the fraction of ingested carotenoid transferred from food matrix to target tissues where it can be stored or utilized at the cellular level (Zaripheh, 2002). The bioavailability of carotenoids has been shown to be affected by many factors including: physiochemical properties (type of carotenoid, crystalline in chromoplasts or dissolved structure in oil, free carotenoids or protein bound, *trans* or *cis* isomers), food matrix (fruit or vegetable, supplement or natural food), food processing (processing method, particle size), interactions with other dietary components (fiber, protein, lipids, phytosterols and other carotenoids) and subjects characteristics (gut health, nutritional status, physiological status, genotype) (Failla, 2005; Harrison, 2005; Hollander, 1978; West, 1998)

Carotenoids must be released from the food matrix, dissolved in oil droplets of chyme and then partitioned into mixed micelles for uptake by intestinal enterocytes. Prior to absorption during the intestinal digestion phase, lipases degrade hydrophobic compounds in oil into smaller particles

in the presence of bile salts (El-Gorab, 1975). During this process triglycerides are broken down to monoacylglycerols and free fatty acids, phospholipids are broken down to lyso-phospholipids, cholesterol esters are broken down to free cholesterol and carotenoid esters are broken down to free carotenoids. Collectively, these compounds are solubilized within micelles that diffuse across an aqueous layer within the small intestine to deliver lipophilic compounds and carotenoids to the apical surface of intestinal mucosal cells. The fraction of carotenoids transferred from the food matrix to mixed micelles during digestion is referred to as bioaccessibility (Micozzi, 1992).

Delineating factors contributing to carotenoid micellarization is paramount to determining or predicting which fat-soluble compounds are best positioned for ordered packaging within micelles. It has been reported that different components of foods can promote or inhibit carotenoid micellarization and absorption. The association of carotenoids with protein and fiber have been shown to be associated with decreased carotenoid absorption, respectively (Sundaresan, 2005; Riedl, 1999; Rock, 1998; Zanutto, 2002). The presence of sucrose esters and phospholipid emulsifiers such as phosphatidylcholine have also been shown to decrease micellarization of hydrophobic compounds (Weststrate, 1995; Baskaran, 2003; Sugawara, 2001) It is possible that the presence of water-soluble carbohydrates or partial water-soluble emulsifier components interfere with bile salt micelle formation activity thereby shifting the equilibrium to favor partitioning of lipophilic carotenoids into the micellar phase. Conversely, carotenoids consumed or solubilized with oil are reported to increase carotenoid micellarization and absorption (Rich, 2005; Unlu, 2005; Clark, 2000).

Considering what is known about carotenoid physiochemical characteristics, impacts of food matrix processing, and favorable or unfavorable conditions for carotenoid micelle incorporation, exogenous carotenoid processing using both traditional and novel processing or

energy transfer methods to improve carotenoid micellarization potential is possible. Volumetric heating techniques such as microwave-assisted heating has been adapted to food processing applications and commercialized as a faster, more efficient method for food sterilization that minimizes nutrient degradation (Coronel, 2003). This thermal processing method achieves a heightened degree of efficiency by exciting water and polar molecules within a food matrix, creating thermal energy from the inside of a homogenized food matrix slurry to enable faster and more efficient come-up time to achieve sterilization temperature for adequate microbial reduction (Coronel, 2005: Kumnar, 2008).

Due to energy transfer efficiencies of microwave-assisted heating, carotenoid processing has the potential to create conditions for maximizing carotenoid micellarization by inducing carotenoid isomerization. Energy transfer and absorption phenomena are intrinsic to the structural and biological significance of carotenoid antioxidants. In plants, carotenoids are essential components of the photosynthetic system, where they function in the light-harvesting process, photo-protection, singlet oxygen scavenging, and dissipation of excess energy from excited chlorophylls (Frank, 1996). Within the human body carotenoids scavenge or quench singlet oxygen radicals by direct energy transfer to the carotenoid molecule to generate ground state oxygen and an excited carotenoid which then returns to the ground state by dissipating its energy through interaction with surrounding solvent molecules. Lycopene is the most efficient quencher of singlet oxygen, and this physical quenching is related to the number of conjugated double bonds in the molecule (Stahl, 2003).

Lycopene isomerization under acidic conditions has been reported previously to enhance lycopene micellarization and bioavailability (Boileau, 1999; Moraru, 2005; Holloway, 2000). Lycopene in traditional red tomato cultivars exists packaged in stable crystalline form within

chromoplasts as predominantly all-*trans*-lycopene. However, within human serum and tissue, roughly half of lycopene present exists in the *cis*-lycopene form which indicates a lycopene isomerization event takes place pre-absorption, during absorption or post absorption (Stahl, 1992). Due to the fact that lycopene is susceptible to oxidation, isomerization or degradation when exposed to heat, light, oxygen, acid or metal ions, it is within the realm of possibility to identify ideal and perhaps novel processing conditions which may result in an improvement to carotenoid micellarization or relative bioaccessibility of commonly consumed tomato carotenoids. It is with this intent that we proceed with the subsequent studies.

General Hypothesis

Post-harvest processing of tomatoes using traditional mechanical homogenization and microwave assisted heating will add value to homogenized tomato ingredient material by increasing carotenoid isomerization and bioaccessibility.

Specific Aim 1: Determine if processing increases carotenoid bioaccessibility in tomato.

Specific Aim 2: Determine if microwave processing tomatoes with processing aids increases carotenoid bioaccessibility.

Specific Aim 3: Determine if microwave processing increases carotenoid isomerization in tomato.

Specific Aim 4: Determine if processing tomatoes with processing aids will influence carotenoid isomerization.

Materials and Methods

Chemicals. Authentic standards for lutein, β-carotene, *trans*-β-apo-8′-carotenal were obtained from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA) and used for high-performance liquid chromatography (HPLC) calibration, identification, and quantitation. *Cis*-lycopene and

trans-lycopene isomer standards were provided by Dr. Neal Craft (Eurofins-Craft Technologies., Wilson, NC, USA). All solvents, including acetone, petroleum ether, methyl tert-butyl ether (MTBE), methanol, and ethyl acetate were of certified ACS and HPLC grade (Fisher Scientific, Fairlawn, NJ, USA). Butylated-hydroxytoluene (BHT), ammonium acetate, α-amylase (A3176, 10 units/mg solid), mucin (M2378), porcine pepsin (P7125), lipase (L3126), pancreatin (P7545), and bile (B8631) enzymes were sourced from Sigma-Aldrich and used for *in vitro* digestion. Commercial sunflower oil and sunflower lecithin were purchased from a local grocery retailer.

Tomatoes

Tomato Fruit. Roma tomatoes were purchased at the local farmers market (Decatur, GA, USA). Roma tomatoes were purchased from Whole Foods Market (Raleigh, NC, USA).

Tomato Processing. After tomatoes were purchased, they were transported to the laboratory and stored under refrigerated conditions. The following day, each biological replicate of tomato was weighed, washed with deionized (DI) water, dried and held at room temperature. Samples were then homogenized with a food processor, 10032-766 (Radnor, PA, USA) for 90 seconds on high until pureed at room temperature. Aliquots were transferred to stainless steel mixing bowls and held at room temperature for 1 hr. Processing aids sunflower oil (5% w / w), sunflower lecithin (2% w / w) or sunflower oil and sunflower lecithin (7% w / w) were randomly added to treatment aliquots and homogenized for 120 seconds. Thermal treatment samples consisting of 40 g were heated thereafter with MARS 6 microwave digester for 5-10 minutes at 90-95˚C. Treatment aliquots were transferred to screw cap 50 mL Falcon polypropylene tubes (Falcon Plastics, Los Angeles, CA, USA), wrapped in foil and flushed with nitrogen gas and kept frozen at -80˚C until

further processing. Control and heated samples were digested, extracted and analyzed using HPLC for qualitative and quantitative levels of carotenoid.

Carotenoid Bioaccessibility *In Vitro* Digestion

High-Throughput in Vitro Digestion. A three-phase *in vitro* digestion simulating oral, gastric, and intestinal digestion followed a similar procedure used by Garrett et al was performed with modifications as a means to assess the bioaccessibility of lutein, beta-carotene and lycopene from mechanical processed/thermal unprocessed and mechanical processed/thermal processed tomato food matrices (Garrett, 1999). Screening carotenoid bioaccessibility of Roma Tomatoes required a high-throughput *in vitro* digestion model. To achieve this, a static, three-phase *in vitro* digestion was proportionally scaled down from a 50 mL to a 15 mL digestion for use with the Tecan Freedom EVO liquid handling robot (Tecan Group Ltd., Männedorf, Switzerland), which facilitated all of the liquid handling portions of the protocol (Kean, 2011). Homogenized unheated Roma (UR), homogenized heated Roma (HR), homogenized unheated Roma with oil (URO), homogenized heated Roma with oil (HRO), homogenized heated Roma with oil and lecithin (HROL), and homogenized heated Roma with lecithin (HRL) were subjected to digestion with n = 4 replicates (by completing 4 sets of digestions and analyses, each including one of each homogenate condition). Homogenized treated and untreated samples were thawed, and 0.5 g samples were weighed. Briefly, 0.5 g of homogenized tomato was aliquoted into 15 mL tubes with 5% (w/w) canola oil to facilitate micellarization of carotenoids as bioaccessibility of carotenoids is limited in the absence of oil (Goltz, 2013). A control of carotenoid-rich spinach salad with 5% w/w canola oil, was simultaneously digested during each set to confirm repeatability between assays (Huo, 2007). To each sample, 1.8 mL of the oral phase solution was added along with α-amylase (10 units/mg) (Kean, 2011). Each sample was capped under nitrogen, vortexed, and incubated for 10

min at 37 °C, 120 rpm. Following the oral phase, a gastric phase was simulated with the addition of 0.6 mL of pepsin (final concentration of 0.4 g/L) and adjusted to pH 2.5 ± 0.1 with 1 M HCl. The reaction volume was adjusted to 9 mL with a saline solution (0.9% NaCl), blanketed with nitrogen gas, capped, and then incubated for 60 min at 37 °C, 120 rpm. Following the gastric phase, an intestinal phase was simulated by adjusting the pH to 5 ± 0.1 with 1 M $NaHCO_3$, and then, 0.6 mL of pancreatin-lipase solution (final concentration of 0.8 g/L for both pancreatin and lipase) and 0.9 mL of bile solution (final concentration of 1.8 g/L) were added. The solution was then adjusted to pH 7.0 ± 0.1 with 1 M $NaHCO_3$, blanketed with nitrogen gas, capped, and incubated for 120 min at 37 °C, 120 rpm. Following the intestinal phase, an aliquot was transferred into a separate tube for storage at −80 °C to assess digestive stability. The remaining digesta was centrifuged (Beckman Coulter, Allegra X-30R Centrifuge, Indianapolis, IN, USA) at 4255g for 60 min after which the aqueous fraction was collected and filtered through a 0.2 μm cellulose acetate filter to isolate the aqueous fraction containing the micellarized carotenoid compounds of interest.

Extraction of Carotenoids from Tomato Materials and Digestive Fractions. Carotenoid contents in homogenized tomato samples were quantified in 50 mg aliquots using a previously reported method (Lipkie, 2013). Briefly, all samples were extracted under a yellow light to prevent carotenoid degradation. Initially, 1 mL of DI water was added to expand the tomato matrix and disperse the internal standard. Samples were placed on ice for 10 min prior to extraction of carotenoids using 5 mL of chilled acetone (2×), followed by 2 mL of MTBE. Extracts were combined and dried under nitrogen gas, resolubilized in 2 mL of ethyl acetate/methanol (1:4), and then filtered using a 0.45 μm cellulose acetate filter for quantification using HPLC with a photo diode array detector (HPLC−PDA). Two mL aliquots of both aqueous and digesta fractions were extracted with a 1:3 ratio of acetone and petroleum ether (0.1% BHT) (×3), dried under nitrogen,

resolubilized in the 1:4 ratio of ethyl acetate and methanol, and filtered with 0.45 μm cellulose acetate filters for HPLC analysis of carotenoid contents. Extraction recovery of aqueous and digesta was 94%, determined by using *trans*-β-apo-8′-carotenal spiked into samples.

Analysis

Carotenoid Analysis. Lutein, β-carotene, and lycopene were analyzed by HPLC−PDA using a Waters Alliance 2695 LC system (Waters, Milford, MA, USA) equipped with a model 2998 PDA (Milford, MA, USA). The separation was attained using a YMC C30 column (3 μm 150 mm × 2 mm) thermostat at 35 °C and a gradient elution method starting with 95% solvent A (methanol/ammonium acetate, adjusted to pH 4.6, 98:2) and 5% solvent B (ethyl acetate) for 3 min, 85% solvent A and 15% solvent B for 5 min, 20% solvent A and 80% solvent B for 1 min, 100% solvent B for 4 min, and 95% solvent A and 5% solvent B for the remaining 4 min of the 17 min run. The flow rate was 0.37 mL min^{-1} with an injection volume of 10 μL. All compounds of interest were quantified at 450 nm. Compound concentrations were determined using external standard calibration curves developed in the concentration range of 0.01−7.5 μM for the carotenoids.

Calculations and Statistical Analysis. Analyses were performed on three biological replicates for each homogenate condition, and the results are expressed as the mean ± standard deviation. The relative bioaccessibility (micellarization efficiency) was calculated as follows:

$$\text{Relative Bioaccessibility: } \frac{\text{concentration in aqueous fraction}}{\text{concentration in digesta fraction}} \times 100$$

Bioaccessible content was calculated as follows:

Bioaccessible content =

relative bioaccessibility % x quantitative bioactive content (nmol/g FW).

JMP (SAS Institute Cary, NC, USA) software was used to analyze the data. Means were compared using a one-way analysis of variance (ANOVA) to determine a significant difference ($p < 0.05$) among carotenoid bioaccessibility for unheated Roma tomato control and treatment conditions. The subsequent Tukey-Kramer analysis and Dunnett test were run when ANOVA identified a significant difference in the data set.

Results

Carotenoid profile in untreated control and processing treatments in Tomato

Variation in carotenoid content was observed across 6 different tomato homogenate treatments. Processing methods including homogenization, homogenization with processing aids and microwave-assisted thermal processing were standardized; therefore, reported carotenoid species ranges were reflective of tomato treatment conditions. The lutein (LUT) content ranged from 0.35 to 0.72 µg/g, *cis*-LUT 0.051 to 0.1203 µg/g, and total-LUT 0.42 to 0.77 µg/g. The *trans* beta-carotene (BC) content ranged from 1.17 to 1.55 µg/g, *cis*-BC 0.328 to 0.806 µg/g, and total-BC 1.64 to 2.36 µg/g. The *trans* lycopene (LYC) content ranged from 38.7 to 55.9 µg/g, *cis*-LYC content ranged from 3.67 to 5.09 µg/g, and total-LYC content ranged from 43.8 to 59.6 µg/g (Table 1). The three carotenoids of interest were detected and quantified in raw material, digesta and filtered aqueous (micelle) fractions. *Trans* and *cis* isomers for LYC, BC and LUT

were identified, but levels of LUT and its *cis*-LUT isomers were relatively low within the filtered aqueous fraction. There was a statistically significant increase ($p < 0.05$) observed for *cis*-LUT content in HRL processing treatment compared to unheated control. Lutein content in raw material expressed a decreasing trend in treatment samples compared to UR control, however the differences were not statistically significant.

Table 1: Lutein content changes in Tomato Raw Materials derived from process treatment conditions. Lutein Isomer content in raw material extract was increased in HRL>HR> HROL, treatments compared to control UR sample. Lutein content was lowest in URO treatment while expressing the highest concentration in HR treatment conditions behind UR control. Total Lutein content expressed highest in UR, HR and HRL, while conversely representing lowest values for URO and HRO. Changes due to processing treatments were not statistically significant. [1,2,3]

Lutein Content µg/g			
Treatment	cis-Lutein	Lutein	Total Lutein
UR	0.053 ± 0.004	0.72 ± 0.23	0.77 ± 0.23
HR	0.092 ± 0.021	0.56 ± 0.03	0.65 ± 0.05
URO	0.065 ± 0.005	0.35 ± 0.02	0.42 ± 0.03
HRO	0.051 ± 0.003	0.38 ± 0.007	0.43 ± 0.004
HROL	0.091 ± 0.011	0.39 ± 0.01	0.49 ± 0.02
HRL	0.12 ± 0.012 [A]	0.44 ± 0.02	0.56 ± 0.03

[1] Changes in lutein isomer concentrations during microwave-assisted sterilization of Roma Tomatoes.[2] Data are presented as mean ± standard error of the mean (SEM). Significant difference between treatments on Lutein content based on Tukey-Kramer HSD analysis within each column.[3] Different letters indicate significant differences ($p < 0.05$) between treatment groups within lutein isomers. Roma Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, URO = Unheated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

Table 2: β-carotene content changes in Tomato Raw Materials derived from process treatment conditions. β-carotene Isomer content in raw material extract was increased by 2x in HR and close to 2x in HRL treatments compared to control UR sample. β-carotene content decreased in treated samples compared to UR control. Changes due to processing treatments were not statistically significant. [1,2,3]

	Beta Carotene Content µg/g		
Treatment	cis-Beta Carotene	Beta Carotene	Total Beta Carotene
UR	0.387 ± 0.05	2.26 ± 0.75	2.65 ± 0.803
HR	0.806 ± 0.23	1.55 ± 0.19	2.36 ± 0.417
URO	0.467 ± 0.013	1.17 ± 0.04	1.64 ± 0.05
HRO	0.585 ± 0.026	1.35 ± 0.012	1.94 ± 0.036
HROL	0.472 ± 0.04	1.26 ± 0.016	1.74 ± 0.05
HRL	0.749 ± 0.214	1.39 ± 0.17	2.14 ± 0.38

[1] Changes in β-carotene content concentrations during microwave-assisted sterilization of Roma Tomatoes.[2] Data are presented as mean ± standard error of the mean (SEM). Significant difference between treatments on β-carotene content based on Tukey-Kramer HSD analysis within each column.[3] Different letters indicate significant differences ($p < 0.05$) between treatment groups within β-carotene isomers. Roma Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, URO = Unheated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

Table 3: Lycopene content changes in Tomato Raw Materials derived from process treatment conditions. *Trans*-Lycopene content in raw material extract was decreased in treatments compared to control UR sample. Total and *cis*-lycopene content was relatively stable in treated samples compared to UR control. [1,2,3]

	Lycopene Content µg/g		
Treatment	cis-Lycopene	trans-Lycopene	Total Lycopene
UR	5.15 ± 1.21	68.8 ± 10.5	73.9 ± 11.7
HR	4.18 ± 0.15	51.06 ± 1.33	55.2 ± 1.47
URO	3.77 ± 0.12	45.52 ± 0.53	49.3 ± 0.64
HRO	5.09 ± 0.11	38.72 ± 0.46 [A]	43.8 ± 0.56 [A]
HROL	5.05 ± 0.23	46.66 ± 2.14	51.7 ± 1.93
HRL	4.67 ± 0.23	48.15 ± 2.08	52.8 ± 1.89

[1] Changes in lycopene content concentrations during microwave-assisted sterilization of Roma Tomatoes.[2] Data are presented as mean ± standard error of the mean (SEM). Significant difference between treatments on Lycopene content based on ANOVA Dunnett's test analysis within each column.[3] Different letters indicate significant differences ($p < 0.05$) between treatment groups and control. Roma Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, URO = Unheated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

Tested processing conditions resulted in the identification of a change in lycopene isomerization in treated raw material compared to control, suggesting isomerization due to treatment conditions had taken place. When comparing the percentage of *cis*-LYC content by dividing *cis*-LYC (µg / g) by total-LYC (µg / g) in treated and untreated samples, maximal increase in levels of *cis*-lycopene isomers detected were highest for HROL and HRO samples, compared to control (UR) (Figure 1). *Cis*-Lycopene isomerization was inversely proportional to *trans*-lycopene content detected in treated tomato homogenate samples compared to untreated control (UR) (Figures 1, Figure 2). An additional observation supporting *cis*-isomerization is tomato homogenate HPLC chromatograms displayed an additional third *cis*-lycopene isomer peak in HROL treated samples compared to only 2 *cis*-isomer peaks (11.29 retention time), *cis*-lycopene

isomer (11.73 retention time) in untreated control samples (Figure 3, Figure 5).

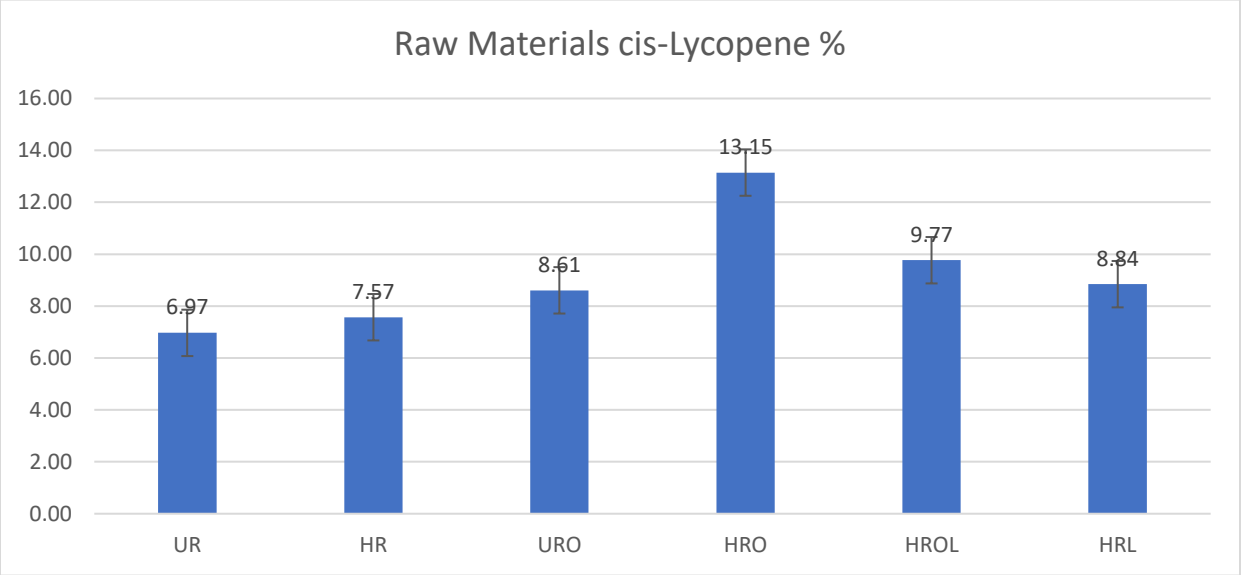

Figure 1. Percentage of *cis*-Lycopene compared to total Lycopene in control and treated raw material samples. *Cis*-Lycopene percentage increases suggesting isomerization in processing aid and thermal processed samples. Heated Roma (HR), Unheated Roma/Oil (URO), Heated Roma/Oil (HRO), Heated Roma/Oil/Lecithin (HROL), Heated Roma/Lecithin (HRL).

