Determination of Lycopene Isomers in Model Food Systems and Their Effectiveness as Antioxidants

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CHAPTER I

Introduction

"Watermelon is the leading U. S. melon crop in terms of acreage, production, and per capita consumption", and the consumption of watermelon in the United States in 2000 was estimated to be 3.9 billion pounds, an increase of 59% compared to consumption rates in 1980 (Lucier and Lin, 2001). Sales of fresh-cut produce account for approximately 10% of the fresh fruits and vegetables marketed through food service and retail channels in the United States (Cantwell and Suslow, 2002), and cut watermelon represents approximately 10% of all watermelon sales (Perkins-Veazie and Collins, 2004). Unfortunately about 20% of the annual watermelon crop is left in the field as culls because of imperfections or undesirable appearance (Fish and others, 2009).

However, there is an opportunity to utilize these culled watermelons in value-added further processed products where the appearance of the watermelon is not an issue. Additionally, watermelons are an excellent source of lycopene. Lycopene (gamma, gamma-carotene) is the pigment responsible for the characteristic red color of tomatoes and watermelons, and is thought

to aid in the prevention of certain types of cancers and chronic diseases (Nguyen and Schwartz, 1999).

The presence of lycopene may help make processed products more attractive to health conscious consumers. However, the majority of research involving lycopene is conducted on tomatoes because they are a larger part of the western diet (Anguelova and Warthsen, 2000). Thus, there is very little information available regarding processing parameters to effectively extract lycopene from the matrix of the watermelon tissue or for determining the effect of those parameters on the lycopene, its antioxidant capacity, or its isomeric forms. This information is crucial for establishing procedures for the manufacture of value-added foods from watermelon.

Therefore, our goal was to create model foods that could be used to determine this information. Specifically, our objectives were:

Experiment 1

1. Evaluate the effect of common food processing techniques on the extraction of lycopene from watermelon.
2. Determine their effect on lycopene stability, isomeric form, and antioxidant activity.

Experiment 2

1. Develop a watermelon-based product that is an analogue to tomato sauce.
2. Measure the effect of thermal processing on lycopene stability, isomeric form, and antioxidant activity

Experiment 3

1. Develop a watermelon-based wine.
2. Measure the effect of wine-making techniques on lycopene stability, isomeric form, and antioxidant activity.

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CHAPTER II

REVIEW OF LITERATURE

Health benefits associated with consumption of fruits and vegetables

The consumption of fruits and vegetables is associated with a reduced risk of cancer, and the National Cancer Institute along with the National Research Council recommend consuming at least five servings of fruits and vegetables daily; additionally, the consumption of fruits and vegetables has been associated with a lowered risk of ischemic stroke, and decreased blood pressure; however, only 17% of 15000 Americans surveyed consume five servings per day (Vinson and others, 2001).

These beneficial effects may be due the presence of nutritional compounds that are traditionally thought of as being healthy, such as antioxidant vitamins C and E. However, Vinson and others (2001) state that compounds known as phenols and polyphenols that are also present in fruits and vegetables are actually stronger antioxidants than vitamin C and E, so these molecules may also play some part in human health. Therefore, it is most likely that the beneficial effect of fruit and vegetable consumption is due to the combination of all of these nutrients and not just a single type. Collins and others (2006) state that measured antioxidant activity can vary between

aqueous and solvent extracts of the same food, indicating that compounds such as vitamin C, vitamin E, and polyphenols also contribute to a food's total antioxidant profile .

Health benefits associated with the consumption of wine

German and Walzem (2000) describe wine as, "a diverse commodity class composed of the yeast fermentation products of the must, or juice, pressed from grapes, the fruit of the genus *Vitis*." However, specialty wines made from others fruits can also be found.

The hypothesis that a diet containing a high concentration of wine, in addition to cheese and chocolate, can lead to improved cardiovascular health is known as the French Paradox (Hasler, 2009). There are several components in wine that are thought to be beneficial to health and to reduce the occurrence of certain types of disease in those individuals that consume one to two glasses of wine or 10 to 20 grams of actual ethanol per day (German and Walzem, 2000). These beneficial components are ethanol, polyphenols, and resveratrol (German and Walzem, 2000; Hasler, 2009).

The concentration of ethanol in wine typically ranges from 8% to 15% by weight, and the consumption of small to moderate amounts of ethanol have been shown to improve coronary artery disease (Hasler, 2009). While there is no single unifying standard of what constitutes moderate alcohol consumption, the National Institute on Alcohol Abuse and Alcoholism defines a moderate single serving of wine as 120 mL, providing 12.4 grams of ethanol (German and Walzem, 2000). There are several suggested mechanisms for ethanol's beneficial effects. These include increasing high-density lipoprotein (HDL), reducing low density lipoprotein (LDL), decreasing LDL oxidation, reducing blood fibrinogens, and additional antithrombotic actions (German and Walzem, 2000; Hasler, 2009). The suggested mechanism for polyphenols' beneficial effect are their ability to act as antioxidants (German and Walzem, 2000; Hasler, 2009) and to protect human body tissue against oxidative attack (Gahler and others, 2003). There has been disagreement over whether it is more beneficial to drink wine than to drink beer or spirits;

however a study conducted by anti-inflammatory effects (Hasler, 2009). Klatsky found that while moderate consumption of wine, beer, and liquor all reduced the risk of mortality from coronary disease, the risk was most reduced by wine (Hasler, 2009).

How wine is processed can impact levels of phenolic compounds

The majority of phenolic compounds that are found in grapes are associated with the skins and seeds (Hasler, 2009). Both the wine-making technique and grape variety can influence the total phenolic content of wine. However, the wine-making technique is the more influential of the two (German and Walzem, 2000). For example, red wine contains a higher concentration of phenolics because it is fermented in contact with the skins and seeds while white wine has a lower concentration of phenolics because it has minimal contact with the skins and seeds during fermentation (Waterhouse, 2002).

Wine from commodities other than grapes can contain beneficial compounds

The presence of beneficial antioxidants in wine made from fruit other than grapes, such as the phenolics found in blackberry and raspberry wine, suggest that it may also be possible to maintain the beneficial compounds in wine made from watermelon. Watermelon contains antioxidants such as lycopene and smaller amounts of phenolic compounds.

Watermelon

"Watermelon is the leading U. S. melon crop in terms of acreage, production, and per capita consumption", and the consumption of watermelon in the United States in 2000 was estimated to be 3.9 billion pounds, an increase of 59% compared to consumption rates in 1980 (Lucier and Lin, 2001). Sales of fresh-cut produce account for approximately 10% of the fresh fruits and vegetables marketed through food service and retail channels in the United States (Cantwell and Suslow, 2002), and cut watermelon represents approximately 10% of all

watermelon sales (Perkins-Veazie and Collins, 2004). A greater awareness of the link between better health and the inclusion of fruits and vegetables in the diet could be partially responsible for the increase in watermelon consumption. However, there may be additional benefit from the consumption of watermelon beyond conventional nutritional uptake because of its lycopene content. Unfortunately about 20% of the annual watermelon crop is left in the field as culls because of imperfections or undesirable appearance (Fish and others, 2009). The creation of value-added products from these melons could be beneficial to growers, because the extraction of lycopene for use as either dietary supplements or as natural food colorants is valued at $34 million in the United States (Collins and others, 2006).

Processed products from watermelons

The creation of further processed products might help increase consumer demand for watermelons. However, there is little information available regarding these types of items or how they are manufactured. The available literature tends to focus on breeding efforts to make modifications to the fresh melon itself. Examples include, developing a watermelon without seeds (Andrus and others, 1971) for consumers who prefer not swallowing them, developing a miniature or "icebox" water melon (Elmstrom and Crall, 1985) for consumers who have limited storage space or simply prefer small melons, and developing a melon with medium brix and high lycopene (Davis King, 2007) for consumers with diabetes.

There are additional products such as pickled watermelon rind and roasted watermelon seeds, yet these products represent minor uses of watermelon (Gusmini and Wehner, 2004). Pickled watermelon rinds would typically be made from cultivars that have thicker rinds; however current consumers prefer watermelons with thin rinds, so many of these thick rind cultivars have been discontinued from use in the market (Gusmini, G. Wehner, 2004). Additionally, there is little or no safety and science data available for the production of pickled watermelon rinds (Simonne and others, 2003).

However, Fish and others (2009) state that nominally a watermelon contains about 60% flesh, which in turn is comprised of about 90% juice containing 7 to 10% (w/v) sugars; therefore over 50% of a watermelon is fermentable liquid. This suggests that it might be feasible to manufacture wine from watermelon juice.

Lycopene

Lycopene is the pigment that is responsible for the characteristic red color of watermelon (*Citrullus lanatus*), and the consumption of lycopene is also thought to aid in the prevention of certain types of cancers and chronic diseases (Nguyen and Schwartz, 1999). However, there are many additional foods that contain lycopene, including tomatoes, condiments containing tomatoes, carrots, persimmons, peaches, red grapefruit, and spices (Nguyen and Schwartz, 1999; Perkins-Veazie and Collins, 2004). While tomatoes (*Lycopersicon esculentum*) are the major source of lycopene in the western diet (Anguelova and Warthsen, 2000), comprising approximately 80%, the average year-round concentration of lycopene in tomatoes is only 3025 ug/ 100g compared to the average concentration of lycopene in watermelons which is 4868 ug/ 100g (Edwards and others, 2003).

Lycopene occurs predominantly in the chromoplasts of plants (Nguyen and Schwartz, 1999) where it provides protection by absorbing light during photosynthesis which shields the plant against photosensitization; however, it can also be found in bacteria, fungi, and algae (Collins and others, 2006). Additionally, carotenoids contribute to the colors of flamingos, canaries, shrimp, lobsters, and salmon (Shi and Le Maguer, 2000). It is theorized that lycopene also increases the attractiveness of fruits and vegetables to herbivores, thereby increasing the fruits' chances for consumption, and ultimately aiding in the dissemination of their seeds (Collins and others, 2006).

Lycopene is classified as a carotenoid and is an aliphatic hydrocarbon with 11 conjugated carbon-carbon double bonds which are responsible for both its red color and lipid solubility

(Nguyen and Schwartz, 1999). The conjugated structure makes lycopene an extremely affective antioxidant and may also be responsible for its possible role in the prevention of certain types of cancer (Anguelova and Warthsen, 2000). However, this conjugated structure also makes lycopene susceptible to oxidative degradation, and like other carotenoids, sensitive to factors such as oxygen, exposure to light, and extremes in pH (Nguyen and Schwartz, 1999). Rotation around any of lycopene's double bonds may cause the formation of *cis* geometrical isomers; however, in tomato-based foods 79% to 91% of the lycopene is in the *trans* form and in red watermelon 92% to 95% of the total lycopene is in the *trans* form (Collins and others, 2006).

The majority of research regarding lycopene has been conducted on tomatoes and tomato-based products. There was no information found during the review of literature regarding the manufacture of processed products from watermelon and the subsequent effect on lycopene. Given this lack of information, there is an opportunity for research in this area to help establish basic processing parameters.

Initial research would be conducted on model food systems. However, it should be noted that the characteristic flavor of watermelon is volatile and is lost during heating while a bitter off-flavor is formed. An excellent way to minimize this may be to process watermelon into a product that requires little or no heat, such as wine. This would also have the added benefit of removing a potential cause of lycopene degradation.

Effect of heating on lycopene in tomato juice

There is some disagreement within the literature regarding the effect of thermal processing on lycopene from tomatoes. Nguyen and Schwartz (1999) state that typical temperatures used to process tomatoes have no effect on lycopene concentration or isomer content. Additionally, Nguyen and others (2001) conducted an experiment in which tomato slices were heated at 100 °C for 30 min in either distilled water or an 80:20 mixture of water and olive oil and found that this did not cause a significant increase in *cis*-isomer formation.

Agarwal and others (2001) measured the stability, isomeric form, and bioavailability of lycopene from tomatoes during different processing stages as the tomatoes were being made into juice. Samples were pulled and analyzed during the following stages: raw tomatoes, scalded pulp, salting tank, sterilizing tank, and processed juice. The researchers reported that after an initial lycopene concentration of 125.4 +/- 1.6 ppm in the fresh tomatoes there was a loss of 8% to 15% lycopene as the samples progressed to the scalded pulp stage. During this stage the tomatoes reached a maximum temperature of 76 ºC. There was no significant loss in lycopene observed in the subsequent processing operations, including the sterilizing tank where the tomato samples reached a maximum temperature of 120 ºC and the lycopene concentration was reported at 102.6 +/- 0.4 ppm. The lycopene concentration was reported at 102.3 +/- 0.8 ppm in the processed juice.

Agarwal and others (2001) also studied the effect of storage temperature on lycopene concentration in commercially canned tomato juice and found little change. Commercial canned tomato juice samples were stored for 0, 1, 3, and 12 months and held at 4 ºC, 25 ºC, and 37 ºC then analyzed. The researchers reported that there was no significant loss in lycopene concentration for up to 12 months of storage. Lycopene concentrations were reported as 91.1 +/- 0.8 ppm in the fresh juice, 89.5 +/- 0.6 ppm in the juice stored at 4 ºC for 12 months, 92.0 +/- 0.7 ppm in the juice stored at 25 ºC for 12 months, and 92.6 +/- 0.6 ppm in the juice stored at 37 ºC for 12 months.

Agarwal and others (2001) also studied the effect of heat processing on the formation of *cis*- isomers in tomato juice. Tomato juice samples with 10% corn oil were heated on a stovetop for 1 hour. The researchers found that these samples contained approximately 30% *cis*- isomers as compared to uncooked tomato juice without corn oil which contained 5.8% *cis*-isomers. Additionally, the researchers found that there was no significant change in the concentration of *cis*-isomers in the tomatoes cooked with corn oil after 4 months of storage at 4 ºC. They also found that heating did not change the concentration of total lycopene in the tomato juice.

While these results are in conflict with the results of Nguyen and others (2001), it should be noted that Agarwal and others (2001) did not indicate the temperature at which their tomato juice samples were cooked on the stovetop. Additionally, the cook time for their tomato juice was 60 minutes rather than 30 minutes which was the cook time used by Nguyen. This suggests that a higher cooking temperature, a longer cooking time, or some combination of both these factors, along with the addition of fat is necessary to favor the formation of *cis*-isomers in tomato juice.

Unlu and others (2007) conducted an experiment where high-lycopene variety tomatoes (FG99-218) were processed into two tomato sauces. Both contained 10% corn oil; however, sauce A was heated to 75 °C and hot-filled into cans while sauce B was filled into cans and then processed in a steriotort at 127 °C for 40 minutes. The researchers found that sauce A was rich in all-*trans* lycopene and contained a relatively small amount of *cis*-isomers (5%) while sauce B had an almost 9-fold increase in *cis*-isomers (45%). Their conclusion was that fat needed to be present in the product so that the lycopene could be solubilized and released from the food matrix to enhance isomerization by the thermal treatment.

Boskovic (1979) reported that the degradation of lycopene occurs first through isomerization then by autooxidation because of the unsaturated double bonds found in the structure of the lycopene molecule. However, there is disagreement in the literature as to which temperatures that this occurs. Hackett and others (2005) found that the lycopene present in tomato oleoresin would degrade mainly through oxidation at storage temperatures of 25 C and 50 C, and when the storage temperatures were increased to 75 C and 100 C, the lycopene degraded through isomerization.

Mayeaux and others (2006) investigated the thermal stability of lycopene standard. Twenty four 1 milliliter aliquots of 10 ppm lycopene in hexane were dried at 60 °C. One group each of eight samples was placed in a volumetric flask in a sand bath at 100, 125, and 150 °C, respectively. Samples were pulled at 10, 20, 30, and 60 minutes. They found that the lycopene

exhibited decreasing stability as the temperature increased from 100 to 150 °C. After 10 minutes of heating, 90%, 70%, and 30% of the original concentration of lycopene remained, respectively. After 60 minutes of heating the percentage of retained lycopene decreased to 53.5%, 20.9%, and 5.3%, respectively.

Mayeaux and others (2006) also investigated effect of microwave heating (twenty 25 gram samples heated at high power, 1000 w for 10, 15, 30, 45, or 60 seconds), frying (three 25 gram samples mixed with 30 milliliter soybean oil and heated at approximately 145 °C or 165 °C for 60 or 120 seconds), and baking (twelve 25 gram samples heated at 177 °C or 218 °C for 15, 30, or 35 minutes) on the lycopene found in a tomato slurry. They found that the samples that were heated in the microwave retained approximately 65% of their lycopene. Samples that were baked at 177 °C for 15, 30, and 45 minutes retained 64.1%, 45.6%, and 37.3%, respectively compared to 51.5%, 41.3%, and 25.1% for the samples baked at 218°C. Samples that were fried at 145 °C for 2 minutes retained 30% of the lycopene compared to 25% retained in the samples that were fried at 165 °C for 2 minutes.

Mayeaux and others (2006) suggested that the moisture contained in the tomato slurry may have provided the lycopene with some degree of protection during thermal processing, which resulted in the significant difference in stability of lycopene found between the tomato slurry and the lycopene standard. They conclude that lycopene is not stable when cooked at temperatures above 100° C.

Dewanto and others (2002) conducted an experiment in which tomato slurry was processed in a sealed pressure Stephan cooker and processed at 88 °C for 2 min, processed at 88 °C for 15 min, and processed at 88 °C for 30 min. The researchers reported that total *trans*-lycopene content in the tomatoes increased with increased heating time at 88 °C compared to an uncooked control. They also found that total *cis*-lycopene content in the tomatoes also increased with increased heating time at 88 °C compared to an uncooked control. This suggests the thermal processing helped to release both *cis*- and *trans*- isomers from the tomato tissue.

Takeoka and others (2001) others found that the processing of fresh tomatoes into paste caused the concentration of total lycopene to decrease by 9 to 28%; however, lycopene in tomatoes was relatively resistant to thermal degradation compared to pure lycopene in model systems. They suggest that the presence of other constituents in the tomatoes, such as tocopherols, ascorbic acid, and phenolic compounds, may help stabilize lycopene during processing.

Seybold and others (2004) suggested it may be possible that in some cases the differences in thermal processing effects may be due to researchers not taking moisture loss into account when calculating the lycopene concentration of samples that have been heat treated.

It should be noted that Edwards and others (2003) found that lycopene from watermelon was bio-available without heat processing. This suggests that any heat treatment that might be applied to the watermelon would only be necessary as a means to reduce the moisture content and increase the stability of a watermelon feedstock that could be used in further processed products.

Distribution of lycopene in the human body

The concentration of carotenoids is not equally distributed throughout human body tissue. While the adipose tissue has been thought of as the primary deposit center for these molecules, some organs, such as the liver, adrenals, and reproductive tissue, can actually have 10-fold higher concentrations of carotenoids (Erdman, 2005). High concentrations of lycopene have been found in the adrenal glands, testes, liver, prostate (Roldán-Gutiérrez and Luque de Castro (2007) and concentrations of lycopene have also been found in the breast, skin, cervix, and lung (Collins and others, 2006). Additionally, there can be as much as a 30 to 100 fold difference in lycopene concentration between individuals (Roldán-Gutiérrez and Luque de Castro, 2007).

Roldán-Gutiérrez and Luque de Castro (2007) state that the mechanism through which carotenoids are transported through the human body is that after absorption in the intestines the they are carried by chyclomicrons through the lymphatic system to the bloodstream. Once the

carotenoids are in the plasma they are transported by lipoproteins through the body. Carotenoids that have polar groups are carried on the lipoprotein surface at the aqueous interface. However, lycopene is carried in the hydrophobic portion of low density lipoproteins (LDL) because it is a hydrophobic molecule.

The variations in lycopene concentration and distribution may be due to either the relative number of LDL receptors contained within the tissue or the relative uptake of lipoproteins between the different body tissues (Kaplan and others, 1990; Schmitz and others, 1991). These variations may also be due to differences in metabolic or oxidative breakdown of the carotenoids within individuals (Erdman, 2005). Additionally, there may be some interaction between lycopene with other carotenoids and/ or interactions between these two sets of molecules along with other bioactive compounds present in fruits and vegetables that could influence or antagonize the absorption and bioavailability of lycopene (Roldán-Gutiérrez and Luque de Castro, 2007).

The concentration of *cis*- and *trans*- isomers of lycopene is not evenly distributed throughout the human body. The *cis*-isomeric form of lycopene appears to be present in higher concentration than the all-*trans* isomeric form in human tissue. Boileau and others (2002) found that there were as many as 18 different *cis*-isomeric form of lycopene in human prostate and serum, and that while all-*trans* and 5-*cis* isomers were the most abundant, 12-41% and 28% respectively, the sum of all *cis*-isomers made up the majority of total lycopene (55-88%).

Factors affecting the bioavailability of lycopene

The Food and Drug Administration defines the bioavailability of a drug "the rate and extent to which the active substances or therapeutic moiety is absorbed from a drug product and becomes available at the site for action" (Benet and Shiner, 1985). Bioavailability may also be thought of as the portion of a consumed nutrient that is available to the body through absorption

that can be used for normal physiological and metabolic function (Macrae and others, 1993; Jackson, 1997).

According to Shi and Le Maguer (2000), both of these definitions are useful ways of thinking of the uptake of lycopene by the human body. However, it should also be noted that the absorption of lycopene can be influenced by several dietary factors and food properties such as the level of intactness of the food matrix that incorporates the lycopene, the particle size of the source material, co-ingestion of fat, and *cis*- / *trans*- molecular linkage in the lycopene molecule.

The bioavailability of lycopene from processed tomatoes may be higher than from fresh tomatoes because both heating and mechanical disruption of the plant tissue can break down the structure of the cell wall as well as disrupt chromoplast membranes; this allows the lycopene to be more easily released from the plant matrix (Gartner and others, 1997; Shi and Le Maguer, 2000). Additionally, heating and mechanical disruption of the tomato matrix also helps to decrease the particle size of the plant tissue which in turn increases its surface area and the bioavailability of lycopene; however, excessive mechanical destruction of the plant tissue can also destroy lycopene so optimal processing parameters must be utilized to maximize the disruption of the matrix while minimizing the degradation of lycopene (Shi and Le Maguer, 2000).

Stahl and Sies (1992) reported that the bioavailability of lycopene increased when co-ingested with lipids. They found that test subjects that consumed tomato juice cooked with 1% corn oil at 100 C for 1 hour had increased levels of serum lycopene 1 day after consumption as compared to unprocessed controls. Gartner and others (1997) suggest that this is due to more of the lycopene being extracted from the food matrix into the fat during the thermal processing which increased the amount of lycopene available for absorption. The fat is also necessary for the formation of mixed micelles which in turn allow the carotenoids to be absorbed by enterocytes and transferred to body tissues by plasma lipoproteins (Frohlich and others, 2006).

The *cis*-isomeric form of lycopene appears to be more bioavailable or is preferentially absorbed compared to the *trans*- form (Collins and others, 2006; Stahl and others, 1992; Stahl and Sies, 1992; Unlu and others, 2007). It has been suggested that the all *trans* form of lycopene is a larger, more bulky molecule that tends to aggregate and form crystals and is therefore less soluble in bile acid micelles; however the *cis* form of lycopene is able to move across plasma membranes easier because the *cis* linkages make it a shorter molecule that can fit more easily into micelles (Boileau and others, 2002; Erdman, 2005) and therefore more easily transported within cells or between tissues (Roldán-Gutiérrez and Luque de Castro, 2007). However, the effect of the difference in isomeric form of lycopene on human health is unknown (Colllins and Veazie, 2006).