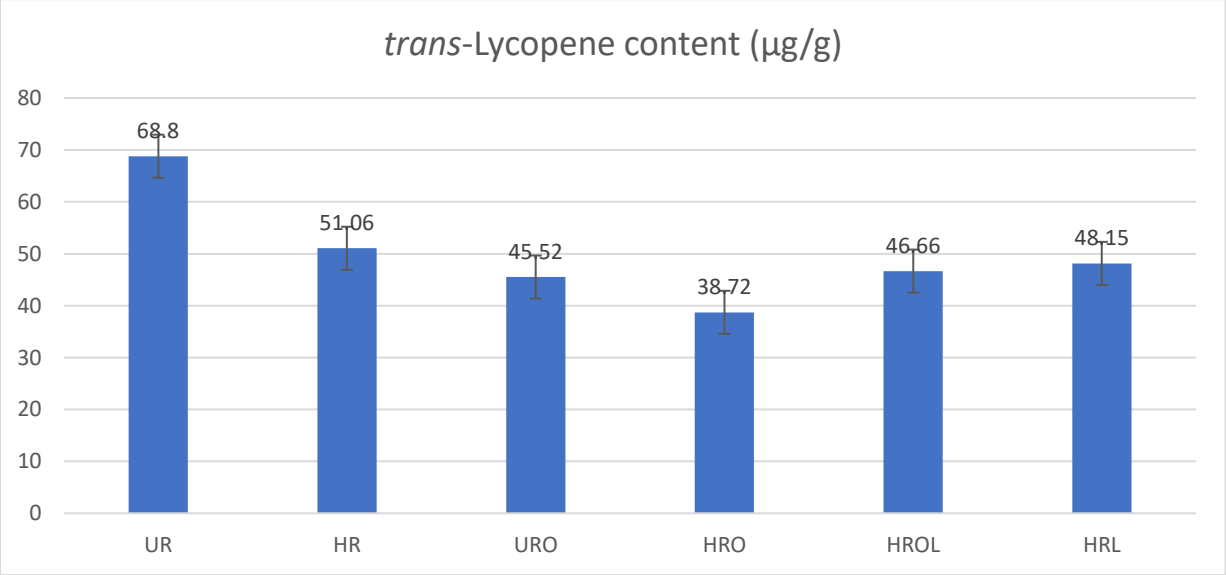

Figure 2. *Trans*-lycopene content in raw material extract illustrated in descending order based upon treatment samples compared to Control-UR>HR>HRL>HROL>URO>HRO.

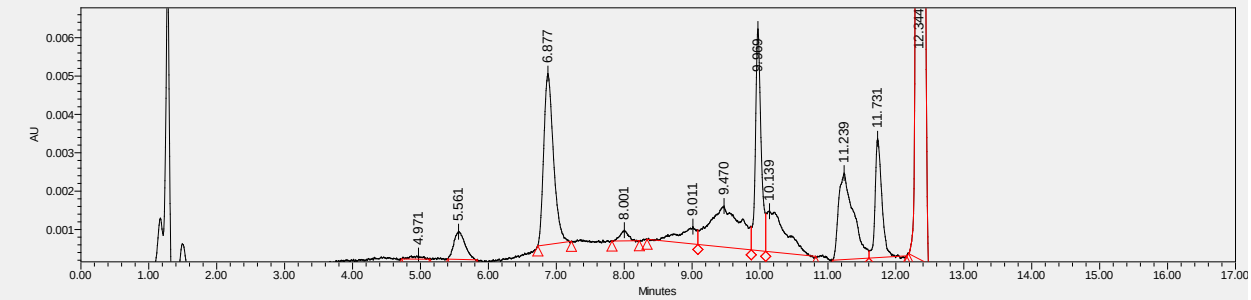

Figure 3. Chromatogram of major carotenoid species in untreated Roma tomato (UR) homogenate analyzed by HPLC with diode-array detection at 450 nm. Peak identification: lutein isomer (4.97 retention time), lutein (5.56 retention time), β-apo-8′-carotenal internal standard (6.87 retention time), *all-trans*-β-carotene (9.98 retention time), 9-*cis*-β-carotene (10.1 retention time), *cis*-Lycopene isomer (11.29 retention time), *cis*-Lycopene isomer (11.73 retention time), *trans*-Lycopene (12.34 retention time).

Carotenoid Bioaccessibility

Relative bioaccessibility is a measure of the proportion of carotenoids released from the tomato matrix and transferred into bile salt− lipid micelles during digestion and therefore is made available for intestinal uptake (Ellison, 2017).

UR vs URO. The percentage amount of *cis*-LYC, *trans*-LYC, total LYC, *cis*-BC, *trans*-BC, and total-BC transferred to the filtered aqueous fraction during digestion of purée containing sunflower oil (URO) was 9.81 ± 1.2%, 8.07 ± 2.8%, 8.28 ± 2.6%, 4.24 ± 2.29%, 15.43 ± 2.9%, 10.7 ± 2.6%, respectively (Table 4). In contrast, when sunflower oil was not added (UR) prior to digestion the relative amounts of *cis*-LYC, *trans*-LYC, total LYC, *cis*-BC, *trans*-BC, and total-BC in the aqueous fraction were 5.76 ± 0.7%, 1.96 ± 0.48%, 2.35± 0.4%, 6.47 ± 0.29%, 21.59 ± 1.9%, 17.2 ± 1.1%, respectively (Table 4).

UR vs HR. Thermal processing tomato homogenate (HR) did not generate a change or significant difference in relative bioaccessibility for *cis*-BC, *trans*-BC, total-BC, *cis*-LYC, *trans*-LYC or total-LYC compared to control (UR) (Table 4).

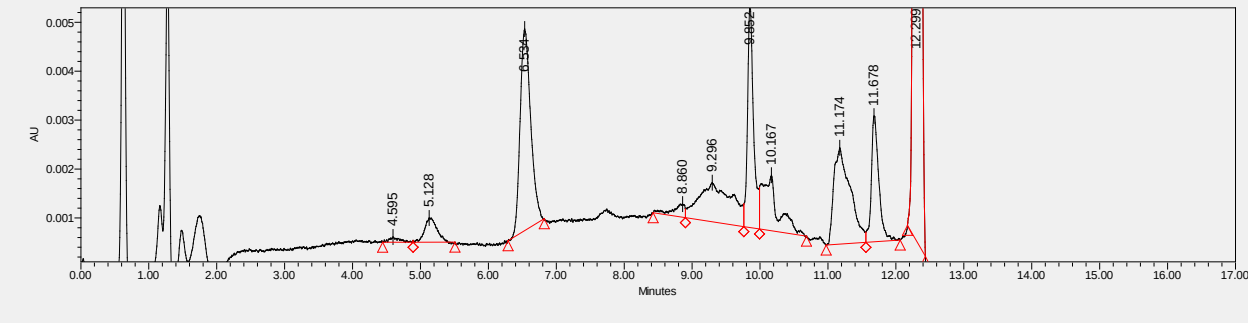

Figure 4. Chromatogram of major carotenoid species in untreated Roma tomato with oil (URO) analyzed by HPLC with diode-array detection at 450 nm. Peak identification: lutein isomer (4.97 retention time), lutein (5.56 retention time), β-apo-8′-carotenal internal standard (6.87 retention time), *all-trans*-β-carotene (9.98 retention time), 9-*cis*-β-carotene (10.1 retention time), *cis*-Lycopene isomer (11.17 retention time), *cis*-Lycopene isomer (11.67 retention time), *trans*-Lycopene (12.29 retention time).

UR vs HRO. Thermal processing tomato homogenate with oil (HRO) generated an increase in relative bioaccessibility for *cis*-BC ($p < 0.05$) compared to baseline control (UR) (Table 4). HRO treatment also generated a significant increase in relative bioaccessibility for *cis*-LYC ($p < 0.01$), *trans*-LYC ($p < 0.05$) and total-LYC ($p < 0.05$), respectively; but no significant difference for *trans*-BC and total-BC compared to baseline control (UR). Bioaccessible content for *cis*-LYC was increased ($p < 0.05$) in HRO treatment compared to control (UR) (Table 5).

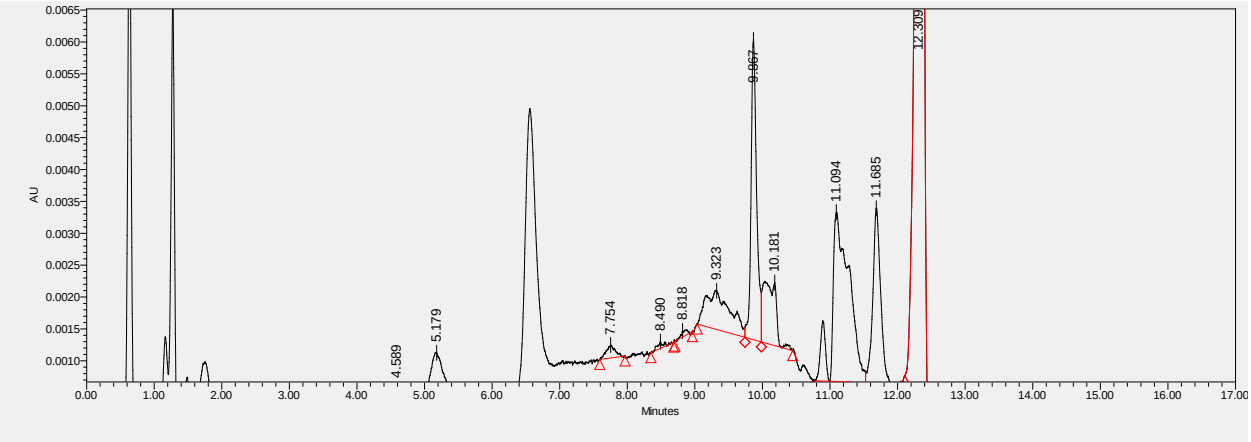

Figure 5. Chromatogram of major carotenoid species in heat treated Roma tomato with oil + Lecithin (HROL) analyzed by HPLC with diode-array detection at 450 nm. Peak identification: lutein isomer (4.97 retention time), lutein (5.56 retention time), β-apo-8′-carotenal internal standard (6.87 retention time), *all-trans*-β-carotene (9.98 retention time), 9-*cis*-β-carotene (10.1

retention time), *cis*-Lycopene isomer (11.09 retention time + additional peak), *cis*-Lycopene isomer (11.73 retention time), *trans*-Lycopene (12.34 retention time).

UR vs HRL. Thermal processing of tomato homogenate with lecithin (HRL) resulted in an increase in *trans*-BC ($p < 0.0001$) relative bioaccessibility compared to UR control. An increase in total-BC ($p < 0.0001$) relative bioaccessibility was observed compared to control UR (Table 4). HRL treatment expressed an increase in *cis*-LYC ($p < 0.01$) relative bioaccessibility, but no difference was observed for *trans*-LYC or total-LYC compared to control (UR).

UR vs HROL. Comparing untreated control UR to HROL treatment, relative bioaccessibility increased for *cis*-BC ($p < 0.0001$), *trans*-BC ($p < 0.0001$) and total-BC ($p < 0.0001$) by 322%, 114% and 121%, respectively. Relative bioaccessibility increases for *cis*-LYC ($p < 0.0001$), *trans*-LYC ($p < 0.0001$), total-LYC ($p < 0.0001$) rose by 413%, 918%, 805%, respectively in HROL compared to UR. Chromatogram analysis in HROL samples showed an additional *cis*-LYC isomer peak (Figure 5) compared to URO (Figure 4) and UR (Figure 3).

HR vs URO. Thermal processing without oil (HR) increased *trans*-BC ($p < 0.05$) relative bioaccessibility compared to URO. Conversely, *cis*-LYC, *trans*-LYC, total-LYC, *cis*-BC and total-BC relative bioaccessibility did not change significantly in HR treatment compared to URO treatment.

HR vs HRO. Thermal processing tomato homogenate with oil (HRO) generated significant increases in relative bioaccessibility for *cis*-LYC ($p < 0.05$), total LYC ($p < 0.05$), and *cis*-BC ($p < 0.01$), respectively compared to thermal processed tomato homogenate without oil (HR) (Table 4). Conversely, the addition of oil and thermal processing resulted in no difference in *trans*-LYC, *trans*-BC or total BC relative bioaccessibility compared to heated w/o oil (HR) treatment.

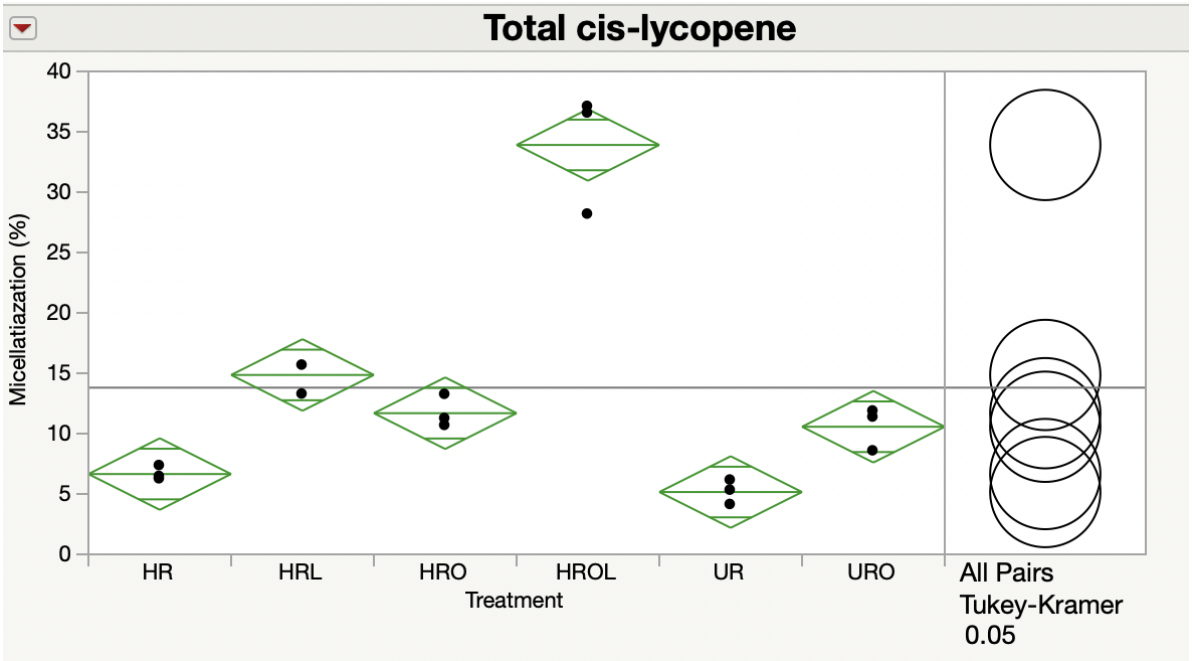

Figure 6. Representative figure of One-way Analysis of Variance showing differences in Total *cis*-lycopene micellarization percentage by Treatment.

HR vs HRL. Thermal processing of tomato homogenate with lecithin (HRL) generated a significant increase in relative bioaccessibility for *cis*-BC ($p < 0.05$), *trans*-BC ($p < 0.001$), total-BC ($p < 0.001$) and *cis*-LYC ($p < 0.01$), respectively compared to heat treated (HR) (Table 4). There were no significant changes for *trans*-LYC and total-LYC relative bioaccessibility when HRL treatment was compared to HR.

HR vs HROL. Thermal processing of tomato homogenate with oil and lecithin (HROL) produced an increase in *cis*-LYC ($p < 0.0001$), *trans*-LYC ($p < 0.0001$) and total-LYC ($p < 0.0001$) in relative bioaccessibility compared to thermal processing without oil and lecithin (HR) (Table 4). HROL treatment also increased *cis*-BC ($p < 0.0001$), *trans*-BC ($p < 0.001$) and total-BC ($p < 0.0001$) relative bioaccessibility compared to HR (Table 4).

URO vs HRO. Mechanical and thermal processing Roma with oil (HRO) compared to only mechanical processing with oil (URO) displayed no statistical difference in relative bioaccessibility of *cis*-LYC, *trans*-LYC, total-LYC, *trans*-BC and total-BC. However, *cis*-BC

relative bioaccessibility was increased significantly ($p < 0.01$) in HRO treatment compared to URO treatment.

URO vs HRL. Mechanical and thermal processing with lecithin (HRL) did not show any difference in relative bioaccessibility for *cis*-LYC, *trans*-LYC, total-LYC compared to unheated with oil (URO) treatment (Table 4). HRL treatment resulted in increases in relative bioaccessibility for *trans*-BC ($p < 0.0001$) and total-BC ($p < 0.0001$) compared to URO treatment. HRL showed an increase in *cis*-BC relative bioaccessibility compared to URO, however the increase was not statistically significant (Table 4).

URO vs HROL. Mechanical and thermal processing with oil and lecithin (HROL) resulted in an increase in relative bioaccessibility for *cis*-LYC ($p < 0.0001$), *trans*-LYC ($p < 0.001$), total-LYC ($p < 0.001$), respectively compared to mechanical processing with oil (URO) (Table 4). HROL treatment produced an increase in relative bioaccessibility for *cis*-BC ($p < 0.0001$), *trans*-BC ($p < 0.0001$) and total-BC ($p < 0.0001$), respectively compared to URO treatment (Table 4).

HRO vs HRL. Thermal processing of HRO and HRL resulted in no statistically significant difference in relative bioaccessibility of *cis*-LYC, *trans*-LYC, total-LYC, and *cis*-BC (Table 4). Relative bioaccessibility was increased significantly for *trans*-BC ($p < 0.0001$) and total-BC ($p < 0.001$) in HRL treatment samples compared to HRO treatment (Table 4).

HRO vs HROL. Thermal processing tomato homogenate with oil and lecithin (HROL) resulted in significant increases in relative bioaccessibility for *cis*-LYC ($p < 0.0001$), *trans*-LYC ($p < 0.001$), total-LYC ($p < 0.001$) compared to HRO treatment (Table 4). HROL treatment produced an increase in relative bioaccessibility for *cis*-BC ($p < 0.01$), *trans*-BC ($p < 0.0001$) and total-BC ($p < 0.0001$), respectively compared to HRO treatment (Table 4).

% Relative Bioaccessibility	*cis*-LYC	*trans*-LYC	Total-LYC	*cis*-BC	*trans*-BC	Total-BC
UR	5.76	1.96	2.35	6.47	21.59	17.2
URO	9.81	8.07 [A]	8.28 [A]	4.24	15.43	10.7
HR	5.68	1.53	1.96	3.63	26.49 [A]	18.55
HRO	11.82 [A]	8.32 [A]	8.81 [A]	14.46 [A]	18.84	17.03
HRL	12.37 [A]	5.17	5.91	11.76 [A]	46.51 [A]	35.63 [A]
HROL	29.58 [A]	20.04 [A]	21.32 [A]	27.29 [A]	46.29 [A]	38.03 [A]

Table 4. Relative Bioaccessibility (micellarization) for carotenoids, *cis*-Lycopene (*cis*-LYC), *trans*-Lycopene (*trans*-LYC), total-Lycopene (Total-LYC), *cis*-Beta-Carotene (*cis*-BC), *trans*-Beta-Carotene (*trans*-BC), and total Bet-Carotene (Total-BC) in processing tomato homogenate. Significant differences using Dunnett's test between control (UR) and other conditions, (A) equals significant differences ($p < 0.05$).

HRL vs HROL. Thermal processing tomato homogenate with oil and lecithin (HROL) resulted in significant increases in relative bioaccessibility for total-LYC ($p < 0.0001$), *cis*-LYC ($p < 0.0001$), *trans*-LYC ($p < 0.0001$) and *cis*-BC ($p < 0.001$), respectively compared to thermal processed tomato homogenate with lecithin (HRL) (Table 4).

Table 5: Lycopene isomers and total Lycopene content changes in absolute bioaccessibility (bioaccessible content) in Tomatoes derived from process treatment conditions. [1,2,3]

	Lycopene Bioaccessible Content µg/g		
Treatment	*cis*-Lycopene	*trans*-Lycopene	Total Lycopene
UR	0.298 ± 0.07	1.42 ± 0.39	1.81 ± 0.45
HR	0.238 ± 0.01	0.778 ± 0.02	1.08 ± 0.02
URO	0.371 ± 0.03	3.69 ± 0.63	4.09 ± 0.63
HRO	0.603 ± 0.03 [A]	3.22 ± 0.305	3.85 ± 0.27
HROL	1.49 ± 0.09 [A]	9.504 ± 1.57 [A]	11.15 ± 1.59 [A]
HRL	0.581 ± 0.05	2.511 ± 0.377	3.14 ± 0.37

[1] Changes in lycopene isomer concentrations during microwave-assisted sterilization of Roma Tomatoes.[2] Data are presented as mean ± standard error of the mean (SEM). Significant difference between treatments on lycopene content based on Dunnett test analysis within each column.[3] (A) = significant difference from control (UR) ($p < 0.05$). Roma Control = Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

Table 6: β Carotene isomer and total β Carotene content changes in Absolute Bioaccessibility or Bioaccessible content in Tomatoes from microwave processing treatment conditions. [1,2,3].

		Beta Carotene Bioaccessible Content µg/g	
Treatment	*cis*- β Carotene	β Carotene	Total β Carotene
UR	0.025 ± 0.002	0.475 ± 0.14	0.445 ± 0.12
HR	0.028 ± 0.006	0.402 ± 0.02	0.435 ± 0.05
URO	0.019 ± 0.005	0.182 ± 0.02	0.177 ± 0.02
HRO	0.084 ± 0.005	0.255 ± 0.018	0.33 ± 0.02
HROL	0.128 ± 0.018 [A]	0.586 ± 0.029	0.661 ± 0.05
HRL	0.087 ± 0.023	0.654 ± 0.099	0.782 ± 0.17

[1] Changes in β-carotene isomer concentrations during microwave-assisted sterilization of Roma Tomatoes.[2] Data are presented as mean ± standard error of the mean (SEM). Significant difference between treatments on β-carotene content based on Dunnett test analysis within each column.[3] Different letters indicate significant differences ($p < 0.01$) between treatment groups within lycopene isomers. Roma Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

Discussion

Food processing can enhance bioavailability of carotenoids by disrupting the food matrix. This can be accomplished through a mechanical shear stress homogenization process that breaks up plant cell walls and the linkages between the carotenoids and protein or fiber, dissolution of the crystalline carotenoids in oil droplets, and increasing surface area for greater access of hydrolytic enzymes and bile salts to oil droplets during digestion (You, 1996; Kostic, 1995; Livny 2003; van het Hof, 2000; Edwards, 2002). In red tomatoes, lycopene containing 11 linear conjugated carbon–carbon double bonds typically exist in a crystalline state within chromoplasts encompassed by a compartmentalized acidic (pH 3.6) water-filled environment (Page et al., 2012) (Figure 9). According to some studies, this hydrophobic crystalline lycopene structure, predominantly in the *trans*-form, is in its most thermodynamically stable conformation within this aqueous environment. However, during a shear stress mechanical homogenization process

along with the presence of oil or hydrophobic substances the crystalline carotenoid structure can become solubilized or destabilized.