Health Benefits of Lycopene

Consuming foods that contain lycopene is thought to be beneficial because lycopene may help prevent certain types of diseases from occurring (Roldán-Gutiérrez and others, 2007). However, lycopene lacks the β–ionone ring structure that is present in β-carotene, and therefore does not have provitamin activity (Yeh and Hu, 2000). Therefore the health benefits of lycopene must occur through some activity or mechanism other than conventional nutrition.

The prooxidant/ antioxidant theory suggests that prooxidants, such as superoxide free radicals, influence the development of different types of chronic disease, such as cancer and cardiovascular disease (Meydani and others, 1998). Normal respiration produces superoxide free radicals as a byproduct, and approximately 1 to 3% of the oxygen taken into the body is converted into these superoxide species or other reactive oxygen species (Collins and others, 2006). These free radicals can then interact with macromolecules and cause damage to proteins, lipids, and DNA (Roldán-Gutiérrez and Luque de Castro (2007). The damage caused by the radicals to protein and DNA can result in mutations, while the damage caused to lipids can result in the formation of lipid peroxyl radicals which can cause a propagating chain of cellular damage (Collins and others, 2006).

A healthy human body will produce enzymes that can either slow or halt free radicals; however, as the body ages it becomes less efficient in suppressing the radicals which causes an increase in damage to the body and subsequently an increase in the incidence of disease (Wei and Lee, 2002). Most of the benefits associated with consuming lycopene are theorized to come from its ability to act as a scavenger of singlet oxygen and peroxy radicals and deactivating excited molecules (Roldán-Gutiérrez and others, 2007; Stahl and others, 1997). Singlet oxygen is not a free radical, but it can still form oxygen species that react with biomolecules to negative effect (Clinton, 1998).

Lycopene quenches singlet oxygen by either chemical or physical processes (Roldán-Gutiérrez and others, 2007). When lycopene physically quenches singlet oxygen, excitation energy is transferred from the oxygen, which returns to the ground-state, to the carotenoid which is then in the triplet excited state. Excess energy dissipates from the lycopene as heat through rotational and vibrational interaction with surrounding molecules, such as solvent molecules; when the lycopene cycles back to its ground-state the singlet oxygen is quenched (Roldán-Gutiérrez and others, 2007). During physical quenching the structure of the lycopene remains unchanged; however during chemical quenching the structure of lycopene is decomposed.

While the exact mechanism is unknown, it is lycopene's ability to act as an antioxidant that is thought to play a role in its reputed anti-carcinogenic effect by offering protection to lipids, DNA, and lipoproteins against oxidative damage and stress that are linked to carcinogenesis (Kris-Etherton and others, 2002). Lycopene's ability to act as an antioxidant is also thought to play a role in protecting against cardiovascular disease. Again, the exact mechanism is unknown; however it is theorized that lycopene may may protect lipoproteins and vascular cell against oxidative damage (Clinton, 1998).

Effect of fermentation on lycopene in tomato juice

Koh and others (2010) studied the effect of fermentation by three species of bifidobacteria on the chemical properties of tomato juice. They found that tomato juice that was fermented by either *Bifidobacterium breve* or *Bifidobacterium longum* had both a significantly lower pH and an increased titratable acidity compared to the non-fermented tomato juice control, indicating that these microorganisms could grow in a product such as tomato juice. However, tomato juice fermented by *Bifidobacterium infantis* did not show any significant difference in either pH or titratable acidity compared to the non-fermented tomato juice, indicating that it would not grow in tomato juice. Koh and others (2010) also reported that there was no significant difference in lycopene content in the tomato juice that was fermented by the *Bifidobacterium breve* (112.1 +/- 4.14 ug/g) compared to the tomato juice control that was not fermented (113.0 +/- 4.21 ug/g). Additionally, Fish and others (2009) conducted an experiment in which they determined that the chromoplasts contained in watermelon juice that had been fermented to produce 12% (w/v) ethanol were 97% viable. This indicates that the fermentation had no effect on the lycopene.

It should be noted that in products such as wine, it may be unlikely that carotenoid pigments would play a large role in any antioxidant activity given that they are hydrophobic molecules and would probably be removed during processing unless they were suspended in the wine by using stabilizers. It would be more likely that under typical circumstances phenolic compounds would be at least partially responsible for the antioxidant activity, given that they are hydrophilic molecules. While watermelons are an excellent source of lycopene, they contain moderately low levels of phenolic compounds (Mattila and others, 2006). These molecules are produced in watermelon plants in response to heat or cold stress (Rivero and others, 2001).

Lycopene concentration measured by HPLC

High performance liquid chromatography (HPLC) will be used to identify and quantify the concentration and isomeric forms of lycopene. We will follow the method employed by Perkins-Veazie and others (2006) which they used to identify the carotenoid profile of 50 watermelon cultivars. The method utilizes a YMC C30 carotenoid column, which can separate a wide polarity range of carotenoids and their isomers; however, it requires the use of a gradient reverse mobile phase because of the column's strong retention of the carotenoids (Craft, 2001).

The method (Perkins-Veazie and others, 2006) requires an HPLC equipped with an auto-sampler, photodiode array detector, and integration software. It also requires a C30 YMC carotenoid column (4.6 X 250 mm^2), YMC carotenoid guard column S-3 (4.0 X 20 mm^2), and a column oven. The method for the reverse mobile phase uses 3 solvents. Solvent A consisted of 90% methanol and 10% Milli-Q water containing 0.5% triethylamine (TEA) and 150 mM ammonium acetate. Solvent B consisted of 99.5% 2-propanol and 0.5% TEA. Solvent C consisted of 99.95% tetrahydrofuran and 0.05% TEA. The following solvent gradient was used: initial conditions 90% solvent A plus 10% solvent B; 24 minute gradient switched to 54% solvent A, 35% solvent B, and 11% solvent C; final gradient conditions were 11 minute gradient of 30% solvent A, 35% solvent B, and 35% solvent C and then held for 8 minute. The mobile phases were returned to initial conditions over 15 min.

Antioxidant activity measured by Oxygen Radical Absorbance (ORAC) assay

The Oxygen Radical Absorbance Capacity (ORAC) assay is a method for measuring antioxidant activity and has been used to analyze many different types of foods. The assay measures antioxidant scavenging activity against peroxyl radicals generated by 2,2 '-Azobis(2-Amidinopropane) Hydrochloride (AAPH). This antioxidant activity is measured by comparing the area under the fluorescence decay curve (AUC) of the test sample, a blank sample that

contains no antioxidant, and 6-hydroxy-2 5 7 8-tetramethylchroman-2-carboxylic acid (TROLOX) (Ou and others, 2001).

Several improvements have been made to the assay. These include replacing β -phycoerythrin as the fluorescence probe with 3‘,6‘-dihydroxyspiro[isobenzofuran-1[3*H*],9‘[9*H*]-xanthen]-3-one (fluorescein) because it is not photostable and it reacts variably with the peroxyl radicals which results in inconsistency in the assay (Ou and others, 2001). An additional improvement includes the addition of randomly methylated β-cyclodextrin (RMCD) to the assay to act as a water solubility enhancer, which extends the use of the assay to include lipophilic antioxidants (Huang and others, 2002).

Summary

Watermelons contain high concentrations of lycopene, and the literature suggests that lycopene is beneficial to health. However, watermelons that are otherwise fit for human consumption are often culled because of undesirable appearance. It might be possible to use these melons to develop value-added products; yet there is little information regarding manufacturing processed food products from watermelon. Therefore, the object of our project was to determine how different food processing techniques could affect the content and isomeric form of lycopene in value-added watermelon products.

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CHAPTER III

Experiment 1

Use of different food processing techniques to extract lycopene from watermelon.

Abstract

Samples of red seedless watermelon (cultivar unknown, purchased at local retail market) were processed using four different treatments. The first treatment consisted of unfiltered/ macerated watermelon tissue. The second treatment consisted of unfiltered/ macerated watermelon tissue heated to 80 ºC. The third treatment consisted of pressed/ filtered watermelon juice. The fourth treatment consisted of pressed/ filtered watermelon juice heated to 80 ºC. Significantly ($p<0.05$) higher concentrations of lycopene were measured in unheated and heated unfiltered/ macerated tissue treatments as compared to unheated and heated pressed/ filtered juice treatments (9.22 mg/100 mL and 7.93 mg/100 mL vs 4.01/100 mL and 4.18 mg/100 mL, respectively). No significant difference in lycopene concentration was found between unheated and heated samples within the unfiltered macerated tissue treatments or the pressed/ filtered juice treatments (9.22 mg/100 mL vs 7.93 mg/100 mL and 4.01 mg/100 mL vs 4.18 mg/100 mL,

respectively). No difference in antioxidant activity as measured by lipophilic-ORAC was measured within the unheated and heated maceration treatments or within the unheated and heated press/ filtered juice treatments (0.16 µM TE/ g and 0.11 µM TE/ g vs 0.05 µM TE/ g and 0.07 µM TE/ g, respectively). There was a significant difference between unfiltered/ macerated tissue without heat treatment and pressed/ filtered juice without heat (0.16 µM TE/ g vs 0.05 µM TE/ g). However there was no significant difference between unfiltered/ macerated tissue with heat treatment and pressed/ filtered juice with heat treatment (0.11 µM TE/ g vs 0.07 µM TE/ g). Heating had no effect on the amount of lycopene or the antioxidant activity within either maceration or pressing treatments. None of the treatments alone or in combination resulted in the formation of *cis* isomers. HPLC analysis determined that the lycopene was in the all *trans* form.

The results indicate that more lycopene was measured in the unfiltered macerated tissue treatments than in the press/ filtered juice samples. Because there was no filtered macerated tissue control, additional research is required to determine if the difference between maceration and pressing was due to more lycopene being released by the macerated tissue samples or more lycopene being retained in the filter of the pressed juice samples.

Introduction

Lycopene (gamma, gamma-carotene) is the pigment responsible for the characteristic red color of tomatoes and watermelons. The majority of the research in the United States concerning lycopene and processed food products has been conducted on tomatoes, since they comprise a larger part of the Western diet (Anguelova and Warthsen, 2000). The available literature tends to focus on breeding efforts to make modifications to the fresh melon itself. Examples include: developing a watermelon without seeds (Andrus and others, 1971) for consumers who prefer not spitting them out; developing a miniature or "icebox" water melon (Elmstrom and Crall, 1985)

for consumers who have limited storage space or simply prefer small melons; and developing a melon with medium brix and high lycopene (Davis King, 2007) for consumers with diabetes.

There is little information available that focuses on the effect of heating on lycopene from watermelon; however, the use of heating during the processing of tomatoes, such as the hot break and cold break methods, is common. Thermal treatment of fresh watermelons might be used to concentrate watermelon pulp for transport to be further processed into foods, supplements, or colorants. Heating might also be used to make lycopene from watermelon tissue more bioavailable.

However, different plant matrices may provide different levels of protective effects and influence the stability of lycopene during processing (Xianquan and others, 2005). It may also be possible that different processing treatments prior to heating may influence the stability of lycopene in watermelon. Therefore the objective of this study was to use bench top equipment to mimic the effect of industrial maceration and pressing on watermelon tissue and measure their effect on lycopene extractability, stability, antioxidant activity and isomeric form.

Materials and Methods

Chemicals

HPLC: All solvents used were HPLC grade and were purchased from Fisher Scientific (Fair Lawn, NJ). Triethlyamine and ammonium acetate were also purchased from Fisher Scientific. Deionized water was produced by a Milli-Q unit (Millipore, Bedford, MA, USA). Lycopene standard (Lot number SL09054, Sigma-Aldrich, St-Louis, MO).

ORAC: Potassium phosphate dibasic (K2HPO4) ACS (EM Science, US)), sodium phosphate monobasic (NaHPO4*H2O) ACS (Fisher Scientific NJ, US), AAPH [2,2'-azobis(2-amidino-propane) dihydrochloride] (Waco Chemicals Inc., Richmond, VA), Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Fluka Chemika,

Switcherland), fluorescein disodium (Sigma-Aldrich, St-Louis, MO), Randomly Methylated β-Cyclodextrin (RMCD) (Cyclodextrin Technologies Development Inc., www.cyclodex.com).

Lycopene extraction

Lycopene was extracted using a method modified from Sadler and others (1990). Approximately 2 g of sample was placed in a 250 mL aluminum foil covered Erlenmeyer flask along with 25 mL of ethanol, 25 mL acetone, and 50 mL of hexane. The flask was covered and agitated at 200 rpm in a shaker (Classic C76, New Brunswick Scientific, Edison, NJ) for 10 minutes, then 15 mL of de-ionized water was added. The flask was then agitated in the shaker for an additional 5 minutes and afterwards allowed to sit for 15 minutes. The upper hexane layer was then decanted. This layer was used for subsequent ORAC and HPLC analyses.

ORAC conditions

All ORAC assays were performed at 37° C using the fluorimeter function of a Perkin-Elmer HTS-7000 microplate reader (Perkin-Elmer, Waltham, Massachusetts) set at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

Lipophilic ORAC

Prior to performing the lipophilic ORAC assay (Huang and others, 2002), the hexane extract mentioned in the lycopene extraction procedure above was dried down using a Thermo Savant SPD Speed Vac (Thermo Fisher Scientific Inc., Waltham, MA), re-suspended in 100% acetone,and then diluted accordingly with a mixture of 50:50 acetone:de-ionized water with 7% RMCD. The 50:50 mixture of acetone:de-ionized water with 7% RMCD was used as the blank and Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at 10μM concentration was used as a standard. Fluorescein (FL) was used as a fluorescent probe and a target of free

radical attack, with AAPH [2,2'-azobis(2-amidino-propane) dihydrochloride] being a peroxyl radical generator. Readings were carried out on BD Falcon 48 well clear polystyrene microplates (VWR International Inc., Bridgeport, NJ).

Results for the lipophilic ORAC assay was obtained by calculating the Area Under the fluorescence decay Curve (AUC) for the Blank, Trolox, and Sample using the following equation:

AUC = $f1/f0 + ...fi/f0 + ... + f34/f0 + f35/f0$

where $f0$ = initial fluorescence reading at 0 min and fi = fluorescence reading at time i.

The net areas of the Trolox and Samples are compared after the area of the Blank is subtracted. After adjusting for dilution factors and weight, the results are expressed as μmoles Trolox equivalent (TE) per gram of sample. The plate layout is presented in figure 1.

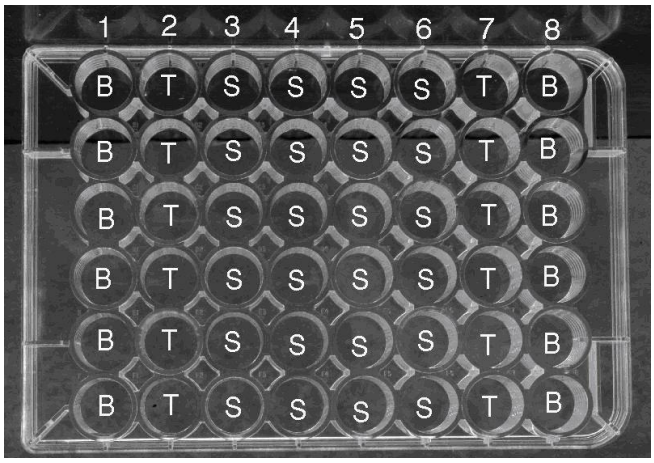

Figure 1. Reagent and sample layout on a 48 well plate.

B = Blank T = Trolox S = Sample

HPLC analysis

Chromatographic conditions

The method (Craft, 2001) for the gradient reverse mobile phase utilized 3 solvents. Solvent A consisted of 90% methanol and 10% Milli-Q filtered water (RG Ultra-pure water system, Millipore Corp.) that contained 0.5% triethylamine (TEA) and 150 mM ammonium acetate. Solvent B consisted of 99.5% 2-propanol and 0.5% TEA. Solvent C consisted of 99.95% tetrahydrofuran and 0.05% TEA. The following solvent gradient was used: initial conditions 90% solvent A plus 10% solvent B; 24 minute gradient switched to 54% solvent A, 35% solvent B, and 11% solvent C; final gradient conditions were 11 minute gradient of 30% solvent A, 35% solvent B, and 35% solvent C and then held for 8 minute. The mobile phases were returned to initial conditions over 15 minutes. One mL of the hexane extract (described above) was filtered using a 0.45μm Polytetrafluoroethylene (PTFE) syringe filter into an amber screw top vial and then loaded onto a Dionex HPLC system (Dionex, Sunnyvale, CA).

The system consisted of a Dionex P680 HPLC pump, a Dionex ASI-100 Automated SampleInjector, a Dionex TCC-100 Thermostatted Column Compartment, and a Dionex Ultimate 3000 Photodiode Array detector. The column used was a YMC C30 carotenoid column (4.6 X 250 mm^2) along with a YMC carotenoid guard column S-3 (4.0 X 20 mm^2) (Waters, Milford, MA). The carotenoid column was used because it can separate a wide polarity range of carotenoids and their isomers. The injection volume for treatment samples was 20 μL. Duplicate injections of lycopene standard were made at volumes of 2 μL, 5 μL, 10 μL, 15 μL, and 20 μL to generate a standard curve for sample analysis.

Sample storage and processing

The red seedless watermelons were purchased from a local supermarket in June and stored inside a walk-in cooler at approximately 4 ºC for 3 days before they were processed. Each

melon served as a replicate. The flesh from each melon was collected, chopped, mixed, then vacuum sealed (Multivac C500, Multivac Inc. Kansas City, MO) into plastic bags (8 x 14 inches). Approximately 2 pounds of watermelon sample was placed in each bag which was then covered in aluminum foil and stored in a freezer at -12 ºC. Before each experiment, the samples were allowed to thaw at refrigeration temperatures (2-4 ºC) overnight before being processed.

Sample treatments

In the first treatment, approximately 25 grams of watermelon tissue was macerated for 1 minute using a Waring blender (model 51BL310), then homogenized on ice using a PowerGen 700 homogenizer (Fisher Scientific, Pittsburgh, PA) set at medium speed (setting 3) for 3 sets of 20 seconds each, pausing 10 seconds in between sets. Two 2 gram sub-samples were then pulled and extracted using the Sadler method. In the second treatment, the same procedure was used as the first treatment, except that after homogenization, the 25 gram watermelon sample was placed in a 50 mL beaker that was placed inside a stainless steel pan that was filled with enough water to cover the level of the watermelon sample. The pan was heated on a hot plate until the sample reached a temperature of 80 ºC. The beaker was removed from the hot plate and two 2 gram sub samples were pulled and extracted using the Sadler method. The maceration process in the first and second treatments was meant to mimic the action of an industrial chopper such as a Hobart vertical chopper (see figure 2).

In the third treatment, approximately 50 grams of watermelon sample was pressed through a portion of Miracloth using a manual fruit press (Ito Juicer; www.discountjuicers.com; Rohnert Park, CA) to collect 25 grams of watermelon juice. Two 2 grams sub samples were then pulled and extracted using the Sadler method. In the fourth treatment, the same procedure was used as the third treatment, except that after collecting the 25 grams of watermelon juice, the juice was placed in a 50 mL beaker that was placed inside a stainless steel pan that was filled with enough water to cover the level of the watermelon sample. The pan was heated on a stirring hot

plate until the sample reached a temperature of 80 ºC. The sample was agitated during heating using a magnetic stir bar and temperatures were taken with a mercury in glass thermometer. The beaker was removed from the hot plate and two 2 gram sub samples were pulled and extracted by the Sadler method. The pressing process used in the third and fourth treatments was meant to mimic the action of an industrial wine press such as a water-powered bladder press (see figure 3).

Figure 2. Example of a Hobart vertical chopper.

Figure 3. Example of a water-powered bladder press.

Statistical Analysis

Analysis of variance procedures were used to compare the effects of the factors on ORAC and Lycopene. Each experiment utilized a randomized complete block model with subsampling, with melon serving as block. Two subsamples for each melon and treatment combination were taken. The effects of two factors were investigated, treatment and heat. The simple effects of each factor (given the other factor) were investigated with the use of simple effects contrast in the ANOVA. A significance level of 0.05 was used to establish statistical significance. Statistical analysis software (SAS) version 9.2 was used to perform the analysis.

Results and discussion

HPLC analysis determined that unheated and heated unfiltered macerated tissue treatments had significantly ($p<0.05$) higher concentrations of total lycopene than unheated and heated pressed/ filtered juice treatments (see figure 4). It is possible that the unfiltered maceration treatments released more lycopene for extraction from the plant tissue than the press treatments. Unheated and heated unfiltered maceration treatments in combination had an average of 109% more lycopene than unheated and heated press/ filtered juice treatments in combination.

Additionally, Shi and Le Maguer (2000) stated that mechanical tissue disruption enhances the bioavailability of lycopene in tomatoes by breaching cell walls, disrupting chromoplast membranes, and reducing cellular integrity. However, Seybold and others (2004) found that homogenization (70 bar, 62.5 kg/h) of canned tomatoes caused a decrease in the lycopene content of the samples. They propose that the pressure (70 bar) destroyed carotenoid-protein complexes and the membranes of chromoplasts which allowed the lycopene to be degraded by oxygen and high temperatures. The much lower and shorter-time pressures experienced by the watermelon samples in our experiments, approximately one bar for one minute, may have been insufficient to cause the kinds of lycopene degradation observed by Seybold and others.

However, it should be noted that there was no filtered control for the maceration treatments. Therefore it is possible that the press/ filtered treatments had lower lycopene concentrations because some portion of the lycopene was retained by the Miracloth during pressing and only a portion made it through to the juice.

HPLC analysis determined that the lycopene present in all of the samples was in the all *trans* form, regardless of treatment. Samples heated to 80 °C, unfiltered macerated tissue samples, and pressed/ filtered juice samples either alone or in combination were insufficient to

cause the formation of *cis*-isomers (see figures 6, 7, 8, and 9). However, a small unidentified peak occurred at a retention time of 38 minutes.