The bioavailability of carotenoids solubilized in oil exceeds that of crystals since the solubilization in oil droplets facilitate transfer to micelles (Rich, 2003). As it relates to *in vitro* digestions performed within this study, micellarization of total-BC, *trans*-BC, and *cis*-BC into aqueous fraction during digestion of tomato homogenate containing sunflower oil (URO) appeared to decrease when compared to UR control. Conversely, lycopene relative bioaccessibility for total-LYC, *trans*-LYC and *cis*-LYC increased, suggesting an inverse relationship between LYC and BC for micelle incorporation when processing aid oil was added (URO). However, according to statistical analysis, the change was not significant when carotenoid species and isomers within UR and URO were directly compared. Although the addition of oil appeared to increase micellarization of lycopene and its isomers, additional experimental replicates are needed to explore potential micellarization relationships between LYC and BC when mechanically processing tomatoes with sunflower oil processing aid. It has been previously reported that the presence of BC improves lycopene absorption and bioavailability (Tyssandier, 2002; Tyssandier, 2003; Johnson, 1997; White, 1993). Nevertheless, the addition of oil during carotenoid processing produced expected results of lycopene micellarization enhancement.

Microwave-assisted thermal processed Roma tomatoes (HR) show no significant increase in relative bioaccessibility for total-LYC, *trans*-LYC, *cis*-LYC, *trans*-BC, *trans*-BC, *cis*-BC compared to unheated Roma tomatoes (UR). These results were unexpected but not surprising. It was hypothesized that the volumetric heat generation mechanism influenced by excitation of water and polar molecules within the fluid tomato homogenate matrix would be localized enough to disrupt the crystalline lycopene structures and perhaps influence lycopene isomerization

(Kumnar, 2008; Venkatesh, 2004). However, the data disproved this hypothesis. The data corroborate Schwartz lab's findings that *trans*-lycopene isomer is relatively stable to isomerization at temperatures 50 and 100 °C (Nguyen, 1999). The data also indicate that micellarization of carotenoids from the cultivar lot harvested and tested was not influenced solely by mechanical shear-stress and/or microwave-assisted thermal processing conditions.

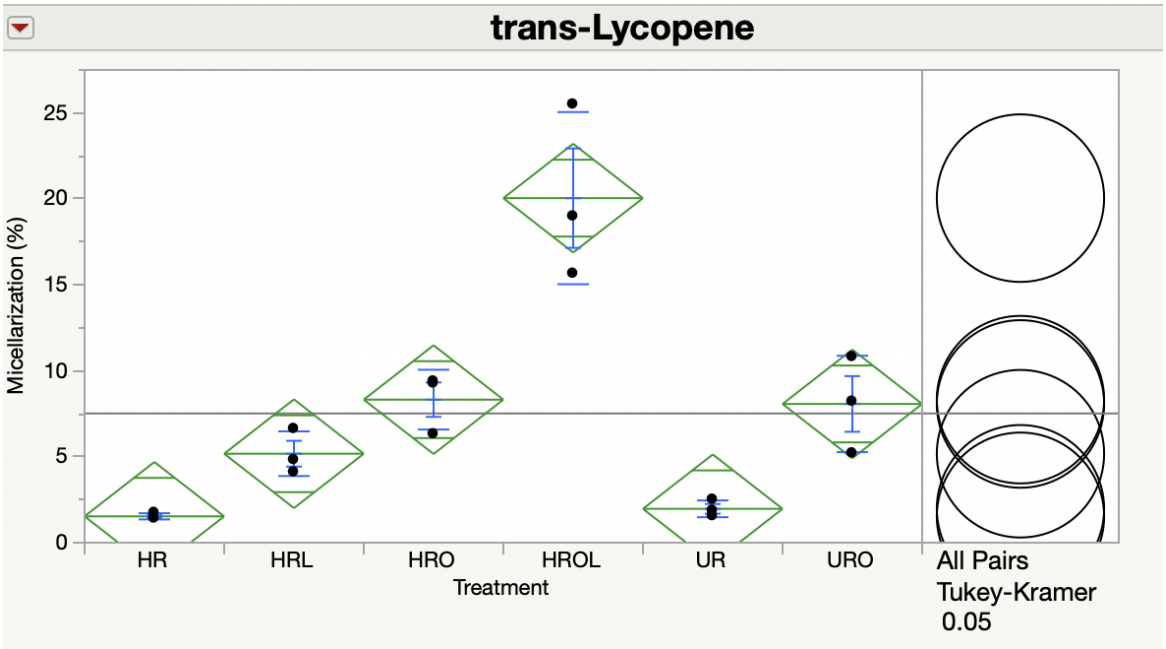

Figure 8. One-way Analysis of Variance for *trans*-Lycopene micellarization percentage by Treatment.

Compared to *trans*-lycopene, the absorption potential of *cis*-lycopene is enhanced due to the lower tendency to self-aggregate and form crystals (Britton, 1995). The solubility of *cis*-isomers is high in the lipophilic phase; therefore, they are more efficiently incorporated into bile acid micelles, and pass across the intestinal barrier via passive diffusion (Cooperstone, 2015; Bohm, 1999; Boileau, 2002; Cartner, 1997). As tomatoes are processed and/or cooked, the *trans*-lycopene is converted to the more bioavailable *cis*-lycopene isomer form, which has been shown to increase absorption by 2 to 3-fold. (Gartner,1997; Stahl, 1992; Rao, 2002). Thermal

processing carotenoid foodstuffs in oil has been shown to increase carotenoid micellarization (Colle, 2013).

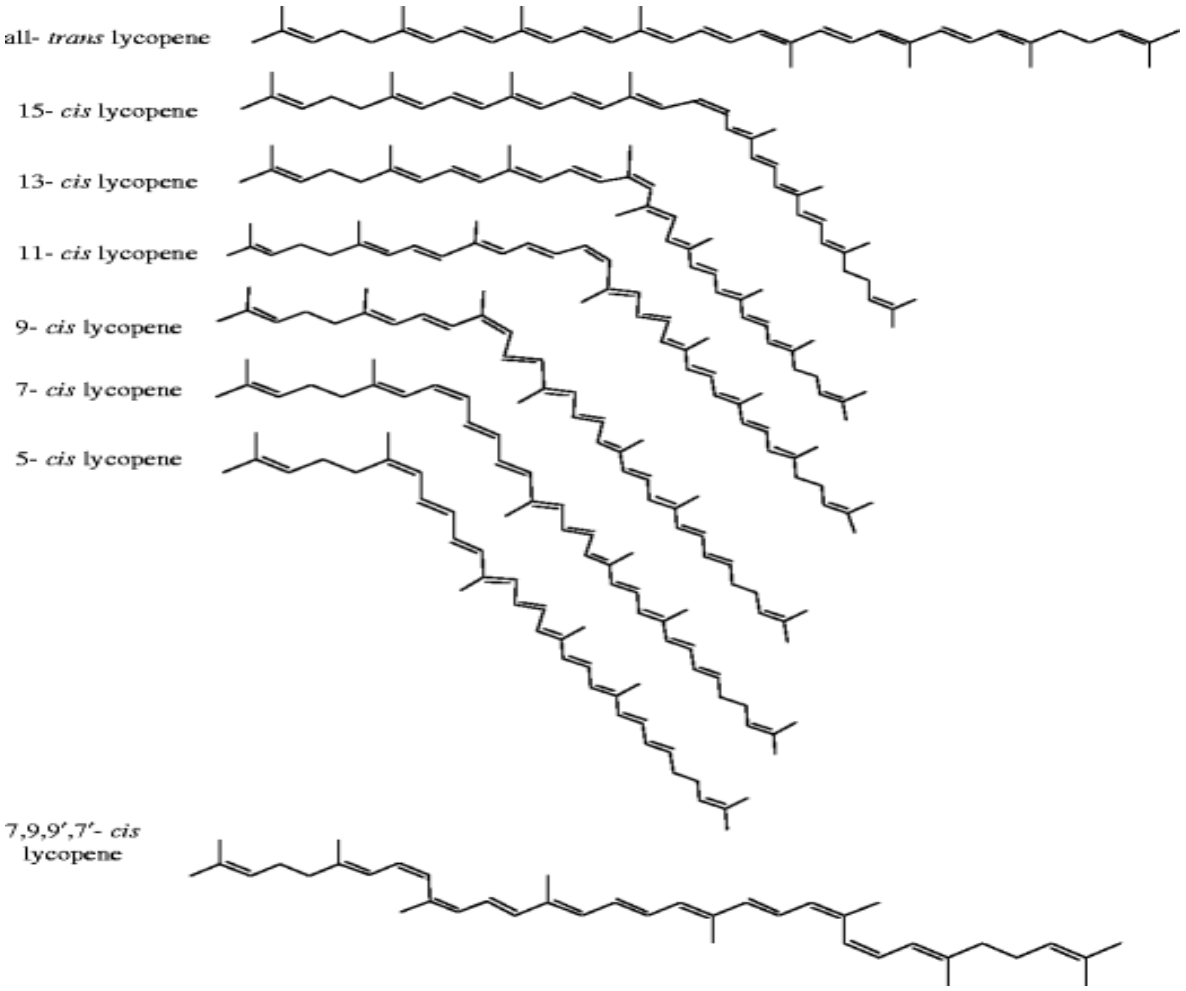

Figure 9. Geometric Isomers of Lycopene (Srivastava, 2015).

Thermal processing tomato homogenate with oil (HRO) generated significant increases ($p < 0.05$) in relative bioaccessibility for *cis*-LYC, *trans*-LYC, total-LYC, and *cis*-BC compared to thermally processed tomato homogenate without oil (HR) (Table 4). Conversely, the addition of oil and thermal processing resulted in a decrease in *trans*-BC micellarization (Table 4). The data suggests, under the treatment conditions carotenoid isomerization of LYC and BC occurred and may contribute to increased micellarization when Roma tomatoes are mechanically

processed with sunflower oil and thermally processed using microwave-assisted heating. During shear stress mechanical homogenization, the stable lycopene form equilibrium may have shifted to favor *cis*-LYC following processing with oil, at the expense of *trans*-LYC (Figures 4 & 5).

Lycopene being a 40-carbon atom highly unsaturated straight chain hydrocarbon containing 11 conjugated and 2 non-conjugated double bonds (Figure 9), the abundance of conjugated carbon-carbon double bonds in its backbone enables lycopene to theoretically assume 211 or 2048 geometrical configurations (Omani 2005). Lycopene biosynthesis in plants leads to the all-

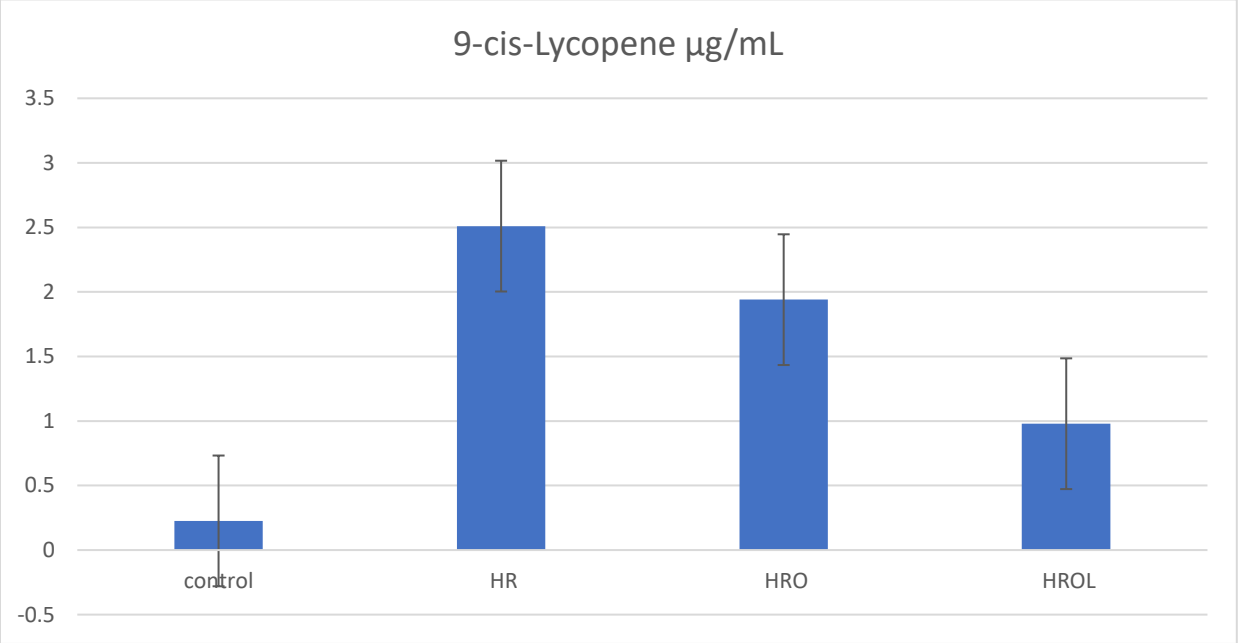

Figure 10. Quantification of 9'-cis-lycopene detected in processed Roma Tomatoes for UR, HR, HRO, HROL. The data shows a 50% increase of 9'-cis-lycopene in HRO compared to control (unheated).

trans-form, but the most commonly identified isomeric forms of lycopene are all-*trans*, 5-*cis*, 9-*cis*, 13-*cis* and 15-*cis* (Figure 9) with a stability sequence being 5-*cis*>all-*trans*>9-*cis*>13-*cis*>15-*cis*>7-*cis*>11-*cis* (Agarwal 2000). The data suggest it is possible for 5-*cis* LYC to be more

thermodynamically stable than all-*trans*-LYC under certain conditions (Agarwal 2000) (Figure 7). Therefore, it is also possible the oil and shear stress solubilized *trans*-LYC enabling more efficient energy transfer through microwave-assisted heating to isomerize lycopene to a favorable form, based on processing conditions, leading to an increase in micellarization of *cis*-LYC, *cis*-BC and total-LYC.

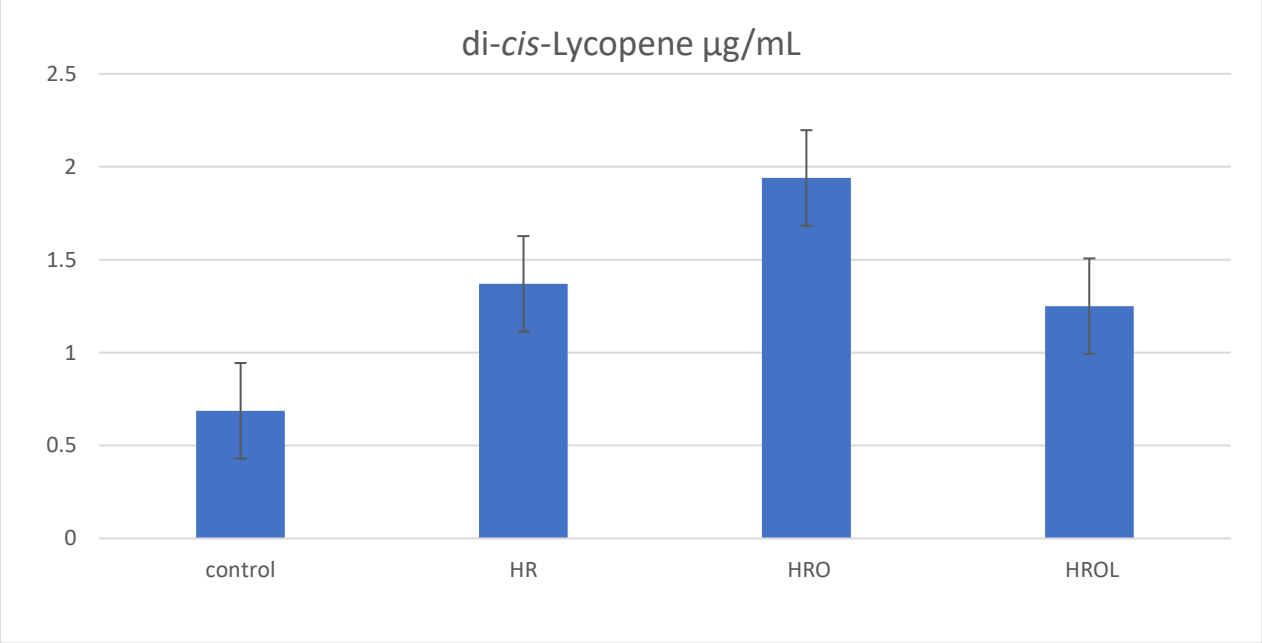

Figure 11. Quantification of di-*cis*-lycopene detected in processed Roma Tomatoes for unheated control, heated, heated with oil and heated with oil and lecithin. The data shows a 99.4% increase of di-*cis*-Lycopene isomer in HR compared to control (unheated). Data show a 182% increase of di-*cis*-Lycopene isomer in HRO compared to control. Data show an 81.9% increase of di-*cis*-Lycopene isomer in HROL compared to control.

In Figure 5 we characterize a decrease in *trans*-LYC content in HRO samples compared to control and HR in raw material samples. Thermal processing tomato homogenate with oil (HRO) generated a 124% increase in relative bioaccessibility for *cis*-BC compared to baseline control (UR) (Table 4). HRO treatment also generated a significant increase in micellarization of *cis*-LYC and total-LYC, but not *trans*-LYC, *trans*-BC and total-BC compared to baseline control

(UR). These data are consistent with an increase in micellarization of *cis* isomers for LYC and BC in comparative HR samples (Table 4).

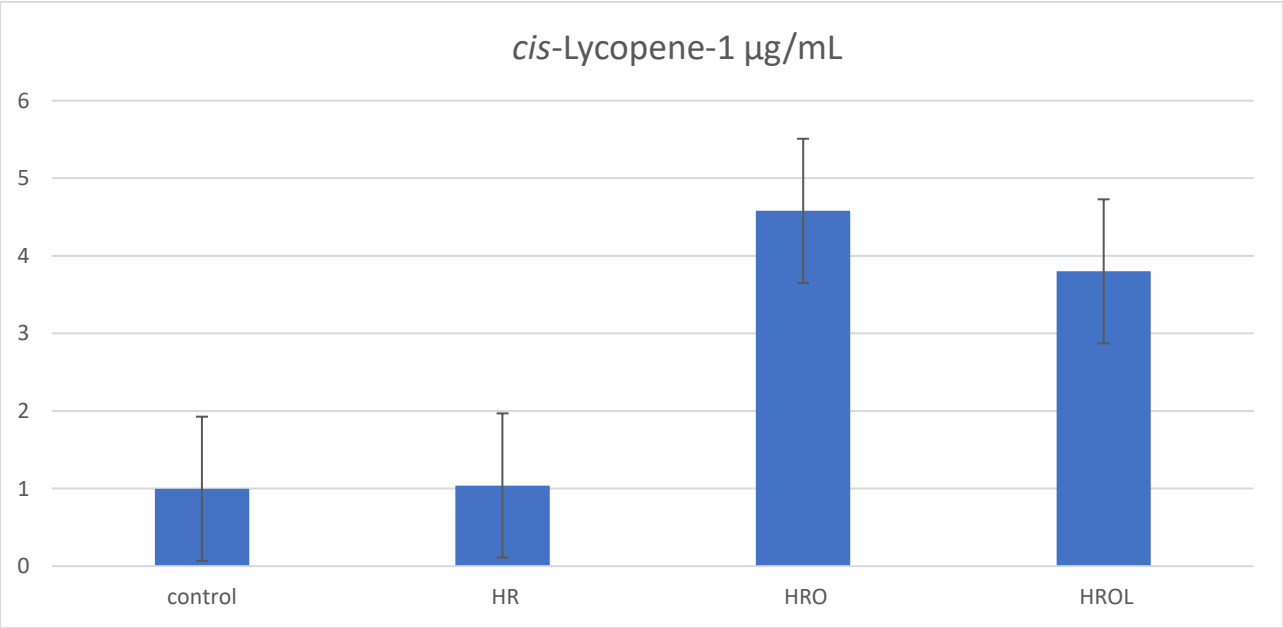

Figure 12. Quantification of *cis*-lycopene-1 detected in processed Roma Tomatoes for unheated control, heated, heated with oil and heated with oil and lecithin. Data show a 350% increase of *cis*-Lycopene-1 in HRO compared to control; Data display a 270% increase of *cis*-Lycopene-1 in HROL compared to control.

Mechanical and thermal processing Roma with oil (HRO) compared to only mechanical processing with oil (URO) displayed no statistical difference in micellarization of total-LYC, *trans*-LYC, *cis*-LYC, *trans*-BC and total-BC. Conversely, HRO sample showed a significant increase in *cis*-BC relative bioaccessibility compared to mechanically processed Roma with oil (URO) (Table 4). These data suggest thermal processing does not significantly influence lycopene micellarization in the presence of oil. Paradoxically, perhaps the addition of 5% sunflower oil (URO) under sheer stress conditions is adequate for the energy transfer required to influence lycopene micellarization and perhaps lycopene isomerization, when compared to

homogenized Roma without oil (UR). Collectively, the data suggest URO samples may contain a higher degree of *cis*-isomer or perhaps experienced a *trans*-lycopene oxidation/isomerization event compared to UR control sample. This idea is supported by an observed increase in lycopene micellarization in URO samples compared to untreated UR control (Table 4).