Nguyen and Schwartz (1999) and Nguyen and others (2001) state that typical temperatures used in food processing of tomatoes had no effect on lycopene concentration or isomer content. Additionally, Re and others (2002) found that tomatoes that were processed into sauce and paste under the following conditions showed an increase in lycopene concentration as compared to unprocessed tomatoes: hot break (HB)-processed at 90ºC, cold break (CB)-processed at 65ºC and super cold break (SCB)-processed at 65ºC under vacuum. Additionally, Re and others (2002) found that there was no change in the isomeric composition of the lycopene before and after the tomatoes were processed. More than 90% of the lycopene was in the all-*trans* form. Dewanto and others (2002) observed an increase in all-*trans* lycopene (3.11 +/- 0.04, 5.45 +/- 0.02, and 5.32 +/- 0.05 mg of all *trans*-lycopene/g of tomato) as compared to unheated raw tomato (2.01 +/- 0.04 mg of all *trans*-lycopene/g of tomato) after heating tomatoes at 88 °C for after 2, 15, and 30 min. They also observed an increase in *cis* lycopene (0.91 +/- 0.15, 1.01 +/- 0.13, and 1.16 +/- 0.21 mg/100 g of tomato) during heating as compared to unheated raw tomato (0.86 +/- 0.09 mg/100 g of tomato). Also, Takeoka and others (2001) found no consistent changes in lycopene levels when they processed fresh tomatoes into hot break juice. However, Shi and Le Maguer(2000) found that tomato juice that was heated for 7 minutes at either 90 °C or 100 °C resulted in a 1.1 or 1.7% decrease in total lycopene, respectively. Hackett and others (2004) investigated the stability of tomato oleoresins under different storage temperatures and times. They observed that the total lycopene concentration decreased as storage temperatures increased from 25 °C to 50 °C, 75 °C, and 100 °C. They also observed that formations of *cis* isomers increased as storage temperatures increased above 75 ºC. However, their oleoresin samples were generated by solvent extracting lycopene from pureed tomato tissue, removing the solvent and then re-suspending the lycopene in corn oil. By removing the lycopene from the protection of the tomato tissue, it may have been more susceptible to thermal degradation. Additionally, Seybold

and others (2004) observed that when they heated a purified standard of lycopene that had been dissolved in corn oil it readily isomerized; however when they processed fresh tomatoes into soup, juice, and sauce the lycopene was not susceptible to thermal isomerization. Since the times and temperatures investigated in our experiments were below those used by these other researchers, they may have been insufficient to cause significant isomerization of the lycopene.

Lipophilic-ORAC measured significantly ($p<0.05$) higher levels of antioxidant activity in unheated and heated unfiltered macerated tissue treatments than in the unheated press/ filtered juice treatment. Additionally, a significantly higher level of antioxidant activity was measured in the unheated unfiltered macerated tissue treatment than in either unheated or heated press/ filtered juice treatments (see figure 5). No significant difference in antioxidant activity was found between the heated unfiltered maceration treatment and the heated press/ filtered juice treatment, and no significant difference was found within unheated and heated unfiltered maceration treatments or within unheated and heated pressed/ filtered juice treatments.

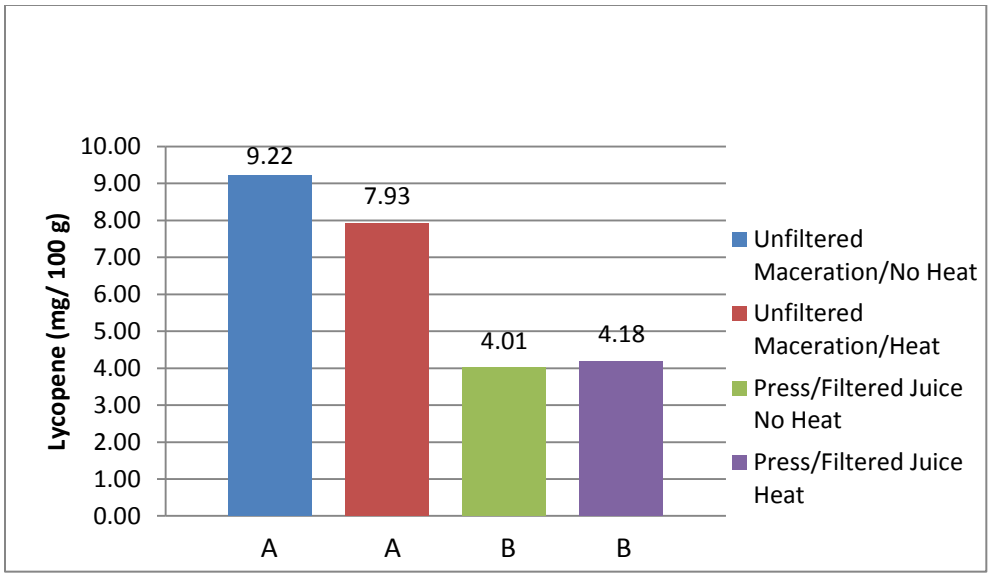

Figure 4. Comparison of lycopene content in watermelon treatments. Numbers represent an average lycopene concentration (mg/ 100 g) of duplicate samples run in 3 replications.

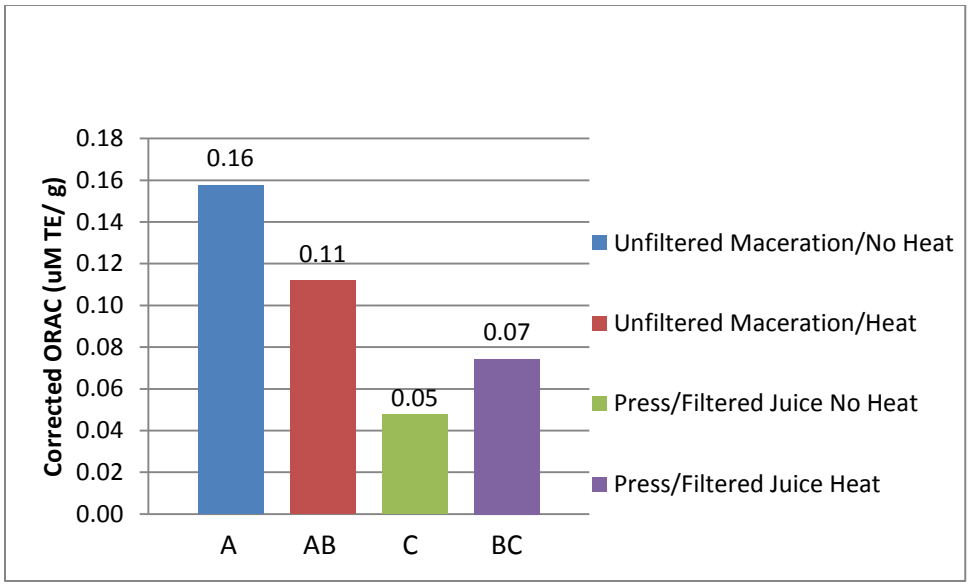

Figure 5. Comparison of antioxidant activity in watermelon treatments. Numbers represent average antioxidant activity (µM TE/ g) of duplicate samples run in 3 replications.

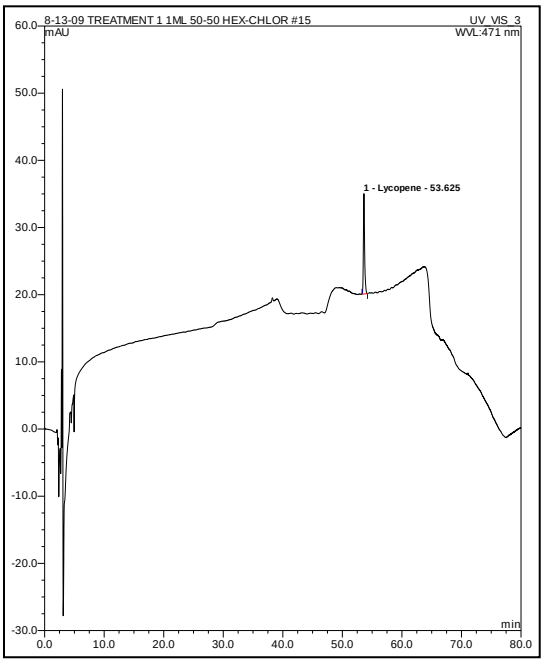

Figure 6. Typical chromatograph for Unfiltered Maceration/ No Heat.

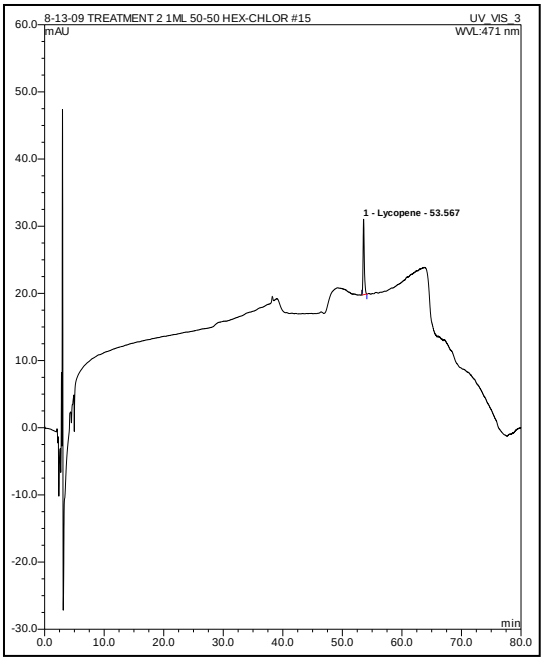

Figure 7. Typical chromatograph for Unfiltered Maceration/ Heat.

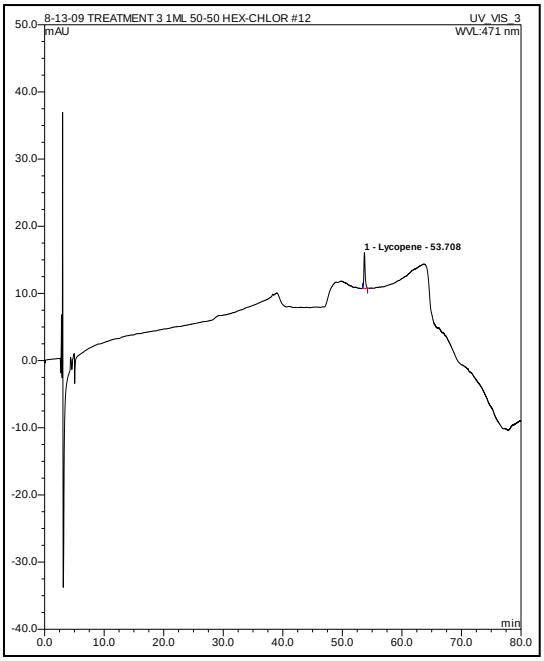

Figure 8. Typical chromatograph for Press/Filtered Juice No Heat.

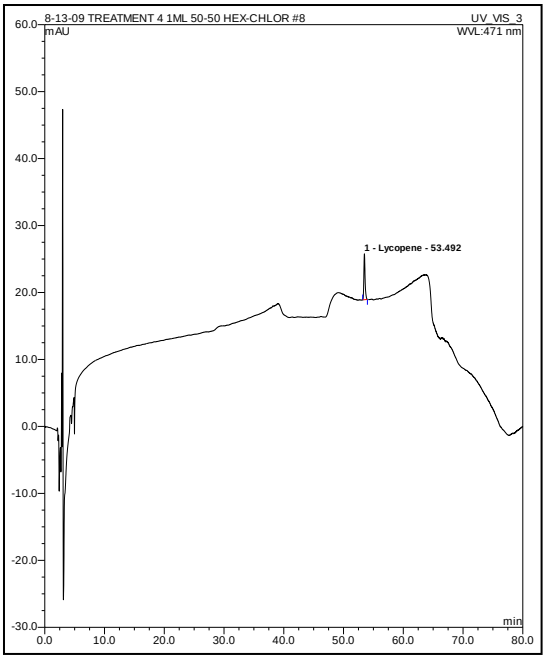

Figure 9. Typical chromatograph for Press/Filtered Juice Heat.

Conclusions

The unfiltered maceration treatment appears to be a better method for preparing melon tissue from culled melons for lycopene extraction because this treatment resulted in higher concentrations of lycopene in the extracts compared to the pressed/ filtered juice treatments. However, there was no filtration control in the maceration treatments, so the difference may have been due to a portion of the lycopene being retained by the Miracloth and not being released into the juice. Additional research including a filtered maceration control is necessary to identify the difference between these treatments.

Heating had no effect on the amount of lycopene or the antioxidant activity within either maceration or pressing treatments. This suggests that heating watermelon to 80 °C would not affect the extraction or isomeric form of lycopene from culled watermelons. None of the

treatments, alone or in combination, were sufficient to cause the formation of *cis*-isomers of lycopene.