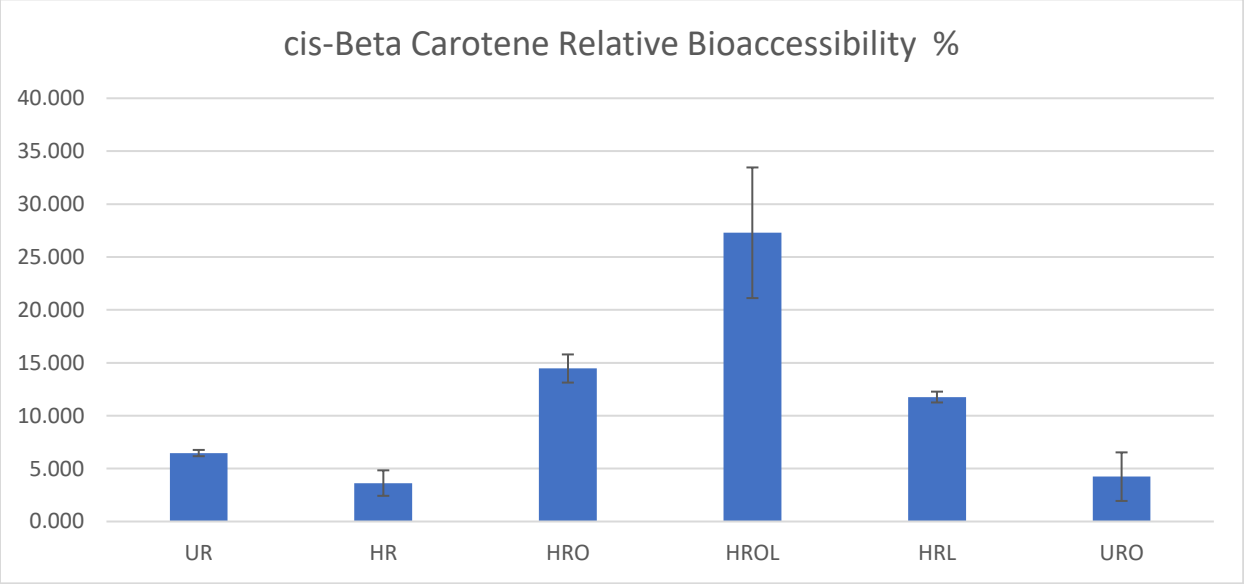

Figure 13. Quantification of *cis*-Beta Carotene isomer micellarized in processed Roma Tomatoes for UR, HR, HRO, HROL. The data shows a 123.5 % increase of *cis*-Beta Carotene relative bioaccessibility in HRO compared to control (unheated) and 241.2 % increase in *cis*-Beta Carotene relative bioaccessibility for HRO treatment compared to URO treatment. HROL processing conditions displayed a 321.8% increase in *cis*-Beta Carotene micellarization compared UR control.

Carotenoid solubilization or crystalline structure destabilization due to the presence of oil or amphipathic surfactant may lower isomerization energy threshold requirements. Isomerization energy is involved with the relocation of the single or double bond of one form of carotenoid into another (Kuki, 1991, ESA, 2009). Data supporting this assertation include URO relative bioaccessibility. According to the data it appears that *cis*-LYC micellization increased without thermal heat energy transfer. If microwave-assisted heating at 90-98 ^{o}C for 5 to 10 minutes is expected to energize or perhaps degrade lycopene, it would be expected for *trans*-lycopene

percentage to decrease. A percent decrease in *trans*-lycopene was observed in all the samples except the unheated Roma (UR) control (Figure 5). However, the untreated Roma with oil (URO) sample decreased *trans*-lycopene content without thermal processing. This would not be explained unless sheer stress accompanied by oil lowered the energy requirement to destabilize *trans*-LYC or energize and/or oxidize into a less stable form; or perhaps a more stable 5-*cis*-LYC isoform (Agarwal et al., 2000). Studies by Agarwal et al., highlight a stability hierarchy for lycopene isomers with *trans*-LYC and 5-*cis*-LYC having similar stability characteristics that may shift their respective ratios based on genotype, environmental conditions, or processing conditions (Agarwal et al., 2000).

In human plasma, lycopene is an isomeric mixture, containing at least 60% of the total lycopene as *cis*-isomers, therefore the phenomenon of lycopene isomerization perhaps influenced by body temperatures (37 ^{o}C), oral mechanical homogenization, acidified stomach peristalsis and digestion, to some degree does exist (Kim, 2012). Previously, an exogenous tomato processing experiment which could be analogous to mammalian food processing expressed an increase in lycopene micellarization after high pressure homogenization and microwave heating tomato pulp with oil at 90 ^{o}C for 2.5-20 min (Colle 2013). Analysis of this study combined with our data presented here using oil as a processing aid suggests that sunflower oil may improve lycopene carotenoid micellarization from shear stress homogenization perhaps without heat application. Conversely, the data suggest microwave heated HRO treatment conditions augmented *cis*-BC micellarization and perhaps BC isomerization compared to URO treatment conditions (Table 4 and Figure 13).

Thermal processing of HRO and HRL resulted in no statistically significant difference in micellarization of *cis*-LYC, *trans*-LYC, total-LYC, and *cis*-BC. Conversely, a statistically

significant increase in *trans*-BC and total-BC relative bioaccessibility was observed with HRL treatment compared to HRO treatment (Table 4 and Figure 14). These data suggest a potential tendency of lecithin as a processing aid to influence *trans*-BC and total-BC micellarization following tested mechanical and microwave-assisted thermal processing conditions compared to utilizing sunflower oil as a processing aid. Thermal processing of tomato homogenate with lecithin (HRL) generated an increase in relative bioaccessibility for *cis*-BC compared to baseline control (UR), however the difference was not significant. Conversely, HRL treatment resulted in 115% increase in *trans*-BC compared to UR control, while 107% increase in total-BC micellarization was observed compared to control UR (Table 4). HRL treatment expressed an increase in *cis*-LYC relative bioaccessibility, but not for *trans*-LYC or total-LYC compared to control (UR).

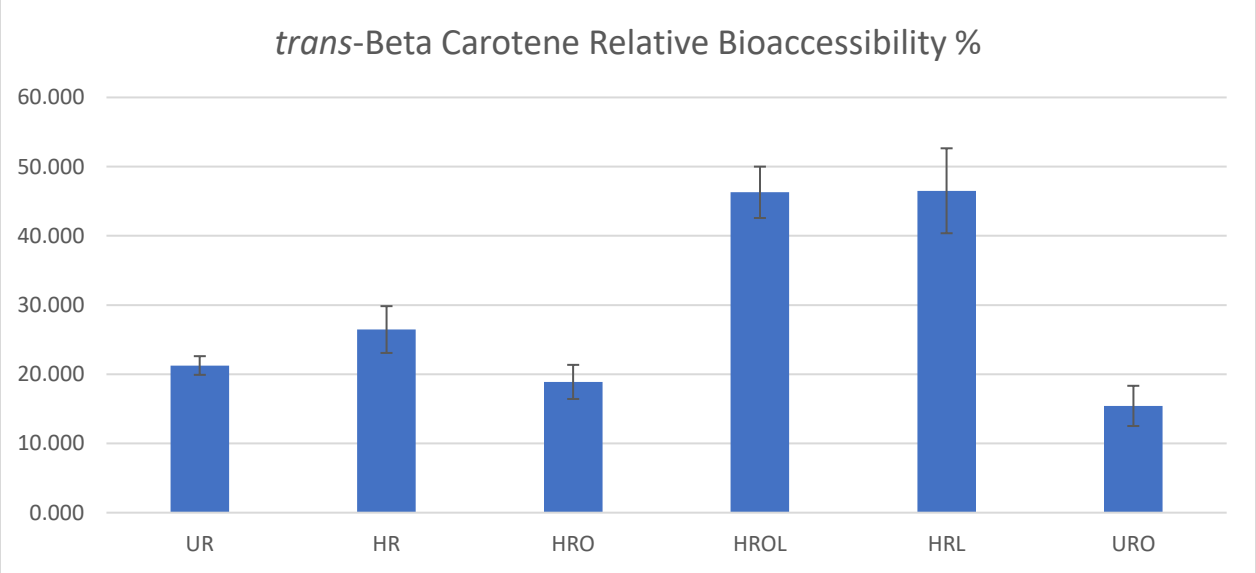

Figure 14. *Trans*-beta carotene increased micellarization in HROL and HRL treatment conditions compared untreated control (UR) in Roma Tomatoes.

Thermal processing of tomato homogenate with lecithin (HRL) generated no significant change for *trans*-LYC and total-LYC micellarization when HRL treatment was compared to HR.

There was, however, a significant increase in relative bioaccessibility for *cis*-BC, *trans*-BC, total-BC and *cis*-LYC compared to heat treated (HR) (Table 4). Studies by Hollander et al reported micellarized BC by intestinal epithelium is affected by factors such as length and saturation of fatty acids, pH, and bile salt composition (Hollander et al., 1978).

It would appear that the addition of sunflower lecithin as a processing aid has more of a consistent impact on increasing *cis*-BC, *trans*-BC and *cis*-LYC micellarization when compared to untreated (UR), thermal treated (HR) and thermal treated with oil (HRO). Comparing HRL to HRO, microwave-assisted thermal processing with sunflower lecithin may have more of an impact on BC micellarization as opposed to LYC. These data correlate with information highlighted by Bengtsson et al., (2009) and Tyssandier et al., (2003) which indicates a more efficient micelle incorporation of isomer 13-*cis*-β-carotene was observed in relation to corresponding *all-trans*-form during digestion. Separate studies by Dhuigue-Mayer et al., reported increased *cis*-BC isomerization and BC bioaccessibility when sweet potato was processed with oil and sterilized for 30 minutes at 100 °C and 120 °C (Dhuique-Mayer, 2018).

Thermal processing tomato homogenate with oil and lecithin (HROL) resulted in significant increases in micellarization for total-LYC, *cis*-LYC, *trans*-LYC and *cis*-BC by 260%, 139%, 287% and 132%, respectively compared to thermal processed tomato homogenate with lecithin (HRL) (Table 4). Conversely, comparing HROL thermal processing conditions to HRL did not significantly change micellarization of *trans*-BC (46.29 – 46.51%) or total-BC (35.63 – 38.03), however when comparing the effects of processing on carotenoids the HRL and HROL test

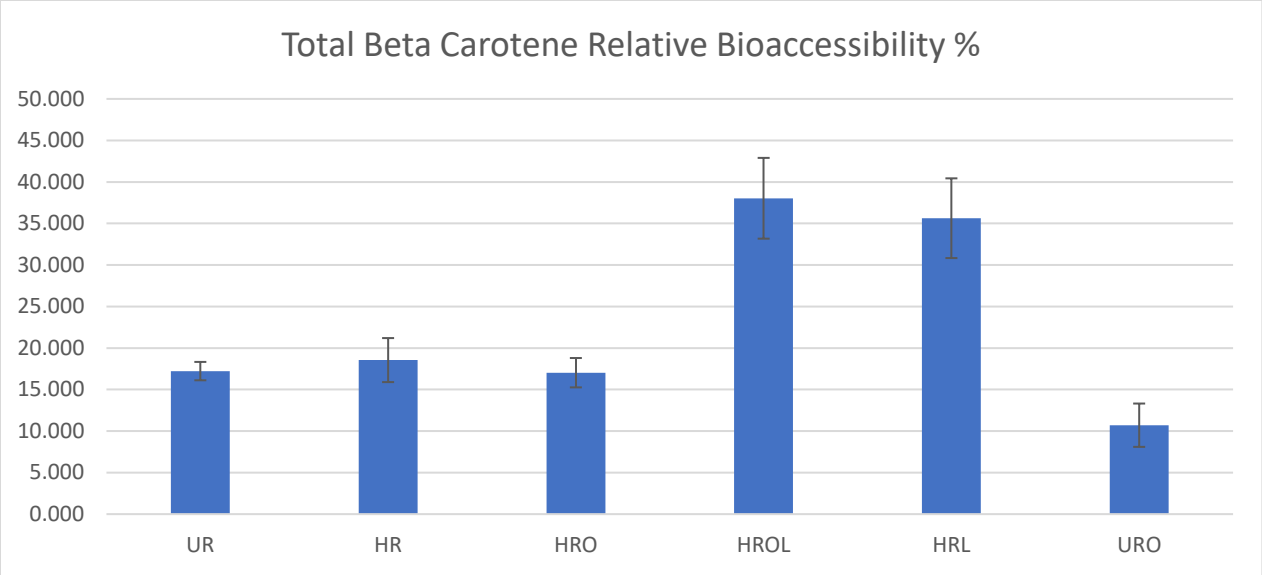

Figure 15. Total Beta Carotene relative bioaccessibility doubled when Roma Tomatoes were processed with HROL or HRL treatments compared to untreated control (UR), HR, HRO and URO treatment conditions.

conditions generated amongst the highest bioaccessibility percentages with *trans*-BC and total-BC of all the carotenoids and isomers evaluated (Table 4). HROL test conditions generated the highest micellarization values for *cis*-LYC (29.58%), *trans*-LYC (20.04%), total-LYC (21.32%), *cis*-BC (27.29%), *trans*-BC (46.29%) and total-BC (38.03%) (Table 4). Compared to heating with oil (HRO), processing tomato homogenate with oil and lecithin (HROL) resulted in significant increases in relative bioaccessibility by 142%, 150%, 141% for total-LYC, *cis*-LYC, *trans*-LYC, respectively (Table 4). HROL treatment produced an increase in micellarization by 123%, 88%, 146% for total-BC, *cis*-BC and *trans*-BC, respectively compared to HRO treatment (Table 4).

Mechanical and thermal processing with oil and lecithin (HROL) resulted in an increase in micellarization by 157%, 201%, 148% for total-LYC, *cis*-LYC, *trans*-LYC, respectively compared to mechanical processing with oil (URO) (Table 4). HROL treatment produced an

increase in micellarization by 2.5-fold (255%), 5.4-fold (545%), 2-fold (200%) for total-BC, *cis*-BC and *trans*-BC, respectively compared to URO treatment (Table 4). Thermal processing of tomato homogenate with oil and lecithin (HROL) produced an increase in total-LYC, *cis*-LYC and *trans*-LYC micellarization by 9.9-fold (987%), 4-fold (420%), 12-fold (1210%), respectively compared to thermal processing without oil and lecithin (HR) (Table 4). HROL treatment also increased total-BC, *cis*-BC and *trans*-BC micellarization by 1-fold (105%), 6.5-fold (651%), and 75%, respectively compared to HR (Table 4). Comparing untreated control UR to HROL treatment, relative bioaccessibility for *cis*-BC, *trans*-BC and total-BC increased by 322%, 114% and 121%, respectively. Micellarization for total-LYC, *cis*-LYC, *trans*-LYC rose by 8-fold (805%), 4-fold (413%), 9-fold (918%), respectively in HROL compared to UR. Similarly, Cooperstone et al., reported an 8-fold increase in lycopene micellarization was observed with tetra-*cis*-LYC expressing orange colored tangerine tomato variety compared to red Roma tomato variety (Cooperstone 2016, Cooperstone 2015). It is interesting to note visual inspection resulting from HR, HRO and HROL processing conditions indicate a chemical change may have occurred resulting in a shift in light absorption for HRO and HROL treated samples that share a light absorption similarity with homogenized orange fleshed tangerine tomato containing predominantly tetra-*cis*-lycopene isomers (Figure 16). Additionally, studies by Burri and Ishida (2009) sponsored by Agricultural Research Services showed the tangerine tomato's tetra-*cis*-lycopene is more efficiently absorbed by human bodies than is the *trans*-lycopene of red tomatoes (Burri, 2009; Cooperstone, 2016).

Figure 16. Roma tomato color changes due to different carotenoid processing conditions. Samples include Heated Roma = HR (A3), Heated Roma w/Oil = HRO (B4), and Heated Roma w/Oil + Lecithin = HORL (D1) treatments in preparation for storage before in vitro digestion.

Data from HROL treatments suggest the processing methods used may improve lycopene micellarization for Roma tomatoes to a level of absorption similar to tangerine tomatoes containing an abundance of tetra-*cis*-lycopene isomers. The presence of sunflower oil and sunflower lecithin under this processing treatment appears to compound the improvements in micellarization observed when processing with oil or lecithin separately (Figures 13 and 17). Significant improvements in micellarization of both *trans*-Lycopene and *trans*-BC when oil and lecithin were used as processing aids (Figures 14 and 26). This was unexpected, as we hypothesized to observe increased micellarization only with *cis* isomers. This treatment process using oil or lecithin or both as processing aids may also induce *cis*-isomerization leading to a significant impact on improving carotenoid micellarization (Figures 10, 11, 12, 13, 17, 19).

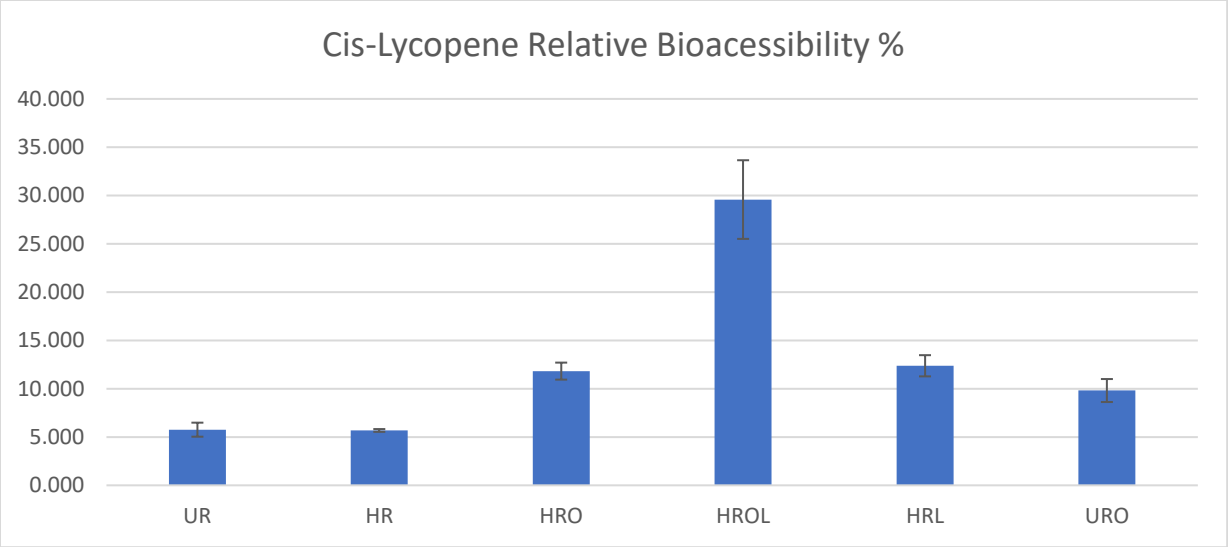

Figure 17. Quantification of Relative Bioaccessibility, an indicator of micellarization, expressed as a percentage of lycopene isomer detected in aqueous phase compared to digesta phase (aqueous/digesta) calculated in mechanical processed Roma Tomatoes for unheated control, heated, heated w/oil, heated w/oil+lecithin. Relative bioaccessibility of *cis*-lycopene increased by 25% in HR compared to control (UR). *Cis*-Lycopene relative bioaccessibility increased in HRO by 100% compared to control. *Cis*-Lycopene in HROL increased by 413.4% compared to control (UR).

Previous studies by other labs indicate processing tomato material with certain levels of intensity promote *trans*-LYC conversion to *cis*-LYC, which influenced increases in lycopene absorption by 2 to 3-fold (Gartner,1997; Stahl, 1992; Rao, 2002).

Conversely, studies by Nguyen et al., reported trans-lycopene isomer was relatively stable to isomerization at temperatures between 50 and 100 ^{o}C (Nguyen et al., 1999). Additional studies by Nguyen and Schwartz (1998) found lycopene to be relatively heat resistant with less than 10% *cis* isomer in the processed tomato products analyzed. It was noted, however that thermal processing influenced isomerization of lutein and beta-carotene, but not lycopene (Nguyen, 2001). Similarly, data generated from this study showed microwave-assisted thermal processing with processing aids influenced isomerization of lutein and beta-carotene.

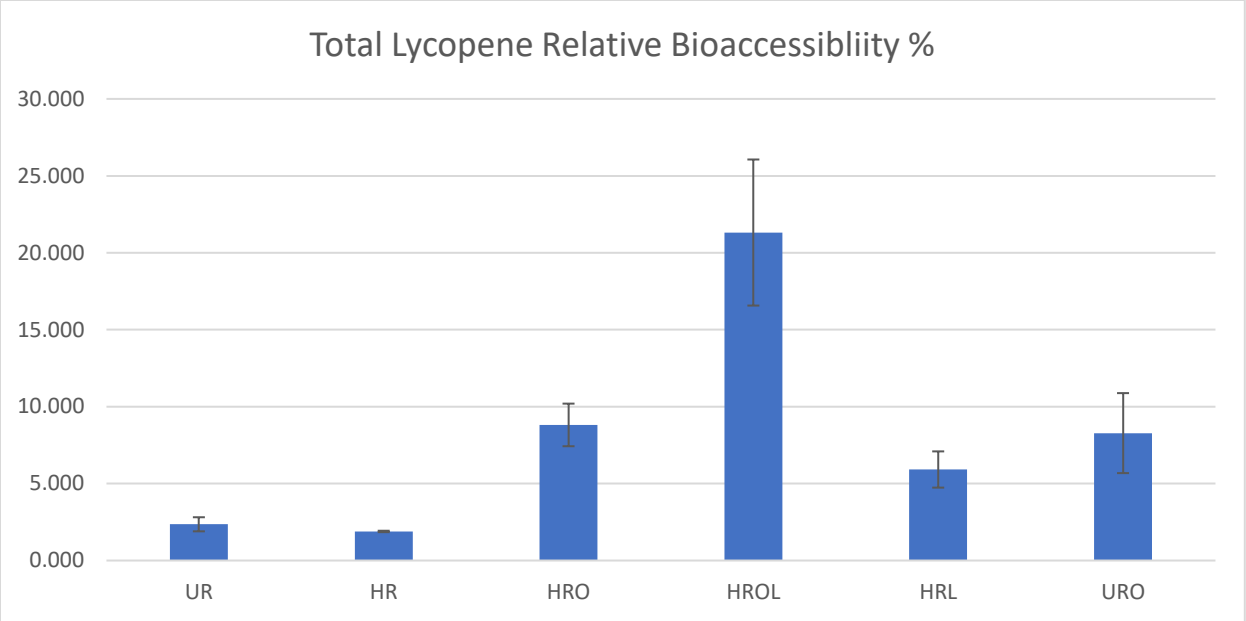

Figure 18. Total-Lycopene relative bioaccessibility remained constant in HR, compared to control (UR) but increased by 315.5% in HRO compared to control (UR), increased by 905.6% in HROL compared to control (UR). Relative bioaccessibility is a measure of the proportion of carotenoids released from the tomato matrix and transferred into bile salt− lipid micelles during digestion and therefore is made available for intestinal uptake (Ellison, 2017).