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CHAPTER IV

Experiment 2

Determining the lycopene stability, isomeric forms, and antioxidant activity of a watermelon-based analogue to tomato sauce.

Abstract

Red seedless watermelon samples (cultivar unknown, purchased at local retail market) were concentrated to viscosities similar to that of tomato sauce either by heating or by evaporation under vacuum. The samples were then analyzed for lycopene concentration, antioxidant activity, and isomeric profile. HPLC analysis revealed that the cooked watermelon samples appeared to have generated a small amount of *cis*-isomers and significantly ($p<0.05$) decreased the amount of lycopene as compared to the uncooked samples. No other differences were found between the heated and unheated samples.

Introduction

Lycopene (gamma, gamma-carotene) is the pigment responsible for the characteristic red color of tomatoes and watermelons. Our previous experiment investigated the effect of a minimal thermal process (80 ºC) on lycopene in watermelon and found that it had no significant ($P<0.05$) effect on lycopene concentration or isomeric profile. Yet many tomato products, such as sauce and paste, are processed at higher temperatures for longer periods of time. For example, canned tomato products may be processed using the hot break method, where the raw tomatoes are heated to 95 ºC to destroy enzymes that are detrimental to quality (Collins and others, 2006). However, different plant matrices may provide different levels of protective effects and influence the stability of lycopene during processing (Xianquan and others, 2005). Therefore, the objective of the current study is to develop a watermelon product that is an analogue to tomato sauce, and determine the effect on lycopene stability, antioxidant activity and isomeric form.

Materials and methods

Chemicals

HPLC: All solvents used were HPLC grade and were purchased from Fisher Scientific. Triethlyamine and ammonium acetate were also purchased from Fisher Scientific. Deionized water was produced by a Milli-Q unit (Millipore, Bedford, MA, USA). Lycopene standard (Lot number SL09054, Sigma-Aldrich, St-Louis, MO).

ORAC: Potassium phosphate dibasic (K2HPO4) ACS (EM Science, US)), sodium phosphate monobasic (NaHPO4*H2O) ACS (Fisher Scientific NJ, US), AAPH [2,2'-azobis(2-amidino-propane) dihydrochloride] (Waco Chemicals Inc., Richmond, VA), Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Fluka Chemika, Switcherland), fluorescein disodium (Sigma-Aldrich, St-Louis, MO), Randomly

Methylated β-Cyclodextrin (RMCD) (Cyclodextrin Technologies Development Inc., www.cyclodex.com).

Lycopene extraction

The lycopene was extracted using a method modified from Sadler and others (1990). Approximately 2 g of sample was placed in a 250 mL aluminum foil covered Erlenmeyer flask along with 25 mL of ethanol, 25 mL acetone, and 50 mL of hexane. The flask was covered and agitated at 200 rpm in a shaker (Classic C76, New Brunswick Scientific, Edison, NJ) for 10 minutes then 15 mL of de-ionized water was added. The flask was then agitated in the shaker for an additional 5 minutes and afterwards allowed to sit for 15 minutes. The upper hexane layer was used for subsequent ORAC and HPLC analyses. The extracts were stored in a freezer at -12 ºC.

ORAC conditions

All ORAC assays were performed at 37° C using a Perkin-Elmer HTS-7000 microplate reader (Perkin-Elmer, Waltham, Massachusetts) set at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

Lipophilic ORAC

Prior to performing the lipophilic ORAC assay, the hexane extract mentioned in the lycopene extraction procedure above was dried down using a Thermo Savant SPD Speed Vac (Thermo Fisher Scientific Inc., Waltham, MA), re-suspended in 100% acetone, and then diluted accordingly with a mixture of 50:50 acetone:de-ionized water with 7% RMCD. The 50:50 mixture of acetone:de-ionized water with 7% RMCD was used as the blank and Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at 10μM concentration was used as a standard. Fluorescein (FL) was used as a fluorescent probe and a target of free radical attack, with AAPH [2,2'-azobis(2-amidino-propane) dihydrochloride] being a peroxyl radical generator.

Readings were carried out on BD Falcon 48 well clear polystyrene microplates (VWR International Inc., Bridgeport, NJ).

Results for the lipophilic ORAC assay was obtained by calculating the Area Under the fluorescence decay Curve (AUC) for the Blank, Trolox, and Sample using the following equation:

AUC = $f1/f0 + ...fi/f0 + ... + f34/f0 + f35/f0$

where $f0$ = initial fluorescence reading at 0 min and fi = fluorescence reading at time i.

The net areas of the Trolox and Samples are compared after the area of the Blank is subtracted. After adjusting for dilution factors and weight, the results are expressed as μmoles Trolox equivalent (TE) per gram of sample. The plate layout is presented in figure 10.

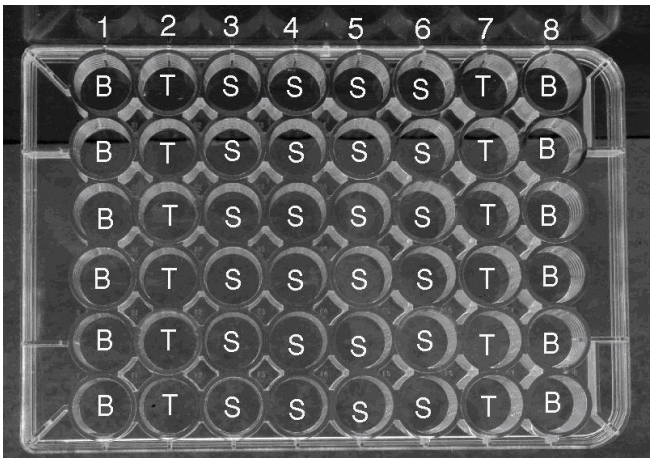

Figure 10. Reagent and sample layout on a 48 well plate.

B = Blank T = Trolox S = Sample

HPLC analysis

Chromatographic conditions

The method (Craft, 2001) for the gradient reverse mobile phase utilized 3 solvents. Solvent A consisted of 90% methanol and 10% Milli-Q filtered water (RG Ultra-pure water system, Millipore Corp.) that contained 0.5% triethylamine (TEA) and 150 mM ammonium

acetate. Solvent B consisted of 99.5% 2-propanol and 0.5% TEA. Solvent C consisted of 99.95% tetrahydrofuran and 0.05% TEA. The following solvent gradient was used: initial conditions 90% solvent A plus 10% solvent B; 24 minute gradient switched to 54% solvent A, 35% solvent B, and 11% solvent C; final gradient conditions were 11 minute gradient of 30% solvent A, 35% solvent B, and 35% solvent C and then held for 8 minute. The mobile phases were returned to initial conditions over 15 minutes. One mL of the hexane extract (described above) was filtered using a 0.45μm Polytetrafluoroethylene (PTFE) syringe filter into an amber screw top vial and then loaded onto a Dionex HPLC system (Dionex, Sunnyvale, CA).

The system consisted of a Dionex P680 HPLC pump, a Dionex ASI-100 Automated Sample Injector, a Dionex TCC-100 Thermostatted Column Compartment, and a Dionex Ultimate 3000 Photodiode Array detector. The column used was a YMC C30 carotenoid column (4.6 X 250 mm^2) along with a YMC carotenoid guard column S-3 (4.0 X 20 mm^2) (Waters, Milford, MA). The carotenoid column was used because it can separate a wide polarity range of carotenoids and their isomers. The injection volume for treatment samples was 20 µL. Duplicate injections of lycopene standard were made at volumes of 2 µL, 5 µL, 10 µL, 15 µL, and 20 µL to generate a standard curve for sample analysis.

Sample storage and processing

Red seedless watermelons were purchased from locally from a local supermarket in July and stored inside a walk-in cooler at approximately 4 ºC for 1 day before processing. Each melon served as a replicate.

Sample treatments

Approximately 1500 grams of tissue from a red seedless watermelon was collected and processed for 1 minute in a Black & Decker Quick and Easy Plus (Walmart, Stillwater, OK) food processor. The liquefied tissue was then weighed into two portions, each approximately 700

grams. The first portion was weighed then placed in a 1 L beaker and heated on a Corning Stirrer hot plate (Fisher Scientific NJ, US) set to the maximum heat setting until the liquefied tissue reached the consistency of tomato sauce. During heating the sample was agitated using a magnetic stir bar. This was accomplished by pulling three samples of the cooked tissue every 20 minutes, allowing them to cool to room temperature, and then testing the consistency of the samples using a Bostwick consistometer (Fisher Scientific) until the samples had reached the same viscosity (8.5 on the Bostwick scale after 30 seconds) as a commercially prepared tomato sauce (Hunt's Tomato Sauce). This took approximately two hours of heating. The sample was then weighed again. The differences in weight were used to calculate the percent of water lost during cooking. The cooked sample was then extracted according to the Sadler method described above.

A two gram sample of the second portion of liquefied watermelon tissue was immediately extracted according to the Sadler method. The weight of the hexane extract was recorded and it was then centrifuged under vacuum using a Thermo Savant SPD Speed Vac. The centrifuge was stopped every 20 minutes and the weight of the extract was measured until the percent weight of solvent lost was approximately the same as the percent weight of water lost in the cooked watermelon samples. The hexane extract of lycopene was then analyzed by lipophilic-ORAC and HPLC according to the methods described above. The hexane extracts were prepared and stored as described in lycopene extraction above. Duplicate samples were run in 3 replicates.

Statistical Analysis

Analysis of variance procedures were used to compare the effects of the factors on ORAC and Lycopene. Each experiment utilized a randomized complete block model with subsampling, with melon serving as block. Two subsamples for each melon and treatment combination were taken. The effects of treatment were assessed. The means and standard errors for each level of the factors concerned are reported for both response variables. A significance

level of 0.05 was used to establish statistical significance. Statistical analysis software (SAS) was used to perform the analysis.

Results and discussion

Analysis by HPLC determined that there was a significantly ($p < 0.05$) higher concentration of lycopene in the uncooked watermelon samples than in the cooked watermelon samples, 17.10 mg/ 100 g versus 13.68 mg/100 g, respectively (see figure 11). Additionally, a very small peak appears before the lycopene peak at a retention time of approximately 51 minutes (see figure 14) that does not appear in the uncooked watermelon (see figure 13). We believe this peak to a *cis*-isomer of lycopene based on observations made by Perkins-Veazie and others (2006) (see Appendices), who used the same HPLC method to characterize carotenoid content in watermelons. This seems to indicate that heating the watermelon samples at boiling temperatures for two hours was sufficient to cause the formation of *cis*-isomers. An unidentified peak occurs at a retention time of 38 minutes.

Our results do not support the observations made by researchers such as Nguyen and Schwartz (1999) and Nguyen and others (2001) who state that typical food processing temperatures have no effect on lycopene concentration, as there was a significant difference between our treatments.

However, our results do support other researchers that have found that thermal processing can reduce the lycopene concentration in tomato products. Mayeaux and others (2006) compared changes in lycopene content in tomato slurry after processing by different cooking methods. They observed that samples that were baked at 177 ºC for 15, 30, and 45 minutes retained 64.1%, 45.6%, and 37.3% lycopene, respectively. However, when samples were baked at 218 ºC, they retained 51.5%, 41.3%, and 25.1% lycopene, respectively. Additionally, Takeoka and others

(2001) found that when they processed fresh tomatoes into tomato juice there was no consistent change in lycopene levels. However, when fresh tomatoes were processed into tomato paste, statistically significant decreases in lycopene levels of 9-28% occurred.

Lipophilic-ORAC analysis measured no significant difference ($p < 0.05$) in antioxidant activity between uncooked and cooked watermelon (see figure 12).

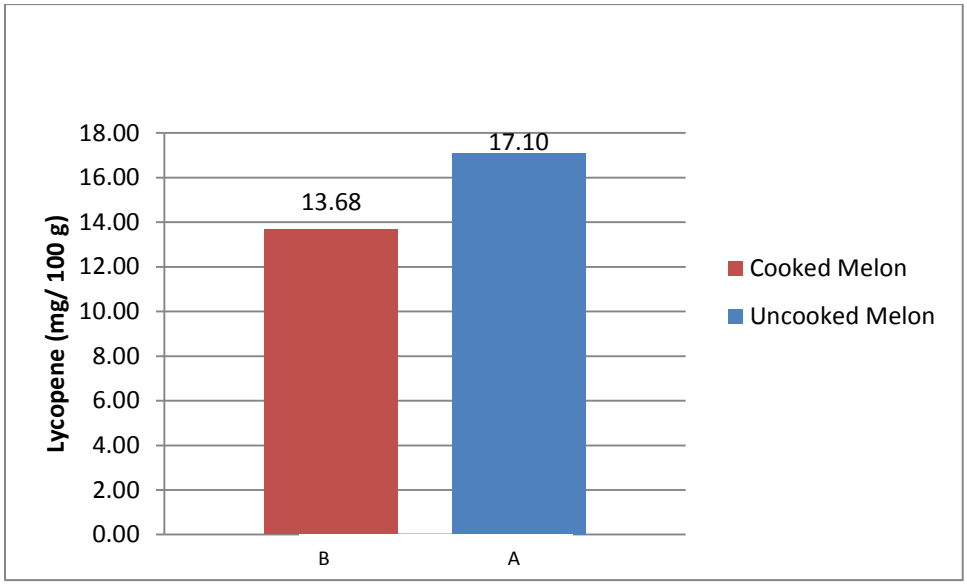

Figure 11. Comparison of lycopene content of cooked and uncooked melon. Numbers represent an average lycopene concentration (mg/ 100 g) of duplicate samples run in 3 replications.

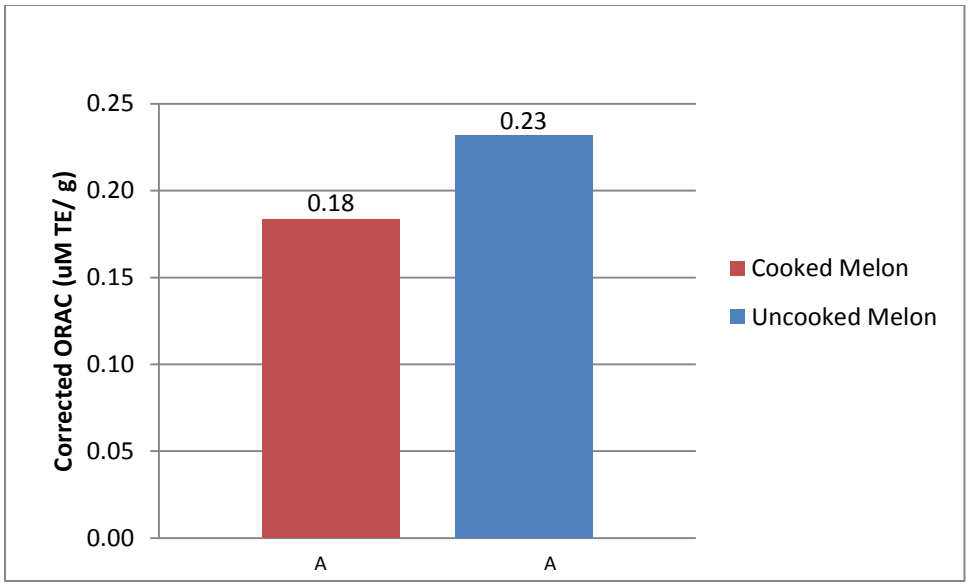

Figure 12. Comparison of antioxidant activity of cooked and uncooked melon. Numbers represent an average antioxidant activity (µM TE) of duplicate samples run in 3 replications.

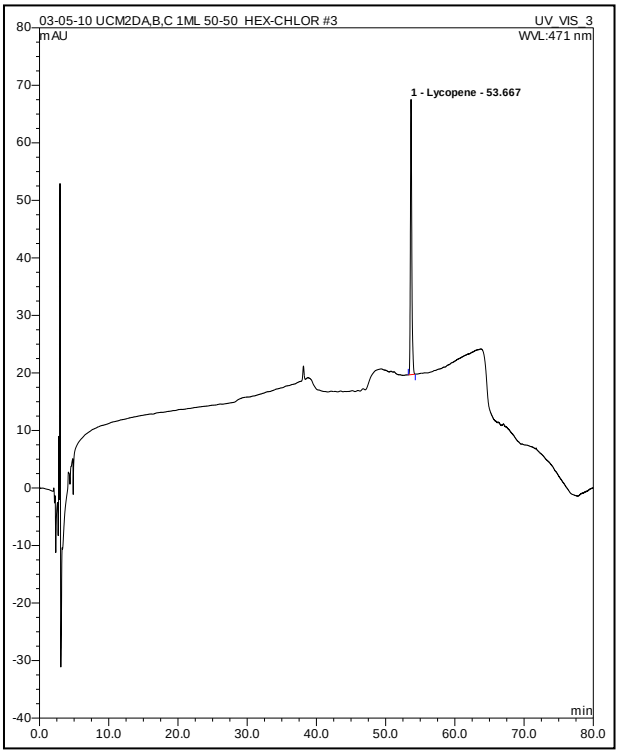

Figure 13. Typical chromatograph of Uncooked melon.

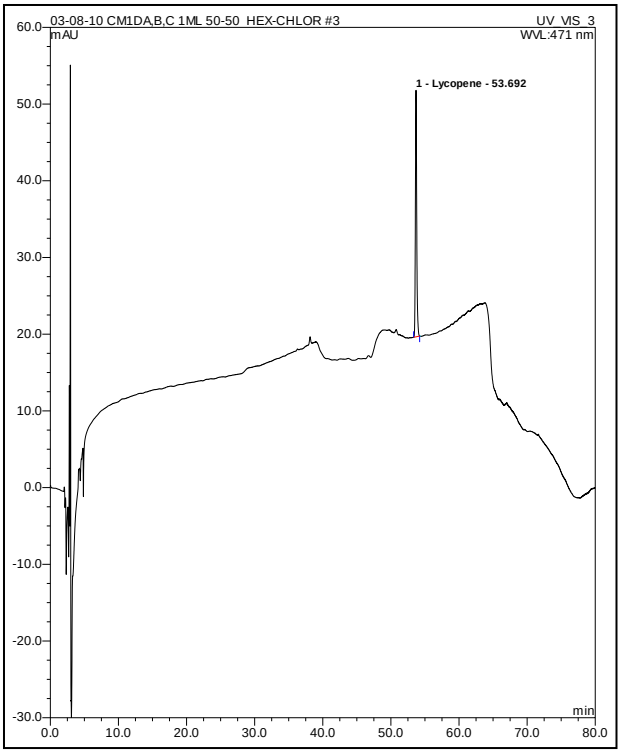

Figure 14. Typical chromatogram Cooked melon.

Conclusions

HPLC analysis revealed a small peak in the heated samples that did not occur in the unheated samples. We believe that this is a *cis*-isomer of lycopene. Additionally, the watermelon samples that were concentrated using heat had significantly ($p < 0.05$) lower amounts of lycopene than watermelon samples that were concentrated using vacuum drying. However, no difference in antioxidant activity was detected by lipophilic-ORAC.

These results suggest that caution is needed when temperatures that are similar to those used in tomato processing are applied to the flesh of culled watermelons. The higher temperatures could cause the loss of lycopene when attempting to make a product that is analogous to tomato sauce. Reduction in lycopene concentration due to heating has also been observed by other researchers such as Mayeaux and others (2006) and Takeoka and others (2001). Additional research establishing the maximum processing temperature that causes no reduction in lycopene concentration is necessary.

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CHAPTER V

Experiment 3

Evaluating lycopene stability, antioxidant activity and isomeric form in watermelon wine.

Abstract

Neither basic wine-making processes nor fermentation by *Saccharomyces cerevisiae* had an effect on the isomeric form of lycopene in watermelon juice. Lycopene recovered from wine lees, the dead yeast cells and other particulate matter that settled to the bottom of the unracked wine, was in the same all-*trans* form as the lycopene from the watermelon juice prior to fermentation. Additionally, fermentation did not inhibit the antioxidant activity of lycopene found in the lees. While no lycopene was detected in the finished wine samples, those samples did have significantly ($p<0.05$) higher antioxidant activity as measured by hydrophilic-ORAC than did the watermelon juice or lees as measured by lipophilic ORAC. The antioxidant activity observed in the wine may have been due to the presence of phenolic compounds, citrulline, or potassium metabisulfite. However, no analysis was performed to measure these compounds; thus, their possible contributions to antioxidant activity were not quantified.

Introduction

Wine is reputed to have beneficial health effects such as protecting against cardiovascular disease, loss of bone density, and dementia (Hasler, 2009). While the ethanol content may play some role in these health benefits, the phenolic compounds present in wine are also thought to be partly responsible by acting as natural antioxidants (Netzel and others, 2003).

The phenolic content of wine can vary due to factors such as the variety of grape and the processing method. Even the progression of fermentation introduces chemical changes to the grape by processes such as altering the conjugation of organic acids and phenolic compounds, extracting and forming co-pigments, and developing an anaerobic and protective redox potential (German and Walzem, 2000). However, an important distinction to note is that the phenolic compounds found in wine, grapes, and many others fruits are hydrophilic molecules while the lycopene that is found in watermelon is a hydrophobic molecule.

Lycopene is the primary carotenoid found in red watermelon and is mainly found in the *trans* geometric isomer in 92 percent to 95 percent of the total lycopene content (Collins and others, 2006). It is also thought that lycopene may aid in the prevention of certain types of cancer (Nguyen and Schwartz, 1999). However, approximately 20% of the annual watermelon crop is rejected for the fresh fruit market and is left in the field because they are misshapen or blemished (Fish and others, 2009). Therefore, it may be possible to produce wine from watermelon culls and waste as an additional source of value-added income to farmers.

There have been studies conducted on utilizing tropical fruits to produce wine (Maldanado and others, 1975), studies conducted on utilizing watermelon for the production of vinegar (Kim and others, 1984), studies conducted on utilizing watermelon juice as a feedstock for bio-fuel production (Fish and others, 2009), and even studies conducted on utilizing watermelon to produce wine (Hwang and others, 2004); however, none of these studies focused on how basic wine-making techniques and subsequent fermentation affect lycopene concentration, the formations of *cis*-isomers, and antioxidant capacity of wine that is

manufactured from watermelons. Therefore the objective of this experiment was to determine the effect of basic wine-making processes on the lycopene content originally present in watermelon juice.

Materials and Methods

Chemicals

HPLC: All solvents used were HPLC grade and were purchased from Fisher Scientific (Fair Lawn, NJ). Deionized water was produced by a Milli-Q unit (Millipore, Bedford, MA, USA). Triethlyamine and ammonium acetate were also purchased from Fisher Scientific.

ORAC: Potassium phosphate dibasic (K2HPO4) ACS (EM Science, US)), sodium phosphate monobasic (NaHPO4*H2O) ACS (Fisher Scientific NJ, US), AAPH [2,2'-azobis(2-amidino-propane) dihydrochloride] (Waco Chemicals Inc., Richmond, VA), Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Fluka Chemika, Switcherland), fluorescein disodium (Sigma-Aldrich, St-Louis, MO), Randomly Methylated β-Cyclodextrin (RMCD) (Cyclodextrin Technologies Development Inc., www.cyclodex.com).

Miscellaneous: Lalvin K1-V1116 yeast (*Saccharomyces cerevisiae*) (Lallemand Inc, Montreal, Canada), Fermaid wine yeast nutrient and potassium metabisulfite (Presque Isle Wine Cellars, North East PA), L-Tartaric acid (Fisher Scientific, Fair Lawn, NJ).