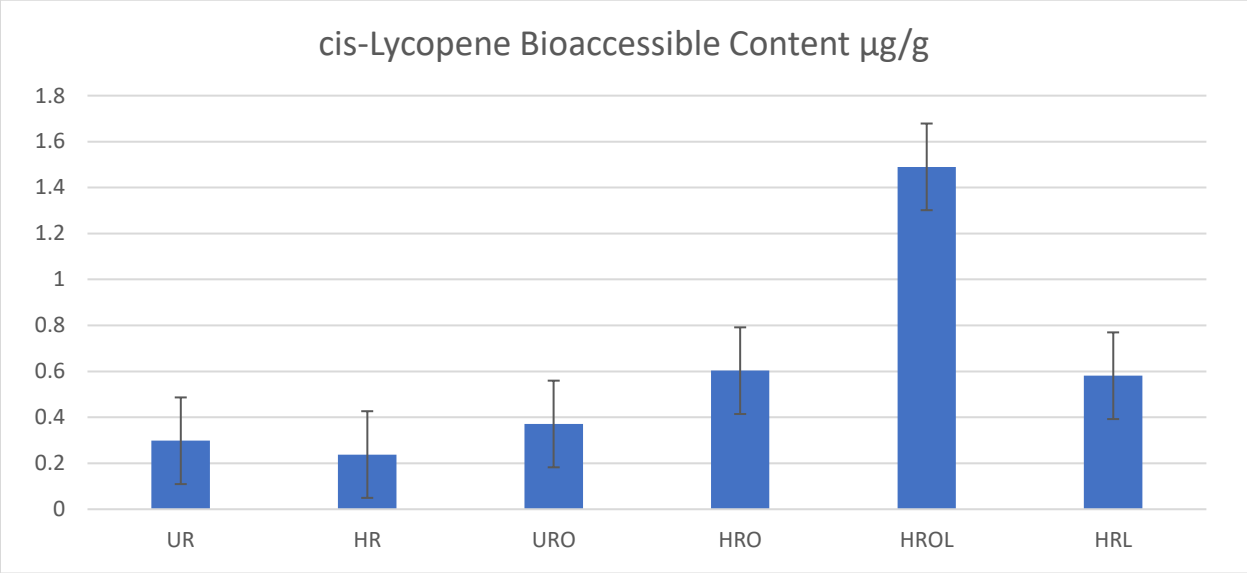

Figure 19. Quantification of *cis*-Lycopene Absolute Bioaccessibility identified in processed Roma Tomatoes for unheated control, heated, heated with oil, heated with oil/lecithin. Data show absolute bioaccessibility of total *cis*-Lycopene increase 200% in HRO compared to control; Data displays a 650% increase in total *cis*-Lycopene absolute bioaccessibility in HROL (Heated Roma Oil/Lecithin) compared to control UR.

Contrary to data reported by Nguyen and Schwartz concerning lycopene isomerization, our data expressed a destabilization suggestive decrease in *trans*-lycopene content (Figure 5), while highlighting a simultaneous rise in *cis*-lycopene isomer percentage ranging ultimately from 7-13% in HRO, HROL and HRL treated samples compared to 6% *cis*-isomer percentage observed in UR control (Figure 1). Additionally, when *cis*-LYC isomers were qualified and quantified following treatments during this study by an independent lab, Eurofins-Craft Technologies, the data showed a consistent *cis*-isomer elevation in treated samples with processing aids compared to untreated controls (Figures 10, 11, 12). Lecithin alone as a processing aid within this mechanical and microwave-assisted thermal processing system appears to favor improving BC micellarization and perhaps BC isomerization (Figures 2 and 13). This observed increase to BC micellarization is consistent with results reported by Dhuique-Mayer et. al, which showed an increase in BC isomerization and bioaccessibility when processing aids oil and lecithin were utilized together followed by thermal processing of sweet potato ingredient material (Dhuique-Mayer, 2018).

Conclusion

The effects of processing on carotenoid bioaccessibility have been identified from the studies within this project. Mechanical and microwave-assisted thermal processing with oil or lecithin increase carotenoid micellarization by 1 to 3-fold. Mechanical and microwave-assisted thermal processing with oil and lecithin increase carotenoid micellarization by 4 to12-fold compared to untreated (UR) or thermal treated (HR). Microwave heating with the combination of sunflower oil and sunflower lecithin used as processing aids have a compounded improvement effect on lycopene and beta-carotene micellarization when compared to processing with either oil (HRO) or lecithin (HRL). The processing conditions utilized in the present study may mimic

optimal physiological conditions for maximum micellarization and carotenoid absorption by the human body. Efficient micellarization is critical to maximizing carotenoid absorption. Carotenoid uptake follows the same digestive route as lipids, and they need to be incorporated into micelles to be absorbable (Bore, 2003). Mixed micelles contain free fatty acids and monoglycerides resulting from triglyceride hydrolysis, bile salts, biliary phospholipids, and carotenoids (Yonekura, 2007).

According to the data presented, the utilization of oil and lecithin as processing aids combined with microwave sterilization conditions created a highly efficient and ordered species of carotenoid/oil substrates/lecithin micelle that may be described as microemulsions or nanoemulsions. Microemulsions, which are transparent and thermodynamically stable, possessing droplet size smaller than 100 nm, are sometimes called "nanoemulsions" (Huang, 2010). The major difference is that microemulsion is thermodynamically stable whereas nanoemulsion is not. However, many nanoemulsion systems possess high kinetic stability because of their characteristic size (Huang, 2010). The microemulsion can be transported through cell membrane more readily to increase concentration of bioactive compounds in plasma and thereby enhance bioavailability (Huang, 2010). Unlike nanoemulsions, micelles can be formed spontaneously by amphiphiles possessing both hydrophilic and hydrophobic groups when the critical micelle concentration (CMC) is exceeded (Figure 20). Then the hydrophilic group forms the shell, and the hydrophobic group forms the core, where lipophilic compounds like lycopene can be trapped (Huang, 2010). The combined use of oil and lecithin may facilitate such ideal and ordered micellarization conditions for carotenoids processed using this protocol (Figure 20). It is also possible that processing conditions result in the creation of a larger liposome type molecule that incorporates *trans*-LYC, *trans*-BC, phosphatidylcholine, *cis*-

isomers, degraded triglycerides, and bile salts resulting in high micellarization efficiency (Figure 20).

Lecithin is a naturally occurring mixture of phosphatidylcholine and other phospholipids with diverse fatty acid side chains such as stearic, oleic and palmitic acids. Data from our studies (not shown) indicate the presence of lecithin consistently lowered lutein bioaccessibility, suggesting phosphatidylcholine may have displaced LUT within micelles following *in vitro* digestion (including lipase and bile salt exposure) in treatment samples compared to control. This is plausible since lutein is a Xanthophyll that is a characteristically polar carotenoid, and therefore more hydrophilic compared to LYC or BC. Polar carotenoids tend to be distributed at the surface of the oil droplets, whereas nonpolar carotenoids (e.g., BC) are in the core (Borel, 1996). During the small intestinal phase of digestion, lipases degrade oil droplets to much smaller particles in the presence of bile salts (El-Gorab, 1975).

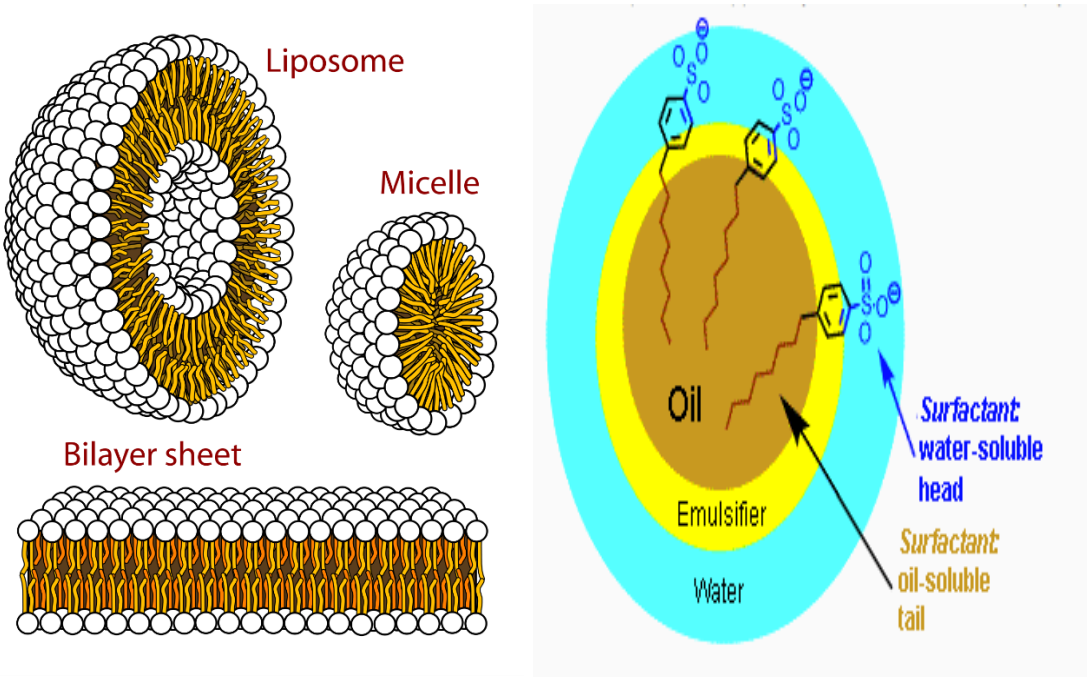

Figure 20. Cross Section view of the structures that can be formed by phospholipids in aqueous solutions (Huang, 2010).

. Perhaps lecithin derived phosphatidylcholine displaced LUT on oil droplets and provided amphipathic properties to the hydrophobic core constituents including *cis*- and *trans* isomers prior to or after bile salt incorporation, that leads to enhanced LYC and BC micellarization as observed in HROL treated samples compared to UR control (Figures 13, 15, 17, 19, 21). Phosphatidylcholine is a significant constituent of nerve and brain tissue and is the predominant phospholipid in most mammalian membranes. It is involved with a myriad of essential metabolic reactions including providing structural stability to cell membranes and constituents of lipoproteins (Schneider, 2001). Lecithin is also widely used in the food industry as an emulsifier, lubricant, viscosity reducer and as an anti-spattering, wetting and release agent (Rossi, 2007).

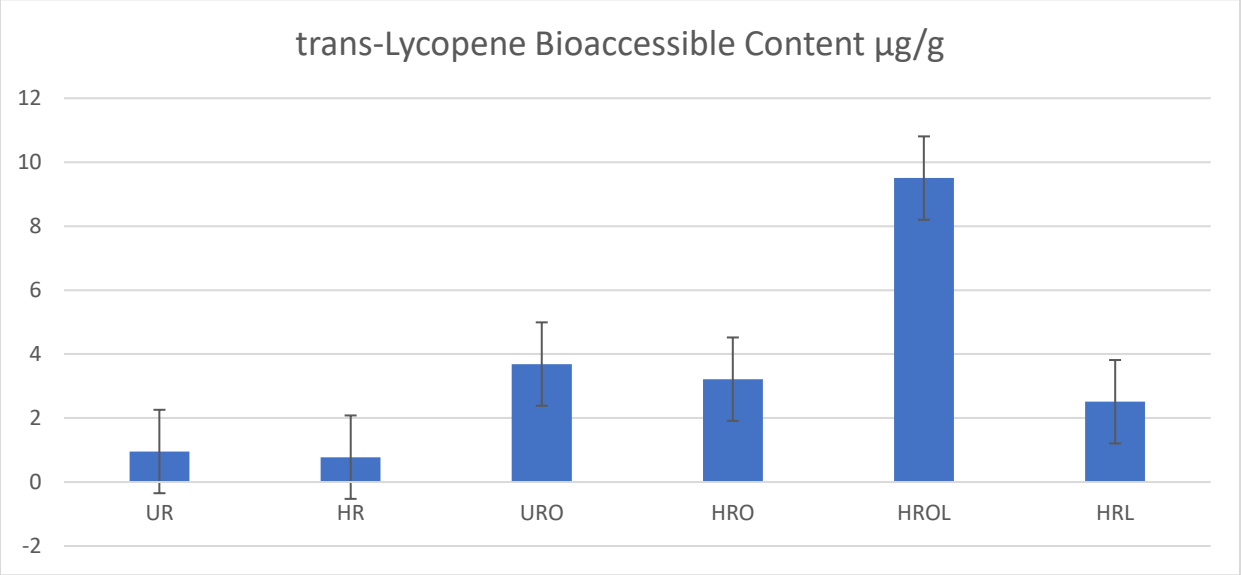

Figure 21. Quantification of *trans*-Lycopene Absolute Bioaccessibility identified in processed Roma Tomatoes for unheated control, heated, heated w/oil, heated w/oil+lecithin. Data shows absolute bioaccessibility of *trans*-Lycopene decreased 20% in HR compared to control, *Trans*-Lycopene absolute bioaccessibility increased 220% in HRO compared to control, and increased 850% in HROL compared to control.

Unexpectedly, *trans*-LYC micellarization increased 9-fold in HROL compared to unheated Roma control (UR) and 12-fold compared to heated Roma control (HR). These data

also suggest a significant role that microwave-assisted thermal processing with processing aids had on improving micellarization of both *cis* and *trans* carotenoid isomers (Figures 17, 18, 19, 21). The proposed microemulsion/nanoemulsion micelle hypothesis, in terms of forming highly efficient micelles, may explain this observation. Additionally, a physical change in light absorption was observed throughout tomato homogenate of processed material, compared to control material, which suggests a carotenoid restructuring or isomerization event occurred (Figure 16). The observed change in color following processing of Roma tomatoes is positively correlated with increases in overall LYC and BC micellarization (Figures 15 & 18). Direct comparisons between tangerine tomato material and HROL and HRO processed material is reported in the next chapter. Follow-up studies will be required to gather more detail on the predominant mechanism of isomerization from a thermal application standpoint (volumetric or convection).

Aspects of data reported through current studies suggest microwave-assisted thermal processing does not have a significant impact on carotenoid micellarization and perhaps isomerization when oil is not used as a processing aid. Micelle analysis will be necessary to determine nano-scale characteristics, micelle size and size distribution. Finally, a lycopene solubilization and/or energy transfer event mechanism is proposed involving utilizing sunflower oil, processing aid and shear stress to affect *trans*-LYC/5-*cis*-LYC stabilization, leading to perhaps improved carotenoid micellarization under non-thermal processing conditions compared to untreated control (UR). Micellarization characterization follow-up studies may shed more light on precise mechanism(s) the profound effects mechanical shear stress homogenization with processing aids oil/lecithin, oil, or lecithin and microwave-assisted heating have on improving relative bioaccessibility of carotenoids in tomatoes. The data also indicates carotenoid

isomerization takes place using conditions mentioned above involving the use of processing aids at practical concentrations. Although *in vitro* digestion models have been shown to be a valuable tool to predict bioavailability of phytochemicals in food, further (in vivo) bioavailability studies are needed to confirm the findings of the current study. The results of this study can be used as a guide for future *in vivo* experiments.

From an industrial standpoint, if the current studies on determining the effects of processing on bioaccessibility can be confirmed by *in-vivo* bioavailability studies, this series of processes may be used as a substitute to the present-day tomato processing industry standard cold-break process that has shown to produce less than desirable product ingredients due to the loss in viscosity from fiber degradation (Page, 2012). The loss of viscosity from cold-break tomato processing is less desirable because it is not conducive to standardized ingredient properties necessary to achieve adequate consistency and mouth feel of high-volume commercial tomato ketchup, pizza sauce and BBQ sauce end products. The further development and scaling of processes derived from this study may lead to novel condiment end products that can serve as healthier alternatives to ketchup, BBQ sauce and pizza sauce. Further research leading to perhaps commercialization of efficiently micellarized carotenoids following digestion may bring to fruition an abundance of healthier ingredients, alternative condiments, and value-added carotenoid-based shelf stable foods.

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Chapter 4

Processing Effects on Lycopene Bioaccessibility and Quality: Roma and Tangerine Tomatoes

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ABSTRACT

Tomato, being one of the most nutrient dense and popular industrially processed fruits, is a rich source of health promoting antioxidants. Variation in the bioaccessible content of these bioactives regarding novel industrial processing conditions and genotype was explored for Roma and Tangerine tomato cultivars. Tomatoes were purchased, washed, homogenized (90 sec) and sterilized using microwave-assisted thermal processing (92 ^{o}C-98 ^{o} C, 5-10 min). Carotenoid bioaccessibility was determined using a high-throughput, three-phase in vitro digestion model. Transfer of *trans*-lycopene and *cis*-lycopene isomers from tomato to micellar fractions were quantified by HPLC to determine bioaccessibility. ANOVA analysis determined content, relative bioaccessibility, and bioaccessible content for lycopene isomers to be significantly different across treatment conditions in Roma genotype compared to control. Tangerine cultivar displayed higher lycopene and relative bioaccessible and absolute bioaccessible content for lycopene isomers compared to Roma, however there was no significant difference in content, relative bioaccessibility and bioaccessible content for Tangerine *cis*-lycopene isomers when comparing microwave-assisted thermal treatment conditions to untreated control. Absolute bioaccessible content ranged from 0.065-0.183, 0.253-1.13, 5.71-6.29, and 0.101-0.188 µg/g for Roma *cis*-lycopene, Roma *trans*-lycopene, Tangerine *cis*-lycopene, Tangerine *trans*-lycopene, respectively. Results suggest that lycopene bioaccessibility may vary based on processing conditions and cultivar genotype.

Background

Producing enough food to feed more than 9.7 billion people, the projected global population by 2050, will be a challenge, particularly given the increasingly restrictive circumstances due to climate change, climate variability, pandemics, and other shocks that put food production at risk. In the face of these challenges, certain crops are vital to global food security. In developed countries such as the United State of America, food security is less of a threat to maintain an overall healthy population compared to malnutrition related to food quality choices common to the domestic Western Diet. The "Western Diet" has been associated with a global rise in metabolic disorders such as cardiovascular disease (CVD), obesity, type II diabetes mellitus, metabolic syndrome, nonalcoholic fatty liver disease (NAFLD), heart disease, and stroke. Interactions between genetic and environmental factors such as diet and lifestyle, particularly over-nutrition and sedentary behavior, promote the progression and pathogenesis of these polygenic diet-related diseases. Their current prevalence is increasing dramatically to epidemic proportions. Nutrition is probably the most important environmental factor that modulates expression of genes involved in metabolic pathways and the variety of phenotypes associated with CVD, obesity, the metabolic syndrome, and type II diabetes mellitus. Furthermore, the health effects of nutrients may be modulated by genetic variants.

Public health campaigns such as the Dietary Guidelines for Americans (USDA-CNPP, 2020) have recommended the general public consume more fruits and vegetables. However, this has not done enough to improve the overall health status of the American general population as metabolic disease rates continue to rise. From a day-to-day practical standpoint, many people do not have time or resources to prepare foods from fresh ingredients every meal, therefore reliance on the convenience of processed foods that are relatively quick and easy to prepare is a reality for many households in the US. Although many of the processed foods currently available are unhealthy

when overconsumed, it is possible to create healthier processed foods today with novel science and technology applications. Exploring innovations in food ingredient processing to improve the nutrient profile of food products provides an opportunity to improve public health. Acceptance of new ingredients will be a key to ensuring a healthier and more sustainable future for the food supply chain as demand for food grows alongside rises in global population.

Introduction

Tomatoes are the most commonly consumed processed vegetable or fruit in the American diet (Canene-Adams, 2004). Tomatoes account for 22% of the vegetable consumption in the United States and over 75% of the tomatoes consumed are in a processed form such as canned, salsa, juice, or ketchup (Burton-Freeman, 2010). The per capita consumption of processing tomatoes in the U.S. is over 73 lb. (farm weight equivalent) and annual processing capacity is between 11 million and 11.5 million tons per season spread across roughly 300,000-400,000 acres of farmland (Carter, 2008).

Lycopene, which is the main carotenoid in tomatoes, is believed to be responsible for some of the positive health effects associated with increased tomato intake. Studies show increased circulation of lycopene improves vascular function and lowers risk factors such as C-reactive protein associated with obesity and cardiovascular disease (Kim, 2011). Consumption of a diet rich in tomatoes has been associated with decreased risk for a variety of chronic diseases, including heart disease and cancer (Giovannucci, 1995; Zu, 2014).

The predominate consumer product application for processed tomato ingredients are soups, sauces, condiments, or flavor enhancement end-products. Domestic consumers purchase 90% or more of the U.S. processing tomato production (Guan, 2017). Recent advances in processing

technologies allows for an improvement in tomato ingredient quality, which may lead to improved functional benefits of processed tomatoes possibly resulting in the establishment of functional tomato foods. Microwave-assisted thermal processing of homogenized fruits and vegetables result in sterile finished food product that retains its taste and nutrition due to the efficiency of volumetric heating. In terms of energy transfer efficiency, close to 80% of electric energy becomes microwave energy and about 95% of microwave energy becomes thermal energy, therefore overall microwave-assisted heating is roughly 78% efficient (Ekezie, 2017; Ishii, 1995). This relatively gentle sterility processing method allows for end products to retain vitamins and antioxidants that can be destroyed by other processing methods such as convection heating powered by steam, which is about 30% efficient (Ekezie, 2017; Ishii, 1995).

Current standardized Hot Break tomato processing involves treating tomato material with methane gas powered steam tubular heaters, which facilitates an elaborate and energy inefficient water evaporation process, to produce a semi-dehydrated 25-30^{o} Brix tomato paste using massive evaporation towers. Takeoka and Ebeler report a 9-28% yield loss in lycopene content from industrial processing of tomatoes into paste (Takeoka, 2001). Tomato paste, the preferred finished ingredient product for consumer-packaged goods (CPG) manufacturers, is produced by the Hot Break method. Hot Break tomato processing utilizes convection heating methods involving evaporation towers that were developed in the 1950s by UC California extension services and corporations with vested interest in the success of the tomato processing industry such as Heinz Ketchup (Hartz, 2008). Since the tomato harvesting and processing methods were developed and standardized between the 1940s and1960s, the industry has become stale in terms of innovation following the exponential growth of large-scale tomato processing facilities in California during the 1970s and 80s (Carter, 2008). Industrially produced tomato pastes and subsequent value-added

end products produced from tomato paste such as standardized tomato ketchup are not considered healthy to the American public or marketed as functional ingredients/foods. Functional foods are developed specifically to promote health or reduce the risk of diseases (Hasler, 2004). They usually look, smell and taste the same as their regular counterparts that can make a positive contribution to health, wellbeing and nutrient quality awareness.

Novel volumetric heat processing technologies originally commercialized for the sweet potato industry in North Carolina enable highly efficient thermal energy transfer which ensures adequate sterility and preservation of homogenized ingredients such as sweet potato, pumpkin, squash, and beets. Volumetric processing in the form of microwave-assisted heating also possesses the potential to improve tomato fruit homogenate material quality through maximizing carotenoid isomerization reactivity during clean and industrial chemical-free tomato sterilization processing. Therefore, investigating the relationship between translatable, potentially industrial, and perhaps scalable tomato processing involving microwave-assisted heating and its impact on absorption potential or micellarization of lycopene is the purpose for this research study. Nguyen and Schwartz suggested in tomato products produced using current industry methods, lycopene was relatively resistant to degradation, including thermally induced *trans-cis* isomerization reactions (Nguyen, 1998; Nguyen, 1999). In raw red tomatoes, approximately 95% of the lycopene present is in the all-*trans* form (Stahl, 1992). However, it is generally accepted that all-*trans*-lycopene is converted to *cis* isomers in *vivo;* and *cis*-isomers account for 58-73% and 79-88% of total lycopene in serum and prostate tissue, respectively (Porrini, 1998; Clinton, 1996; Stahl, 1992). This observation suggests lycopene isomerization not only occurs but can be maximized within the mammalian biological system. Unfortunately, the mechanism(s) involved with this phenomenon are not fully understood. Conflicting reports about bioprocessing induced lycopene isomerization

have been published. Schwartz et al. report commercial processing caused isomerization of lutein and beta-carotene but not lycopene (Nguyen, 2001). Recent studies by our lab have shown promise to a possibility of utilizing microwave-assisted heating to influence *in vitro* lycopene isomerization. Due to the high consumption rate of processed tomatoes in the United States along with the health benefits associated with increased lycopene vascular circulation, potential industrial applications engendered by this work may add value to processing tomato ingredients and manufactured tomato-based consumer packaged goods. In the interest of exploring the prospects of translational research from novel volumetric thermal processing to establish new tomato ingredients with functional benefits, this study will analyze and compare the effects of clean and efficient processing using microwave-assisted heating on the bioaccessibility of lycopene in fresh Tangerine and red Roma tomatoes. Our objective was to compare and contrast processing methods for Roma tomatoes and Tangerine tomatoes and measure isomerization, bioaccessibility and color effects. The outcome of this study could potentially be used to provide an alternative method for industrial tomato processing. The study may identify or provide more insight on the relationship between *in-vitro* microwave thermal treatment and increases in *cis*-lycopene isomer bioaccessibility observed from studies reported in Chapter 3.

General Hypothesis

Post-harvest processing of tomatoes using traditional mechanical homogenization and microwave assisted heating will add value to homogenized Roma tomato ingredient material by increasing lycopene isomerization and bioaccessibility.

Specific Aim 1: Determine if microwave processing increases lycopene bioaccessibility in tomato.

Specific Aim 2: Determine if microwave processing tomatoes with processing aids increases lycopene bioaccessibility.

Specific Aim 3: Determine if microwave processing increases lycopene isomerization in tomato.

Specific Aim 4: Determine if microwave processing tomatoes with processing aids will influence lycopene isomerization.

Materials and Methods

Research methods used to test the hypothesis include carotenoid extraction, carotenoid HPLC analysis, carotenoid in-vitro digestibility assay and colorimeter analysis.

Experimental Design

The experimental design was a randomized complete block design for this translational research project with treatment variables that most represent processing conditions applicable to industrial thermal processing used by the tomato processing industry today (i.e., cold break method). This experiment was replicated 3 different times using tomatoes purchased from different grocery retail locations from 2 different states in Southeast regional grocery stores or local farmers' market.

LOT	Retail	Process Date	Cultiv	Treatmt	Treatmt	Treatmt	Treatmt	Cultiv	Treatmt
1	Raleigh	10-5-20	UR	HR	HRO	HROL	HRL	UT	HT
2	Atlanta	10-6-20	UR	HR	HRO	HROL	HRL	UT	HT
3	Durham	10-7-20	UR	HR	HRO	HROL	HRL	UT	HT

Table 1. Randomized complete block experimental design table. UR= Unheated Roma Tomato, HR = Microwave Heated Homogenized Roma Tomato, HRO = Microwave Heated Homogenized Roma Tomato with Oil, HROL = Microwave Heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave Heated Homogenized Roma Tomato with Lecithin, UT = Unheated Tangerine Tomato, HT = Microwaved Heated Tangerine Tomato.

Chemicals. Authentic standards for lutein, β-carotene, trans-β-apo-8′-carotenal were obtained from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA) and used for high-performance

liquid chromatography (HPLC) calibration, identification, and quantitation. *Cis*-lycopene and trans-lycopene isomer standards were provided by Dr. Neal Craft (Eurofins-Craft Technologies., Wilson, NC, USA). All solvents, including acetone, petroleum ether, methyl tert-butyl ether (MTBE), methanol, and ethyl acetate were of certified ACS and HPLC grade (Fisher Scientific, Fairlawn, NJ, USA). Butylated-hydroxytoluene (BHT), ammonium acetate, α-amylase (A3176, 10 units/mg solid), mucin (M2378), porcine pepsin (P7125), lipase (L3126), pancreatin (P7545), and bile (B8631) enzymes were sourced from Sigma-Aldrich and used for in vitro digestion. Commercial sunflower oil and sunflower lecithin were purchased from a local grocery retailer.

Tomatoes

Tomato Fruit. Roma and Tangerine tomatoes were purchased in the month of October 2020 at the local South Region farmers market (Decatur, GA, USA). Roma tomatoes were purchased at the same time of year from a South Region Food Lion Grocery Store (Durham, NC) and Whole Foods Market Grocery Store (Raleigh, NC, USA).

Tomato Processing. After tomatoes were purchased, they were transported to the laboratory and stored under refrigerated conditions. The following day, each biological replicate of tomato was weighed, washed with deionized (DI) water, dried and held at room temperature. Samples were then homogenized with a food processor, 10032-766 (Radnor, PA, USA) for 90 seconds on high until pureed at room temperature. Aliquots were transferred to stainless steel mixing bowls and held at room temperature for 1 hr. Processing aids sunflower oil (2-5% w / w), sunflower lecithin (2% w / w) or sunflower oil and sunflower lecithin (7 % w / w) were randomly added to treatment aliquots and homogenized for 120 seconds. Thermal treatment samples consisting of 40 g were heated thereafter with MARS 6 microwave digester (CEM Corp., Matthews, NC) for 5-10 minutes at 90-99˚C. Treatment aliquots were transferred to screw cap 50 mL Falcon polypropylene tubes

(Falcon Plastics, Los Angeles, CA, USA), wrapped in foil and flushed with nitrogen gas and kept frozen at -80˚C until further analysis. Control and heated samples were digested, extracted and analyzed using HPLC for qualitative and quantitative levels of carotenoid.

Carotenoid Bioaccessibility *In Vitro* Digestion

High-Throughput in Vitro Digestion. A three-phase *in vitro* digestion simulating oral, gastric, and intestinal digestion followed a similar procedure used by Garrett et al., was performed with modifications to access the bioaccessibility of lycopene and associated isomers from mechanical processed/thermal unprocessed and mechanical processed/thermal processed tomato food matrices (Garrett et al., 1999). Screening carotenoid bioaccessibility of Roma Tomatoes required a high-throughput *in vitro* digestion model. To achieve this, a static, three-phase *in vitro* digestion was proportionally scaled down from a 50-mL to a 15-mL digestion for use with the Tecan Freedom EVO liquid handling robot (Tecan Group Ltd., Männedorf, Switzerland), which facilitated all of the liquid handling portions of the protocol (Kean, 2011). Homogenized unheated Roma (UR), homogenized heated Roma (HR), homogenized heated Roma with oil (HRO), homogenized heated Roma with oil and lecithin (HROL), and homogenized heated Roma with lecithin (HRL) were subjected to digestion with n = 4 replicates (by completing 4 sets of digestions and analyses, each including one of each homogenate condition). Homogenized treated and untreated samples were thawed, and 0.5 g samples were weighed. Briefly, 0.5 g of homogenized tomato was aliquoted into 15-mL tubes with 5% (w/w) canola oil to facilitate micellarization of carotenoids as bioaccessibility of carotenoids is limited in the absence of oil (Goltz, 2013). A control of carotenoid-rich spinach salad with 5% w/w canola oil, was simultaneously digested during each set to confirm repeatability between assays (Huo, 2007). To each sample, 1.8 mL of the oral phase solution was added along with α-amylase (10 units/mg) (Kean, 2011). Each sample was capped

under nitrogen, vortexed, and incubated for 10 min at 37 °C, 120 rpm. Following the oral phase, a gastric phase was simulated with the addition of 0.6 mL of pepsin (final concentration of 0.4 g/L) and adjusted to pH 2.5 ± 0.1 with 1 M HCl. The reaction volume was adjusted to 9 mL with a saline solution (0.9% NaCl), blanketed with nitrogen gas, capped, and then incubated for 60 min at 37 °C, 120 rpm. Following the gastric phase, an intestinal phase was simulated by adjusting the pH to 5 ± 0.1 with 1 M $NaHCO_3$, and then, 0.6 mL of pancreatin-lipase solution (final concentration of 0.8 g/L for both pancreatin and lipase) and 0.9 mL of bile solution (final concentration of 1.8 g/L) were added. The solution was then adjusted to pH 7.0 ± 0.1 with 1 M $NaHCO_3$, blanketed with nitrogen gas, capped, and incubated for 120 min at 37 °C, 120 rpm. Following the intestinal phase, an aliquot was transferred into a separate tube for storage at −80 °C to assess digestive stability. The remaining digesta was centrifuged (Beckman Coulter, Allegra X-30R Centrifuge, Indianapolis, IN, USA) at 4255 x g for 60 min after which the aqueous fraction was collected and filtered through a 0.2-μm cellulose acetate filter to isolate the aqueous fraction containing the micellarized carotenoid compounds of interest.

Extraction of Carotenoids from Tomato Materials and Digestive Fractions. Carotenoid contents in homogenized tomato samples were quantified in 50-mg aliquots using a previously reported method (Lipkie, 2013). Briefly, all samples were extracted under a yellow light to prevent carotenoid degradation. Initially, 1 mL of DI water was added to expand the tomato matrix and disperse the internal standard. Samples were placed on ice for 10 min prior to extraction of carotenoids using 5 mL of chilled acetone (2×), followed by 2 mL of MTBE. Extracts were combined and dried under nitrogen gas, resolubilized in 2 mL of ethyl acetate/methanol (1:4), and then filtered using a 0.45 μm cellulose acetate filter for quantification using HPLC with a photo diode array detector (HPLC−PDA). Two mL aliquots of both aqueous and digesta fractions were

extracted with a 1:3 ratio of acetone and petroleum ether (0.1% BHT) (×3), dried under nitrogen, resolubilized in the 1:4 ratio of ethyl acetate and methanol, and filtered with 0.45-μm cellulose acetate filters for HPLC analysis of carotenoid contents. Extraction recovery of aqueous and digesta was 94-96%, determined by using *trans*-β-apo-8′-carotenal spiked into samples.

Analysis

Carotenoid Analysis. Lycopene was analyzed by HPLC−PDA using a Waters Alliance 2695 LC system (Waters, Milford, MA, USA) equipped with a model 2998 PDA (Milford, MA, USA). The separation was attained using a YMC C30 column (3 μm 150 mm × 2 mm) thermostated at 35 °C and a gradient elution method starting with 95% solvent A (methanol/ammonium acetate, adjusted to pH 4.6, 98:2) and 5% solvent B (ethyl acetate) for 3 min, 85% solvent A and 15% solvent B for 5 min, 20% solvent A and 80% solvent B for 1 min, 100% solvent B for 4 min, and 95% solvent A and 5% solvent B for the remaining 4 min of the 17 min run. The flow rate was 0.37 mL min^{-1} with an injection volume of 10 μL. All compounds of interest were quantified at 450 nm. Compound concentrations were determined using external standard calibration curves developed in the concentration range of 0.01−7.5 μM for the carotenoids.

Calculations. Analyses were performed on three biological replicates for each homogenate condition, and the results are expressed as the mean ± standard deviation.

The relative bioaccessibility (micellization efficiency) was calculated as follows

$$\text{Relative Bioaccessibility: } \frac{\text{concentration in aqueous fraction}}{\text{concentration in digesta fraction}} \times 100$$

Bioaccessible content was calculated as follows

Bioaccessible content:

relative bioaccessibility % x quantitative bioactive content (nmol/g FW)

Color Analysis. Color retention was tested between pre- and post-processed products on the Hunter Color flex Color Reader with a L*a*b* scale. The ΔE value was calculated to quantify the difference between the samples to determine visible difference between the samples tested, particularly thermally processed Roma (HR, HRO, HROL, HRL), nonthermal processed Roma (UR), thermally processed Tangerine (HT) and nonthermal processed Tangerine (UT).

Statistical Analysis. JMP (SAS Institute Cary, NC, USA) software was used to analyze the data. Means were compared using a one-way analysis of variance (ANOVA) to determine a significant difference ($p < 0.001$) among carotenoid bioaccessibility for control and treatment conditions. The subsequent Tukey-Kramer analysis and Dunnett's test were run when ANOVA identified a significant difference in the data set.

Results

Lycopene profile in untreated control and processing treatments in Tomato cultivars

Variation in lycopene content was observed across 5 different Roma tomato homogenate treatments and 2 different Tangerine tomato treatments. Processing methods including homogenization, homogenization with or without processing aids and microwave-assisted thermal processing were standardized; therefore, reported lycopene species ranges were reflective of tomato treatment conditions including unheated control. In Roma tomatoes, the *trans* lycopene content ranged from 20.5 to 26.83 µg/g, *cis* lycopene content ranged from 2.23 to 2.91 µg/g, (Table 2). Tangerine tomato *trans* lycopene content measured from samples tested ranged from 0.28 to 0.32 µg/g, while the observed *cis*-lycopene content ranged from 23.03 to 25.99 µg/g (Table 2). There were no significant differences in *cis*-lycopene or *trans*-lycopene content between treatment groups compared to untreated control in Roma tomatoes. Treatment conditions of Tangerine

tomatoes did not produce a significant difference in *cis*-lycopene or *trans*-lycopene compared to control (Table 2).

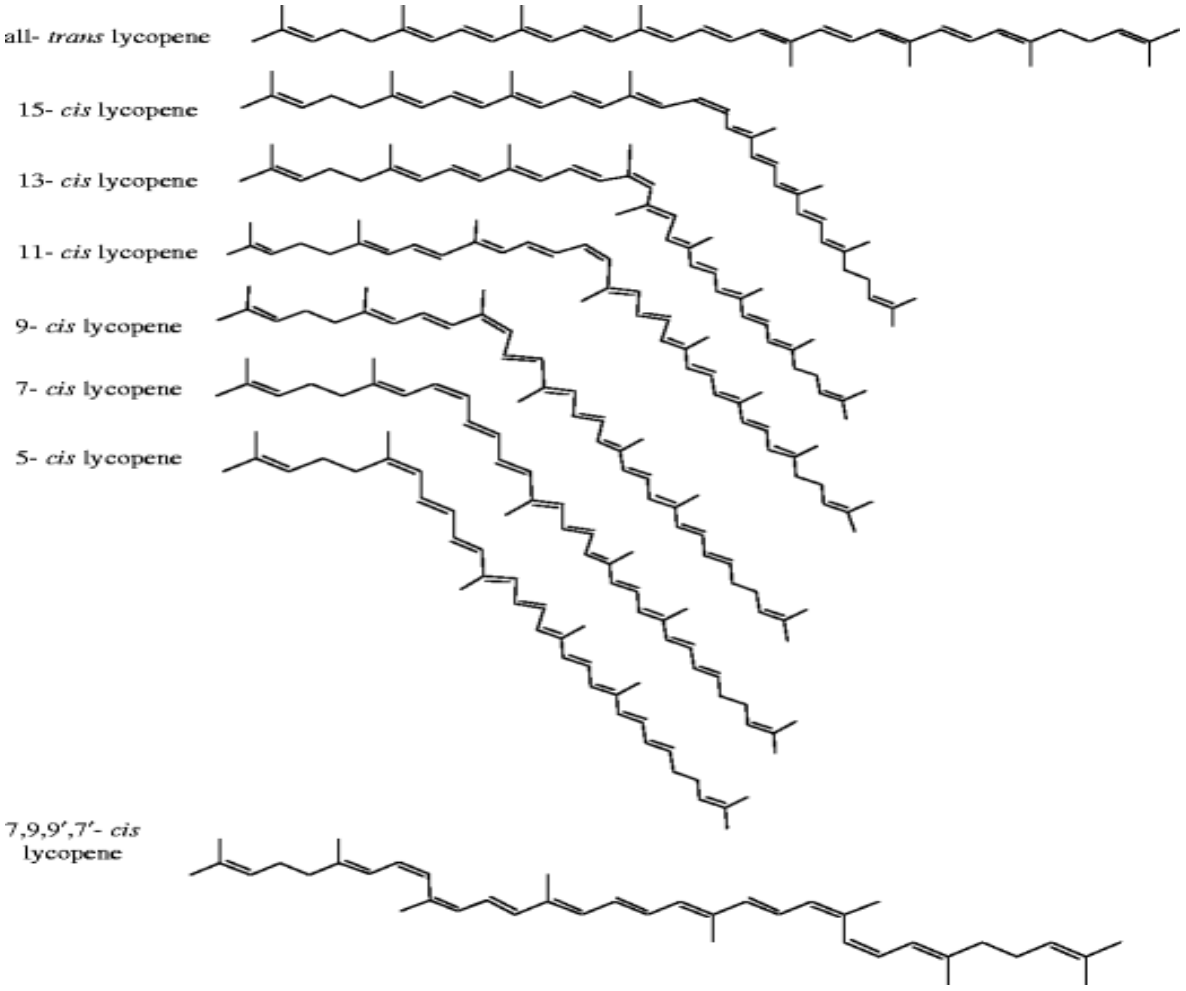


Figure 1. Common Lycopene Isomers (Srivastava, 2015).

Table 2: Lycopene isomer content changes in Tomato Raw Materials derived from process treatment conditions including Unheated Control (UR), Heated Roma (HR), Heated Roma w/oil (HRO), Heated Roma w/oil+lecithin (HROL), Heated Roma w/lecithin (HRL), Unheated Tangerine (UT), Heated Tangerine (HT). [1,2,3]

Raw Material Lycopene Content µg/g			
Treatment	cis-Lycopene	trans-Lycopene	total-Lycopene
Roma Control	2.23 ± 0.11	24.55 ± 3.15	26.78 ± 3.47
HR	2.38 ± 0.06	26.83 ± 2.61	29.21 ± 2.76
HRO	2.41 ± 0.06	20.5 ± 1.67	22.91 ± 1.86
HROL	2.91 ± 0.07	25.08 ± 1.66	27.98 ± 1.86
HRL	2.72 ± 0.09	23.45 ± 2.08	26.17 ± 2.34
UT	25.99 ± 3.27 [a]	0.32 ± 0.104 [b]	28.77 ± 3.66
HT	23.03 ± 2.97 [a]	0.28 ± 0.08 [b]	25.32 ± 3.25

[1] Changes in lycopene isomer concentrations during microwave-assisted sterilization of Roma and Tangerine Tomatoes.[2] Data are presented as mean ± standard error of the mean (SEM). Significant difference between treatments on lycopene content based on Tukey-Kramer analysis.[3] Different letters indicate significant differences ($p < 0.001$) between treatment groups within lycopene isomers. Roma Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin, UT-Tangerine Tomato (control), HT = Microwaved heated Tangerine Tomato.

Lycopene Relative Bioaccessibility in control and thermal processed treatments of Tomatoes

The data gathered show a significant increase ($p < 0.01$) in relative bioaccessibility of *cis*-lycopene in HR, HRO, and HROL treatments compared to control (Table 3). Increases in micellarization of *cis*-lycopene and *trans*-lycopene resulting from Roma processing conditions were between 4-5-fold and 3-4-fold, respectively (Table 3). The highest value measured for relative bioaccessibility of *trans*-lycopene in treatment conditions was 4.91% compared to 1.09% in Roma unprocessed control. Microwave treatment of Roma tomatoes generated similar relative bioaccessibility values for *cis*-lycopene with or without processing aids (Table 3). Microwave processed Tangerine cultivar resulted in no statistically significant increase in relative bioaccessibility of *cis*-lycopene or *trans*-lycopene compared to untreated control (Table 3).

Table 3: Lycopene isomer relative bioaccessibility varies in process treatment conditions for Roma and Tangerine Tomato cultivars. [1,2,3]

Lycopene Relative Bioaccessibility %		
Treatment	*cis*-Lycopene	*trans*-Lycopene
Roma Control	2.59 ± 0.42	1.09 ± 0.14
HR	11.59 ± 1.91 [a]	3.48 ± 0.83
HRO	11.56 ± 1.69 [a]	4.91 ± 0.76 [c]
HROL	10.83 ± 0.84 [a]	4.67 ± 0.52 [c]
HRL	6.68 ± 0.69	2.11 ± 0.24
UT	22.99 ± 1.71 [b]	38.24 ± 3.33 [d]
HT	26.91 ± 2.14 [b]	39.85 ± 3.01 [d]

[1] Changes in lycopene isomer concentrations during microwave-assisted sterilization of Roma and Tangerine Tomatoes.[2] Data are presented as mean ± standard error of the mean (SEM). Significant differences were analyzed using Tukey-Kramer analysis.[3] Different letters indicate significant differences ($p < 0.01$) between treatment groups within lycopene isomers. Roma Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin, UT-Tangerine Tomato (control), HT = Microwaved heated Tangerine Tomato.

Lycopene Absolute Bioaccessibility in control and microwave-assisted thermal processed treatments

Roma tomato microwave-assisted thermal processing resulted in a significant increase ($p < 0.01$) in *cis*-lycopene bioaccessible content for HR, HRO, and HROL treatments compared to UR control. Treatment conditions generated a 4-5-fold increase in *cis*-lycopene bioaccessible content (Table 4). Microwave heating Roma tomatoes with processing aids oil & lecithin produced the greatest increase in *cis*-lycopene bioaccessible content (Table 4). *Trans*-lycopene bioaccessible content in processed Roma tomatoes significantly increased ($p < 0.01$) in HR, HRO, HROL treatments compared to control, but not HRL. The largest fold increase (4.5x) in *trans*-lycopene bioaccessible content was with HROL microwave treatment. Processing of Tangerine tomatoes did not produce a significant increase or decrease in *cis*-lycopene or *trans*-lycopene bioaccessible content.

Table 4: Lycopene isomer absolute bioaccessibility varies in digested process treatment conditions for Roma and Tangerine Tomato cultivars. [1,2,3]

Lycopene Absolute Bioaccessibility µg/g			
Treatment	*cis*-Lycopene	*trans*-Lycopene	total-Lycopene
UR	0.065 ± 0.03	0.253 ± 0.03	0.315 ± 0.04
HR	0.268 ± 0.04 [a]	0.866 ± 0.18 [b]	1.13 ± 0.22 [c]
HRO	0.268 ± 0.04 [a]	0.954 ± 0.14 [b]	1.19 ± 0.17 [c]
HROL	0.308 ± 0.02 [a]	1.13 ± 0.11 [b]	1.41 ± 0.14 [c]
HRL	0.183 ± 0.02	0.487 ± 0.05	0.627 ± 0.07
UT	5.71 ± 0.69 [a]	0.188 ± 0.09	5.95 ± 0.74 [c]
HT	6.29 ± 1.08 [a]	0.101 ± 0.03	6.41 ± 1.11 [c]

[1] Changes in lycopene isomer concentrations during microwave-assisted sterilization of Roma and Tangerine Tomatoes.[2] Data are presented as mean ± standard error of the mean (SEM). Significant differences were analyzed using ANOVA Dunnett test.[3] Different letters indicate significant differences ($p < 0.01$) between treatment groups within lycopene isomers. Figure UR = Roma Tomato, HR = Microwave heated Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Roma Tomato with Lecithin, UT-Tangerine Tomato (control), HT = Microwaved heated Tangerine Tomato.

Lycopene Color Analysis in control and microwave-assisted thermal processed treatments of Roma and Tangerine Tomatoes

Colorimeters are used to measure L*, a*, b* values. Where, value L* (Lightness) indicates the ratio of white to black color, value a* (color index) – the ratio of red to green color, value b* (yellowness index) – the ratio of yellow to blue color (Lopez-Camelo, 2004; Radzevicius, 2009). Lycopene quality and changes in tomato fruit color appearance influenced by processing were best represented by a progression of L* value and a*/b* ratio (Gomez, 2001). Recently, color difference (ΔE), has been used in tomato (Yang, 1990; Habekost, 2013). The L* value in Roma tomatoes ranged from 39.45 in control to 63.45 in HROL treatment (Table 5). Treatment condition HR displayed a statistically insignificant increase in L* compared to control (UR). Compared to control, HRO, HROL, and HRL treatments produced a significant increase in L* value (Table 5, Table 7).

Table 5: Color analysis highlights the effect of processing on Tomato color and light absorption/refraction. [1,2,3]

Color Analysis			
Treatment	L	a*	b*
Roma Control	39.45 ± 0.97^{c}	31.79 ± 1.04^{ab}	29.41 ± 2.66^{c}
HR	39.54 ± 2.14^{c}	31.35 ± 1.86^{ab}	29.60 ± 2.60^{c}
HRO	50.2 ± 1.99^{b}	35.9 ± 1.27^{a}	40.6 ± 1.03^{b}
HROL	63.45 ± 0.23^{a}	24.47 ± 0.79^{b}	28.05 ± 1.54^{c}
HRL	54.2 ± 1.55^{b}	25.69 ± 0.54^{b}	26.77 ± 2.33^{c}
UT	56.17 ± 1.98^{ab}	23.09 ± 3.27^{b}	63.45 ± 2.00^{a}
HT	53.23 ± 0.69^{b}	23.87 ± 2.77^{b}	64.86 ± 1.89^{a}

Table 5: Color analysis highlights the effect of processing on Tomato color and light absorption/refraction [1,2,3]

[1] Changes in color due to light absorption or refraction generated during microwave-assisted sterilization of Roma and Tangerine Tomatoes.[2] Data are presented as mean ± standard error of the mean (SEM). Significant differences were analyzed using Tukey-Kramer HSD test.[3] Different letters indicate significant differences ($p < 0.001$) between treatment groups. Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin, UT-Tangerine Tomato (control), HT = Microwaved heated Tangerine Tomato.

Color index a*/b* ratio decreased with treatment conditions in Roma tomatoes. Color index a*/b* ratio for HR sample generated a lower value compared to UR control (Table 6). Compared to UR the color index a*/b* ratio was lower in HRO, HROL and HRL thus reflecting a color change based on the processing conditions (Table 6). Tangerine tomato UT and HT samples produced lower color index a*/b* ratio figures compared to Roma tomato samples (Table 6). Color index a*/b* ratio did not change significantly between UT and HT conditions (Table 6).

Color difference analysis characterized by ΔL between HR and UR suggests a small positive change reflecting detection of a brighter color in HR compared to UR (Table 7). The ΔL between HRO and UR indicates a relatively positive difference compared to HR/UR, reflecting a greater change in brightness between the samples (Table 7). The ΔL between HROL and UR indicates a greater change compared to HR/UR, reflecting a greater change in brightness between the treatment and control samples (Table 7).

Table 6: Color analysis from various treatments using a*/b* ratio in processed tomatoes.

Color Analysis	
Treatment	*a/*b
UR	1.10 ± 0.12
HR	1.09 ± 0.16
HRO	0.89 ± 0.05
HROL	0.88 ± 0.08
HRL	0.98 ± 0.11
UT	0.36 ± 0.06
HT	0.37 ± 0.05

Table 6. Color changes displayed using a*/b* ratio in thermal processed (HR, HRO, HROL, HRL) Roma tomatoes compared to control unheated (UR) Roma tomatoes and heated (HT) Tangerine compared to unheated (UT) Tangerine tomatoes.

The ΔL between HRL and UR indicates a relatively greater change compared to HR/UR, reflecting a greater change in brightness between the samples (Table 7, Figure 2). The ΔL between HT and UT suggests a negative change value in HT compared to UT following microwave heating indicating a darker product (Table 7).

Color difference analysis characterized by ΔE between UR and HR suggests a relatively small overall change reflecting the magnitude of overall color difference (Table 7, Figure 2). Color differences characterized by ΔE between UR and processed samples HRO, HROL and HRL, respectively indicate a relatively high and therefore visible difference in overall color was observed and calculated (Table 7, Figure 2). The ΔE value for UT/HT was above the 2.7 threshold indicating overall color difference was visible to the human eye (Table 7, Figure 2).

Discussion and Conclusion

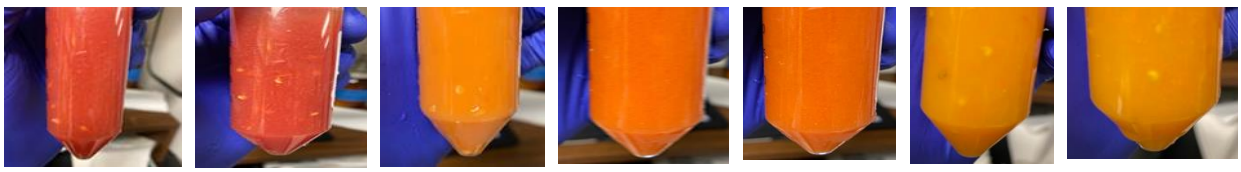
Carotenoids possess unique photophysical properties that enable them to act both as photoprotection agents and light harvesting pigments in regulating the flow of the absorbed energy covering the spectral region 450-550 nm in photosynthetic organisms (Frank, 1996; Christensen,

1999). They transfer absorbed light energy to chlorophyll molecules that funnel energy toward the reaction center where a charge separation occurs (Frank, 1996). Tomato fruit ripening involves differentiation of chloroplasts into chromoplasts, which results in the accumulation of healthy nutrients such as β-carotene, vitamin E, and lycopene (Giovannoni, 2001). Agarwal et al. described a stability hierarchy for lycopene encompassing *trans* and *cis* isoforms in ripened conventional tomatoes, which highlights the heterogeneous nature of lycopene dynamics in tomato homogenate material (Agarwal et al., 2000).

Table 7: Color difference analysis determining changes in brightness and overall color detection.[123]

Color Difference Analysis		
Samples	ΔL	ΔE
UR – HR	0.093 ± 1.18	0.5 ± 0.45
UR – HRO	10.76 ± 1.17 [a]	16.4 ± 0.65 [c]
UR – HROL	24.01 ± 0.99 [b]	25.2 ± 1.1 [d]
UR – HRL	14.75 ± 1.02 [a]	16.24 ± 1.3 [c]
UT – HT	-2.68 ± 1.83	3.77 ± 1.4

Color difference analysis determining changes in brightness/darkness (ΔL) and overall color change (ΔE) between samples detection. Positive figures observed for ΔL indicate an increase in brightness in heated treatment samples compared to control. ΔE above 2.7 indicates a visible change is detected between paired samples.
[1] Changes in lycopene isomer concentrations during microwave-assisted sterilization of Roma and Tangerine Tomatoes.[2] Data are presented as mean ± standard error of the mean (SEM). Significant differences were analyzed using ANOVA Tukey-Kramer HSD.[3] Different letters indicate significant differences ($p < 0.001$) between treatment group comparisons.



UR HR HRO HROL HRL UT HT

Figure 2. Digital photo images taken during color analysis of control (UR, UT) and microwave assisted process samples for Roma and Tangerine tomatoes.

To this end, the effects of microwave processing on lycopene isomerization and bioaccessibility were summarized as follows: 1) Diversity in both relative and absolute

bioaccessibility of lycopene was observed in microwave-assisted processing treatment conditions for Roma tomatoes. 2) Increases in both relative and absolute bioaccessibility of *cis*-lycopene was observed in microwave-assisted heating with or without natural processing aid treatment conditions for Roma tomatoes. 3) Increases were observed in relative and absolute bioaccessibility of *trans*-lycopene in microwave assisted heating with or without natural processing aid treatment conditions for Roma tomatoes. 4) Processing conditions did not negatively impact lycopene content. 5) Processing conditions increased lycopene isomerization in Roma tomatoes with or without processing aid. 6) Changes in lycopene chemical structure producing alterations influencing lycopene's interaction with light resulting in brightness difference and overall color difference compared to control were observed in microwave-assisted processing treatment conditions involving processing aids. 7) Relative bioaccessibility of *cis*-lycopene in microwave-assisted sterilized Roma tomatoes were enhanced to levels comparable with relative bioaccessible *cis*-lycopene in sterilized Tangerine tomatoes.

The microwave processing studies performed suggest that energy transferred into the system of tomato material is absorbed by the system of carotenoids, which are subsequently thrusted into a higher or heightened energy state leading to lycopene isomerization from stable isoforms housed within crystalline structured chromoplasts (Zigmantas, 2002). An increase in higher energy state lycopene isomers may be the predominant factor responsible for the rises observed in relative and absolute bioaccessibility of lycopene in treatment samples compared to control (Figures 3.1, 3.2, 3.3, 3.4). It is possible that the lycopene isomer stability construct characterized by Agarwal was altered by processing conditions to favor enhanced micellarization. The roughly 70-80% energy conversion efficiency of microwave assisted volumetric heating allows for an opportunity to gauge this phenomenon with a higher degree of certainty compared

to convection heat energy transfer, which has an efficiency of around 20% due to excess heat energy loss (Ekezie, 2017; Ishii, 1995).

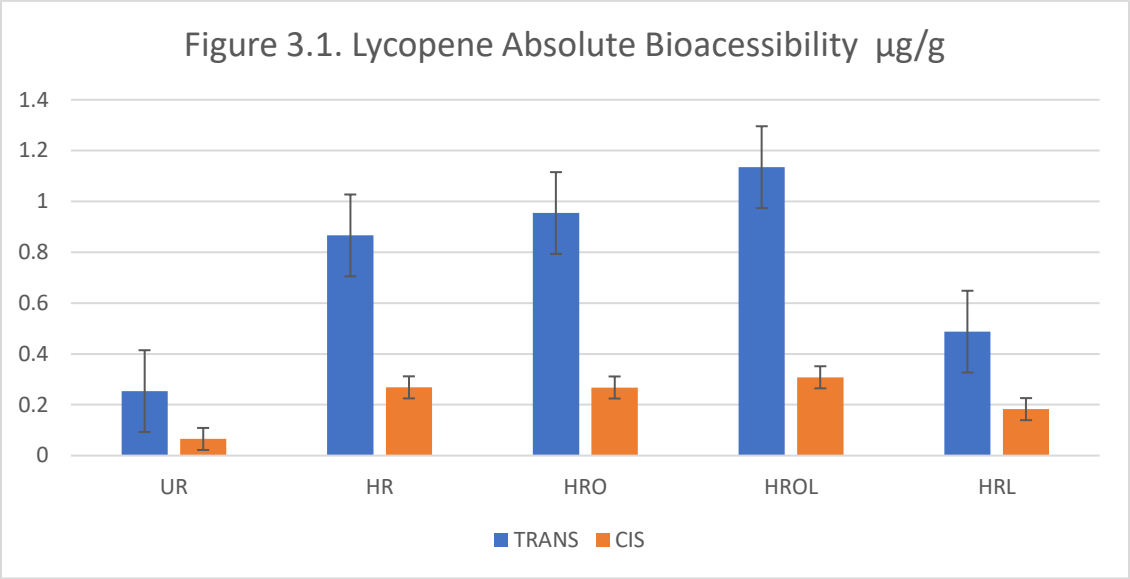


Figure 3.1 Lycopene bioaccessible content changes due to treatment conditions compared to control (UR) for *trans*-lycopene and *cis*-lycopene isomers. Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

Additionally, the data generated in the present work coupled with the randomized complete block experimental design suggests processing aids have the capacity to lower the energy requirement threshold necessary to induce carotenoid isomerization during tomato processing. Processing aids such as natural oils and/or surfactants were used during homogenization of whole tomatoes followed by microwave-assisted sterilization. A 2 to 3.5-fold increase in lycopene micellarization was achieved after thermal processing of Roma tomatoes using microwave heating compared to nonthermal processed homogenized control (Figures 3.1, 3.2, 3.3, 3.4). As it relates to lycopene, *cis* isomerization may have occurred at the expense of *trans* lycopene under certain processing conditions with perhaps influence from tomato genotype and harvest time (Figure 1,

Figure 3.2, Figure 3.5, Figure 3.6). According to published literature, formation of 9-*cis*-lycopene is more favorable at low pH condition while 13-*cis*-lycopene is the major degradation product formed from thermal processing (Moraru et al., 2004). Data generated with our microwave processing test conditions were consistent with findings by Moraru et al., however our methods maintained pH 3.6 from nonacidified homogenate fruit juices derived from whole tomato fruit homogenization. Therefore, it is conceivable that a mechanical shear stress homogenization and microwave heating-imposed energy increases during a sterilization process that could impact the carotenoid structure to a degree that its bonds are altered from *trans* to *cis*-lycopene conformation and result in subsequent increases in *cis*-lycopene micellarization.

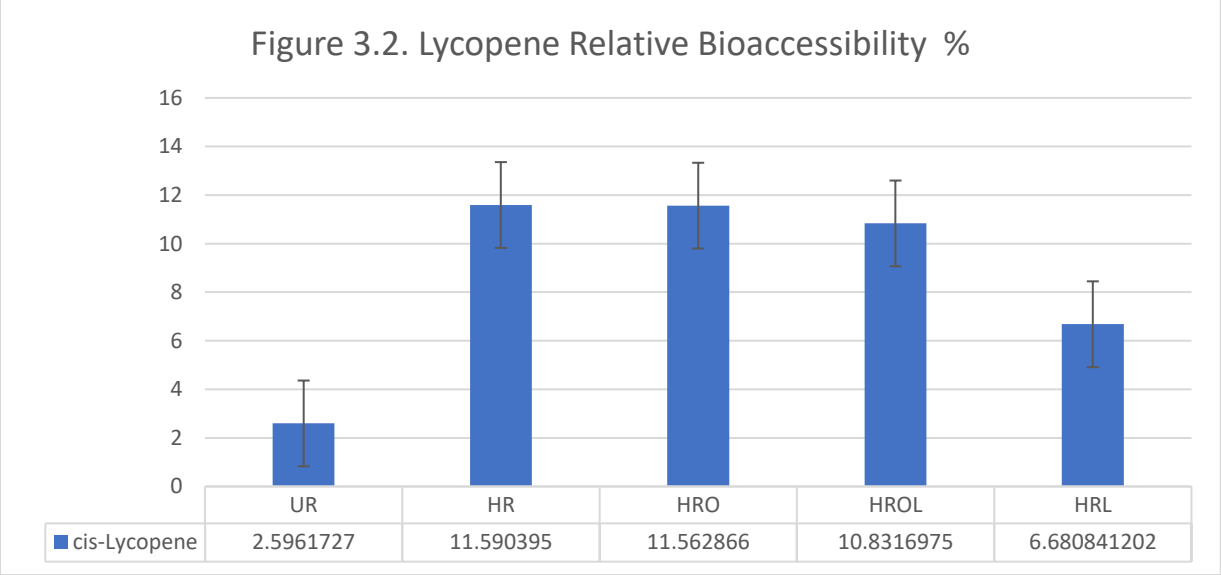


Figure 3.2 Lycopene *cis* isomer Relative Bioaccessibility changes due to treatment conditions compared to control (UR). *cis*-lycopene relative bioaccessibility increased by 158-347%, with statistically significant increases in HR, HRO, HROL compared to UR. Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

Additionally, processing conditions apparently altered Roma tomato carotenoid absorption of light in the red, blue, purple, and green light spectrum giving rise to orange coloration in

processed samples with processing aids (Figure 2). This would suggest a carotenoid structural or chemical change that may be described as an isomerization event or an alteration of conjugated double bonds.

Bonds of predominant lycopene isomers responsible for red color of Roma tomatoes were altered during processing resulting in increased L* (brightness) of processed material and overall color change from red to orange (Figure 2, Table 5, Table 6, Table 7). Most lycopene in Roma tomatoes occur naturally in all-*trans* form and the red color of lycopene is mainly due to numerous conjugated carbon double bonds which enables it to absorb more visible spectrum compared to other carotenes (Boileau, 2002; Schulz, 2005). Roma tomatoes also express β-carotene, which has 11 double bonds, is orange in color and α-carotene that has yellow coloration (Rao, 2007; Takyi, 2001). Lycopene has two more double bonds than β-carotene, hence it appears red as opposed to orange. Lycopene is an open straight chain hydrocarbon consisting of 11 conjugated and two unconjugated double bonds. Of the 13 double bonds, seven of those double bonds can be isomerized to mono-*cis*- or poly-*cis*-isomers (Kong, 2010). It is likely that processing conditions used within this study disrupted *trans*-lycopene double bonds that typically distinguish its coloration from beta-carotene. It is also possible that processing-induced bond disruption resulted in the occurrence of lycopene isomerization. The color change in lycopene from microwave-assisted treated samples with processing aids was associated with increases in lycopene relative and absolute bioaccessibility (Figure 2, Figure 3.1, Figure 3.2, Figure 3.3, Figure 3.4, Table 5, Table 7). Although the data suggests color change and isomerization are positively corelated, the increase in the L* value due to the emulsified lipid induction of light scattering is a possibility. However, light scattering is an unlikely explanation because the UT control treatment without heat has an equivalent amount of lipid as HRO treatment and lycopene in tangerine tomatoes is present

in lipid dissolved droplets (Hempel, 2014; Sitte, 1980). The microwave treatment involving lecithin and oil did increase L* but only thermal treatment with oil was a significant drive of color change in Roma towards the color of Tangerine tomato. Additionally, tangerine tomato *cis*-lycopene absorbs light maximally approximately 35 nm below all-*trans*-lycopene, resulting in tomatoes with an orange color as shown in Figure 2 and Table 5 (Clough, 1979). The data also indicate microwave thermal processing influences lycopene isomerization leading to significant increases in lycopene micellarization without significant color changes or processing aids (Figure 2, Figure 3.1, Figure 3.2, Figure 3.3, Figure 3.4, Figure 3.7, Table 5, Table 7).

The observed effect of processing Roma tomatoes using non-synthetic natural processing aids to presumably lower carotenoid isomerization energy requirements correlate with previous studies performed by two separate labs. Both Schwartz and Boileau labs reported while working with crystalline lycopene in an organic solvent that they noticed that a 50:50 equilibrium mixture of *trans*- and *cis*-lycopene resulted even when all *trans* lycopene fraction was purified by preparative HPLC (Nguyen, 1998; Wasson, 1999). Lycopene in crystalline configuration within a tomato matrix dissolved in petroleum derived solvents are less resistant to thermal degradation/isomerization (Xianquan, 2005). This suggests tomato matrix including crystalline lycopene configuration aids in protecting lycopene from oxidation/isomerization and degradation during conventional thermal processing.

It is possible that processing with specific fat-soluble solvents as well as specific natural oils or surfactants may contribute to carotenoid solubilization and lycopene crystalline structure instability in Roma tomatoes, thereby priming carotenoids and lowering energy requirements to achieve energy excitation engendered isomerization. Increases in carotenoids lycopene and beta carotene (data not shown) isomer content was observed to contribute to enhancements in

micellarization for each treatment processing group (Figure 3.1, Figure 3.2, Figure 3.3, Figure 3.4, Figure 3.7). Collectively, these results underscore the possibility of utilizing microwave assisted thermal processing to sterilize processing tomatoes commercially. In addition to providing higher energy efficiency and potentially producing a more natural and cleaner finished tomato ingredient product, microwave-assisted tomato processing may establish a functional benefit characteristic to the antioxidant material by increasing *cis* lycopene isomer content which was shown in this work to be more bioaccessible as was *trans*-lycopene (Figures 3.1, 3.2, 3.3, 3.4). Results from the current work is contrary to reports by Nguyen et al. indicating commercial processing regularly employed in the food industry or during food preparation could not generate isomerization for lycopene (Nguyen, 1998).

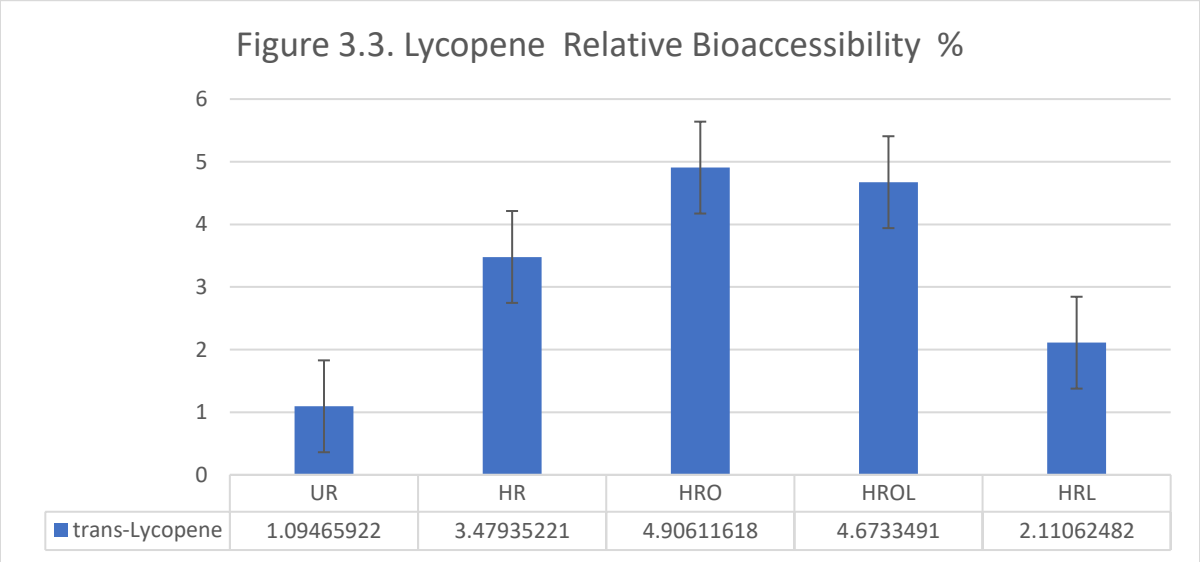


Figure 3.3 Lycopene *trans* isomer Relative Bioaccessibility changes due to treatment conditions compared to control (UR). *Trans*-lycopene relative bioaccessibility increased by 93.6-350%, with statistically significant increases in HR, HRO, HROL compared to UR. Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

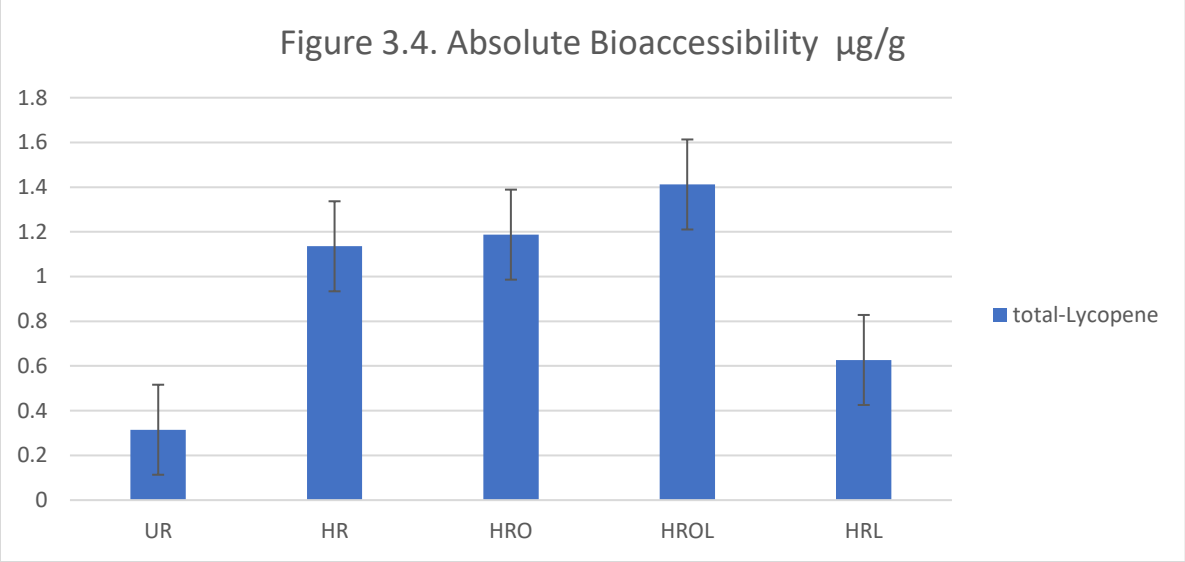


Figure 3.4 Total Lycopene Bioaccessible content changes due to treatment conditions compared to control (UR). Increases in bioaccessible content ranged from 102-355% in tested treatments, with statistically significance expressed in HR, HRO and HROL treatments compared to UR. Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

Originally, the processing test methods utilized within the current studies were designed to mimic physiological conditions identical to processing and digesting of carotenoids by the human body. Therefore, if results were generated proving our hypothesis that tomato processing mimicking industrial processes involving microwave thermal processing increases lycopene isomerization and bioaccessibility; merit could be established to theorize why and how lycopene consumed in *trans* isoform could accumulate in the organs and throughout the vascular system as 60% *cis* isoform as reported by Stahl and Schwarz et al. (Stahl, 1992). According to current literature, the precise mechanism(s) surrounding this phenomenon have yet to be elucidated. Based on the current data, it is possible that mechanical shear stress from mastication within the oral cavity, optimally in the presence of oil and/or surfactant at physiological temperatures carried

through the digestion process maintained at physiological conditions (including stomach acid) and temperatures (37 ^{o} C) throughout the 30-minute digestion process, creates optimal conditions for lycopene isomerization. This change would explain the resulting increased micellarization and absorption of lycopene leading to *cis* isomer ratios identified *in vivo* by Stahl et al. A theory is therefore proposed at this time entitled "human physiology conditioned spontaneous lycopene isomerization" that describes a plausible explanation to the observed phenomenon. This novel theory is supported by the literature which describes pre-digestion processes reported to influence lycopene isomerization such as isomerization augmentation in the acidic environments, increased isomerization tendency in the presence of specific dietary oils, reported *cis*-isomerization thermal temperatures between 85-98^{o}C and a greater micellarization and absorption tendency of *cis*-lycopene isomers (Stahl, 1992; Boileau, 1999; Moraru, 2005; Holloway, 2000; Clark, 2000; Hu, 2000; Lee, 2002; Failla, 2008). Pre-digestion conditions highlighted appear conducive to freeing lycopene from the tomato fruit matrix association with protein and fiber; previous studies show complexation of fiber with lycopene hinders carotenoid bioaccessibility (Castenmiller, 1998). Free form hydrophobic carotenoids assimilated and solubilized with dietary oils maintained at thermal body temperatures optimal for sufficient isomerization energy transfer create conditions conducive to maximizing carotenoid micellarization in the presence of amphipathic bile salts. Our data support this theory by mimicking *in vivo* carotenoid isomerization and digestive processes. The distinction between body temperature and thermal processing temperature related to conditional lycopene or carotenoid isomerization can be explained by the impact pressure has on macromolecules containing an abundance of hydrocarbon chains such as fats or carotenoids as structural components. *In vivo*, stomach peristalsis perhaps incorporates a pressure component to the digestive carotenoid preparation. The influence of pressure on raising the internal temperatures

on systems containing hydrocarbon polymers during non-thermal high-pressure processing was reported by Martinez-Monteagudo et al., (2017). Temperature rises as a function of pressure. During the rise in pressure the temperature within the food system goes up as well, then after the pressure is lowered the temperature lowers. Fat materials in vegetable oils or mayonnaise have high long chain or short chain hydrocarbon molecules that are highly compressible and as a result there is a rearrangement in molecular structure that keep going up in pressure so there is a significant increase in temperature during processing of long chain molecules in particular (Martinez-Monteagudo, 2017; Rasanayagam, 2003). Compression heating factor (^{o} C/100 mPa) for fat is 6-9^{o}C, while compression heating factor for water and hydrophilic molecules such as carbohydrate is 3 ^{o} C per 100 mPa (Patazca, 2007). The magnitude of change depends on compressibility and specific heat of the substance. Due to the intrinsic nature of lycopene (and carotenoids in general) to absorb energy and the orientation of lycopene's isoprene polymer or hydrocarbon chain structures, it is plausible for lycopene to behave in a similar manner to long chain molecules in the presence of pressure. In support of this prospect, it was reported by Vallecilla-Yepez et al. that *cis*-isomerization was achieved during supercritical CO_2 tomato pomace extraction processes, which utilized a combination of heat and pressure to separate polymer biomaterials (Vallecilla-Yepez, 2018). An alternative to this theory of "human physiology conditioned spontaneous lycopene isomerization" would be the existence and activity of some yet to be identified enzyme that catalyzes lycopene isomerization reactions, similar to the manner in which beta carotene oxygenase converts provitamin A to beta-carotene at the enterocyte brush border location of the duodenum (King, 2006).

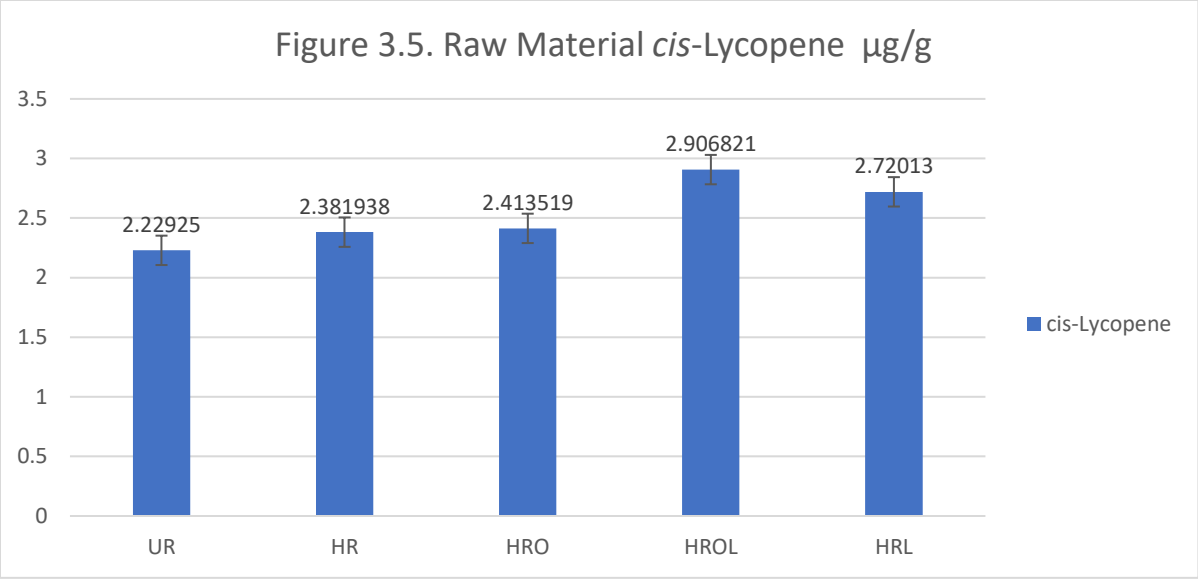


Figure 3.5 Lycopene *cis*-isomer Raw Material content changes due to treatment conditions compared to control (UR). Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

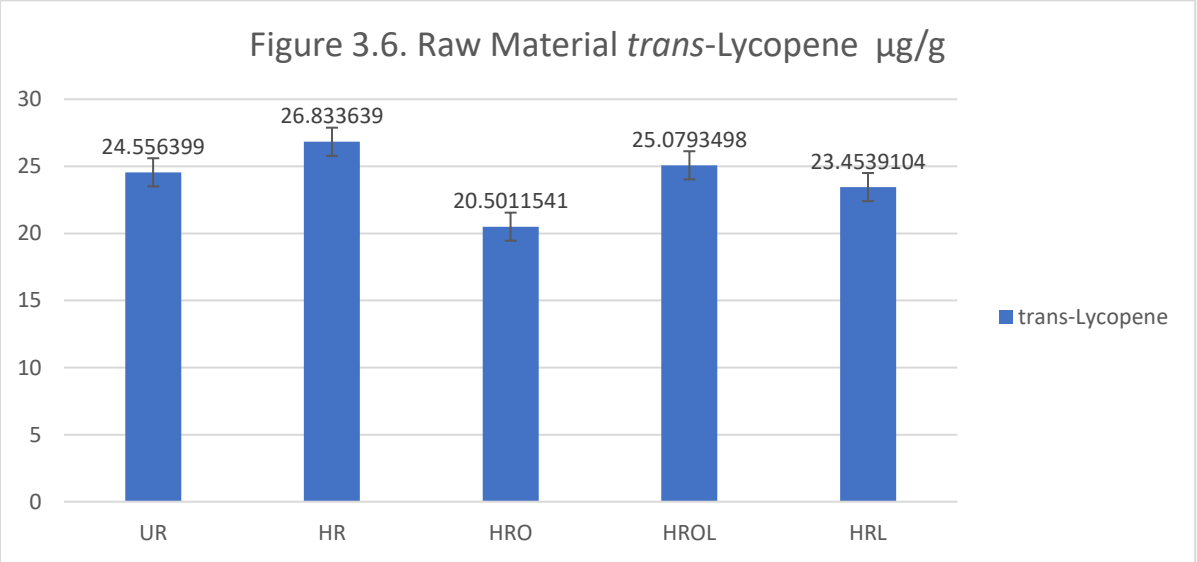


Figure 3.6 *Trans*-Lycopene Raw Material content changes due to treatment conditions compared to control (UR). Control= Homogenized Roma Tomato, HR = Microwave heated Homogenized Roma Tomato, HRO = Microwave heated Homogenized Roma Tomato with Oil, HROL = Microwave heated, Homogenized Roma Tomato with Oil and Lecithin, HRL = Microwave heated Homogenized Roma Tomato with Lecithin.

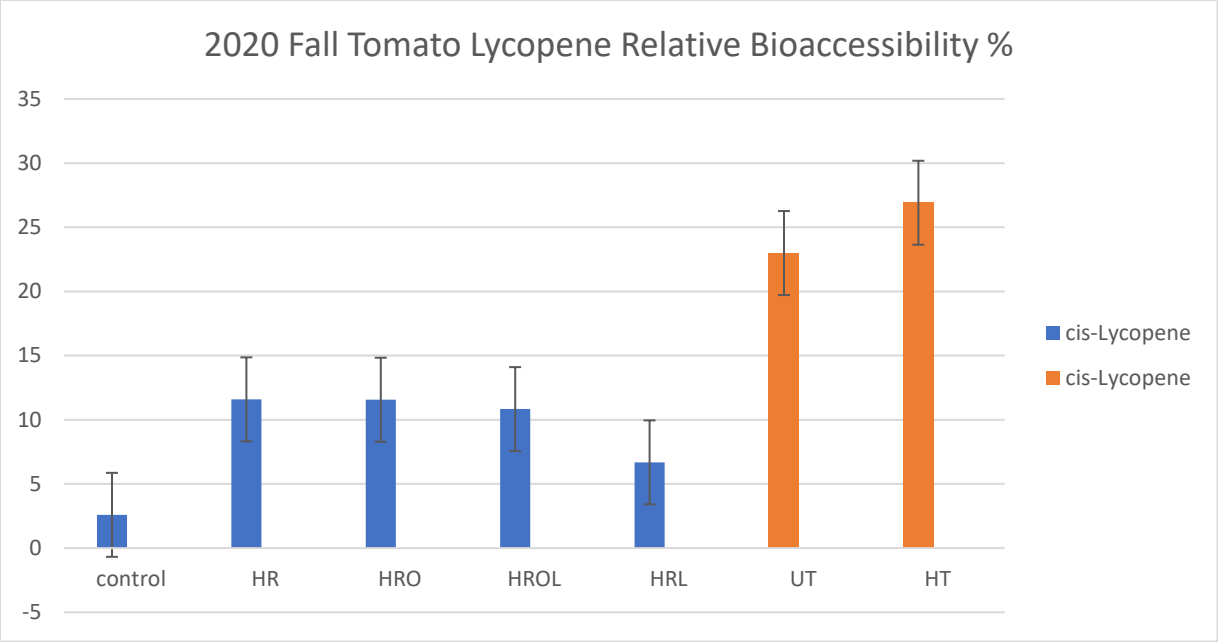


Figure 3.7 Relative Bioaccessibility of *cis*-lycopene in Roma tomatoes increased under microwave heated treatment conditions compared to control. Biofortified tetra-*cis*-Lycopene in tangerine tomatoes display higher relative bioaccessibility of *cis*-lycopene compared to Roma control and microwave processed treatments.

In conclusion, the establishment of product market-fit for academic research is more relevant and important today due to a societal health improvement driven necessity to translate food science research into practical applications for consumer utilization. In today's climate of increasing rates of metabolic and inflammatory diseases in developed nations such as the US, consumers are relying on professionals throughout the Food Science and Food Engineering disciplines to identify solutions to improve food supply chain quality using innovation and technology developed from academic research. The information presented in this summary of industry/academic partnership work represents the opportunity to translate and apply innovative processing methods to a mature tomato processing industry in order to improve the quality of antioxidant fruit ingredients currently consumed at a rate of 73 lb. per capita in the United States. Increasing the availability of more bioaccessible antioxidant ingredients in the market and making

them accessible to consumer product goods manufacturers, may improve consumer access and ultimately contribute to decreasing the prevalence of risk factors positively correlated with common proinflammatory diseases. Perhaps further development of this research effort will lead to a higher volume of product launches, commercialization and marketing of functional ingredients, functional foods and food therapeutics created specifically to promote health or reduce the risk of diseases.

The data from this study suggest the following conclusions:

- In vitro processing methods involving microwave assisted sterilization with industrial potential can be utilized to influence lycopene isomerization without significant yield loss.
- In vitro processing methods involving microwave assisted sterilization with industrial commercialization potential can increase lycopene micellarization by 2-6-fold compared to unheated control.
- Further research is needed to link this increase more definitively in bioaccessibility to health outcomes.

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Recommendations for Future Research

The effect of processing on the bioaccessibility of carotenoids reveals an ability to modulate processing conditions to influence carotenoid isomerization quality and quantity (Figure 3.4). Energy inputs can be calculated and defined to create a predictability model to further understand this area of research. Isomerization energy studies in conjunction with carotenoid speciation analysis would be critical to the development of an accurate model. Isomerization energy is involved in relocation of the single or double bond of one form of carotenoid into another (Kuki, 1991; ESA, 2009).

Model development can be used to expand understanding of micellarization intricacies for fat soluble antioxidants and phytonutrients. Not only does expansion of this research effort have lasting implications in the academic research setting, it also may be applicable to commercial consumer product market fit demographic models. Product market-fit potential can be broken down using demographic data based on skin type.

For example, individuals with type I skin (according to the Fitzpatrick scale) do not express melanin and are more at risk for skin cancers or melanomas caused by UV light exposure from the sun (Orentreich, 2001; Stahl, 2006). According to the CDC, skin cancer is the most common form of cancer in the United States (CDC, 2019). About 77,698 new cases of melanoma occurred in the United States each year, including 45,854 among men and 31,845 among women (CDC, 2019). Type I skin individuals may benefit from the consumption of food ingredients and tomato-based end products containing increased *cis*-lycopene isomers since carotenoids are deposited and accumulate in human skin (Khachik, 2002; Hata, 2000). Studies by Cooperstone et al. suggest consumption of tomato material high in *cis*-lycopene may lower risk factors associated with the development of skin cancer in mammalian models (Cooperstone, 2017). If so, extending our

research by developing commercial processing methods and applying microwave processing technologies designed to improve *cis*-lycopene content could improve tomato supply chain quality, maximize consumer access to higher quality ingredients and provide an ideal product market fit for the U.S. demographic at risk for skin cancers.

Conversely, individuals with type IV skin could benefit from consuming products possessing higher *cis*-lycopene content with enhanced bioaccessibility for different reasons. Type IV skin expresses melanin, which is a natural compound within the skin that protects the human body from the sun's UV rays by absorbing and scattering light (Ortonne, 2002). Therefore, dermal layer abnormalities or dysfunctions related to exposure from sun light are less of a threat to this demographic. However, within the U.S. Type IV skin demographics are often predisposed for acquiring metabolic syndrome related abnormalities such as cardiovascular disease (CVD) and obesity due to a variety of environmental factors. According to the CDC, heart disease is the leading cause of death for men, women, and people of most racial and ethnic groups in the United States (CDC, 2018). About 655,000 Americans die from heart disease each year and roughly 18.2 million adults aged 20 and older have coronary artery disease irrespective of skin type (Virani, 2020; Fryar, 2019). Hallmarks of closely related conditions of CVD and obesity are characterized by low grade chronic inflammation and depressed metabolism. Due to the intrinsic properties of lycopene, within a mammalian system it could directly absorb inflammatory radicals and thereby can protect cells, tissues and functional substances such as LDLs from the harmful effects of inflammatory oxidation cascades generated within the body (Barona, 2011). Lycopene, in elevated vascular circulation, may have the ability to indirectly lower risk factors associated with CVD, obesity and inflammation by inducing mitochondrial biogenesis through the activation of genes that regulate cellular Phase II metabolism enzymes such as superoxide dismutase (SOD),

glutathione (GSH) and biotransformation enzymes, such as glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR) (Velmurugan, 2002; Kim, 2011). In addition to activation of endogenous antioxidant defense system, lycopene activity was also correlated with a significant improvement in endothelial function and a 57% reduction in C-reactive protein (CRP) (Bhuvaneswari, 2002; Kim, 2011). Due to the direct and indirect influence of lycopene; its consumption, enhanced absorption and sustained presence throughout the vasculature and tissues is much to be desired for individuals at risk for CVD and obesity. Therefore, an ideal product market fit can be established with Type IV demographics in the USA.

Foods that target a particular demographic and potentially offer health benefits are often referred to as "functional foods". Functional foods typically look, taste and smell the same as its predecessor counterparts but are developed specifically to promote health or reduce the risk of diseases. Although their consumption does not offer a magic bullet against health problems, they do provide a positive health enhancing addition to an overall balanced diet and active lifestyle. Combined with a healthy lifestyle, functional foods can make a positive contribution to health and wellbeing.

For future studies, it would be worth investigating tomato processing with industrial-scale microwave system frequencies (915 MHz) using identical sterilization parameters developed from the current study, followed by *in vivo* carotenoid bioavailability studies in animal or human clinical models. Expanding upon the current work towards process commercialization could lead to bringing added value to the south region's nascent ingredient bioprocessing industry and create more opportunities for local farmers to provide raw materials to local processors. Establishing tomato processing east of the Mississippi River would have an enormous impact on CO_2 emission carbon footprint in the United States. Currently, industrial scale tomato processing in the United

States occurs in Southern California and sterile tomato ingredients are trucked via interstate highways or transported by rail to high volume customers predominately on the east coast. A diesel truck hauling an avg. 40,000 lbs. (20 tons) of processed tomato ingredients from California to North Carolina to fulfill an order of 250,000 lbs. per year would produce approximately 53.87 MT of CO_2 annually. This result equates to annual estimates of the following CO_2-emitting activities: 59,353 lbs. of coal burned, 2,202 propane cylinders used for home barbecues, and 6,869,709 smartphones charged (EPA regulations, EPA transportation).

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