Sample Collection and Preparation

The first replicate group of 20 red seedless watermelons was purchased from a local supermarket in June and the second replicate group of 25 red seedless watermelons was purchased from a local fruit and vegetable supplier in December. All watermelons were stored inside a walk-in cooler at approximately 4 ºC for approximately 2 days before being processed.

Winemaking and sampling

The flesh was collected from red seedless watermelons by hand using knives and the rinds discarded. The tissue was macerated for approximately 30 seconds in a Hobart vertical chopper (Troy, OH) until a slurry-like texture was reached. The slurry was poured into a water-powered bladder press (Zampelli Enotech JRL, Italy), and pressed until the press cake stopped releasing watermelon juice. The water was at room temperature and the water pressure was at 60 psig. Approximately 15 gallons of watermelon juice was collected for both replicates, and about 15 pounds each was set aside as an unfermented control for subsequent HPLC and ORAC analysis. The remaining watermelon juice was poured into a 25 gallon Letina stainless steel fermentation vessel (Saint Pat's, Austin, Texas) and mixed with sucrose to increase the brix concentration. The brix was adjusted from 8.5° to 24.1° for the first replicate and from 9.57° to 22.1° for the second replicate. The pH of the first replicate was adjusted from 5.35 to 4.5 with 74 grams of tartaric acid. The pH of the second replicate was adjusted from 5.29 to 3.57 with 220 grams of tartaric acid. Additionally, 25 grams of yeast in 250 mL water, 65 grams of fermaid yeast nutrient, and 4 grams of potassium metabisulfite were also added to the first replicate. Thirty grams of yeast in 300 mL of water, 110 grams o fermaid yeast nutrient, and 5 grams potassium metabisulfite were added to the second replicate. The adjustment of the pH and brix as well as the addition of the nutrients and the potassium metabisulfite was to ensure the growth of the *Saccharomyces cerevisiae* and to prevent the growth of wild yeast and/ or bacteria. The fermentation vessel was sealed with an airlock and allowed to ferment. After three weeks, the wine was racked into glass carboys (see figure 15 for process flowchart). The first replicate was racked once into three, 3gallon glass carboys and one, 1gallon carboy. The second replicate was racked once into two, 5gallon glass carboys and two, 1gallon carboys. Approximately 200 grams of sample for subsequent analysis was pulled at the time of racking from both the wine and lees, the dead yeast cells and other particulate matter that settled to the bottom of the wine.

HPLC was used to measure the lycopene content of the watermelon juice, wine, and lees. Lipophilic-ORAC was used to measure antioxidant activity in the watermelon juice, wine, and lees. The hydrophilic-ORAC assay was also used to measure antioxidant activity in the wines.

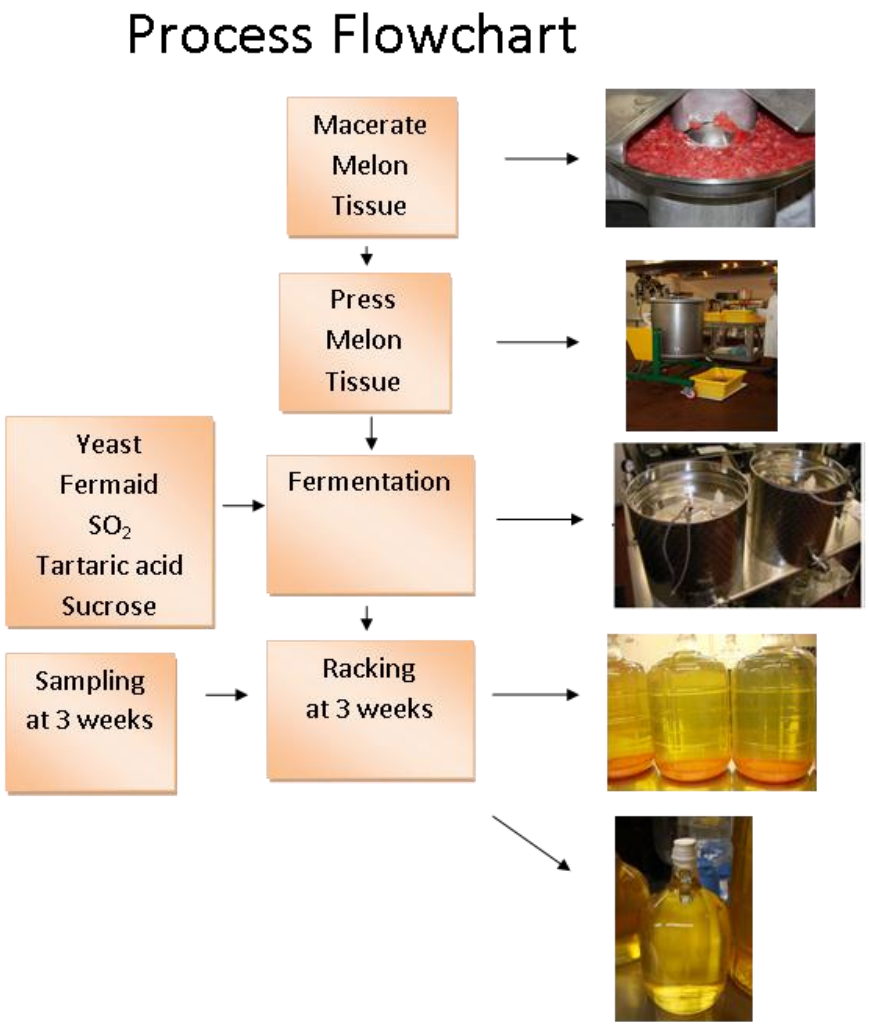

Figure 15. Process flow for wine-making.

Lycopene extraction

The lycopene was extracted using a method modified from Sadler and others (1990). Approximately 2 g of sample was placed in a 250 mL aluminum foil covered Erlenmeyer flask along with 25 mL of ethanol, 25 mL acetone, and 50 mL of hexane. The flask was covered and agitated at 200 rpm in a shaker (Classic C76, New Brunswick Scientific, Edison, NJ) for 10 minutes then 15 mL of de-ionized water was added. The flask was then agitated in the shaker for an additional 5 minutes and afterwards allowed to sit for 15 minutes. The upper hexane layer was then used for subsequent ORAC and HPLC analyses.

ORAC Assay

ORAC conditions

All ORAC assays were performed at 37° C using a Perkin-Elmer HTS-7000 microplate reader (Perkin-Elmer, Waltham, Massachusetts) set at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

The watermelon wine was a source of lipophilic and hydrophilic compounds that both exhibited antioxidant activity. Therefore it was necessary to perform both lipophilic- and hydrophilic-ORAC assays. While the reaction mechanisms of the assays are the same, the dilution of the reagents, standards, and test samples are different. Hydrophilic-ORAC used phosphate buffer as a diluent, while lipophilic-ORAC used a mixture of 50:50 acetone:de-ionized water with 7% randomly methylated cyclodextrin (RMCD). The RMCD was used to enhance the solubility of the lycopene in the samples. Additional details for each assay can be found below.

Hydrophilic ORAC

Phosphate buffer adjusted to pH 7.0 was used as a blank and Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at 10μM concentration was used as a standard.

Fluorescein (FL) was used as a fluorescent probe and a target of free radical attack, with AAPH [2,2'-azobis(2-amidino-propane) dihydrochloride] being a peroxyl radical generator. Readings were carried out on BD Falcon 48 well clear polystyrene microplates (VWR International Inc., Bridgeport, NJ). Wine samples were diluted accordingly with phosphate buffer.

Lipophilic ORAC

Prior to performing the lipophilic ORAC assay, the hexane extract mentioned in the lycopene extraction procedure above was dried down using a Thermo Savant SPD Speed Vac (Thermo Fisher Scientific Inc., Waltham, MA), re-suspended in 100% acetone, and then diluted accordingly with a mixture of 50:50 acetone:de-ionized water with 7% RMCD. The 50:50 mixture of acetone:de-ionized water with 7% RMCD was used as the blank and Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) at 10μM concentration was used as a standard. Fluorescein (FL) was used as a fluorescent probe and a target of free radical attack, with AAPH [2,2'-azobis(2-amidino-propane) dihydrochloride] being a peroxyl radical generator. Readings were carried out on BD Falcon 48 well clear polystyrene microplates (VWR International Inc., Bridgeport, NJ). Juice and lees samples were diluted accordingly with the 50:50 mixture of acetone:de-ionized water with 7% RMCD.

Results for both hydrophilic and lipophilic ORAC assays were obtained by calculating the Area Under the fluorescence decay Curve (AUC) for the Blank, Trolox, and Sample using the following equation: AUC = $f1/f0 + ...fi/f0 + ... + f34/f0 + f35/f0$

where $f0$ = initial fluorescence reading at 0 min and fi = fluorescence reading at time i.

The net areas of the Trolox and Samples are compared after the area of the Blank is subtracted. After adjusting for dilution factors and weight, the results are expressed as μmoles Trolox equivalent (TE) per gram of sample. The plate layout is presented in figure 16.

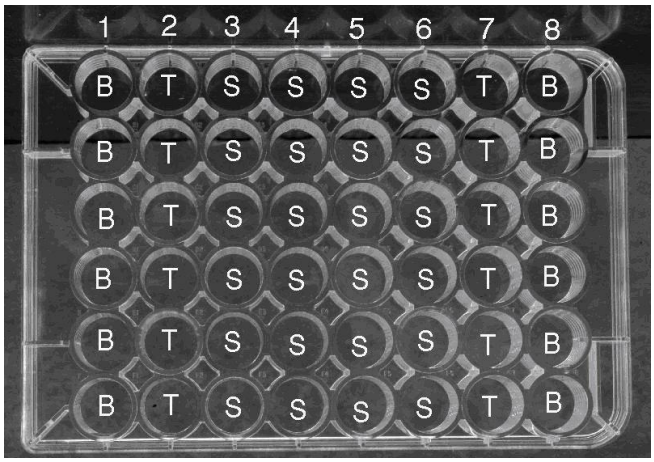

Figure 16. Reagent and sample layout on a 48 well plate.

B = Blank T = Trolox S = Sample

HPLC analysis

Chromatographic conditions

The method (Craft, 2001) for the gradient reverse mobile phase utilized 3 solvents. Solvent A consisted of 90% methanol and 10% Milli-Q filtered water (RG Ultra-pure water system, Millipore Corp.) that contained 0.5% triethylamine (TEA) and 150 mM ammonium acetate. Solvent B consisted of 99.5% 2-propanol and 0.5% TEA. Solvent C consisted of 99.95% tetrahydrofuran and 0.05% TEA. The following solvent gradient was used: initial conditions 90% solvent A plus 10% solvent B; 24 minute gradient switched to 54% solvent A, 35% solvent B, and 11% solvent C; final gradient conditions were 11 minute gradient of 30% solvent A, 35% solvent B, and 35% solvent C and then held for 8 minute. The mobile phases were returned to initial conditions over 15 minutes. One mL of the hexane extract (described above) was filtered using a 0.45μm Polytetrafluoroethylene (PTFE) syringe filter into an amber screw top vial and then loaded onto a Dionex HPLC system (Dionex, Sunnyvale, CA).

The system consisted of a Dionex P680 HPLC pump, a Dionex ASI-100 Automated Sample Injector, a Dionex TCC-100 Thermostatted Column Compartment, and a Dionex

Ultimate 3000 Photodiode Array detector. The column used was a YMC C30 carotenoid column (4.6 X 250 mm^2) along with a YMC carotenoid guard column S-3 (4.0 X 20 mm^2) (Waters, Milford, MA). The carotenoid column was used because it can separate a wide polarity range of carotenoids and their isomers. The sample injection volume was 20 µL.

Statistical Analysis

Confidence intervals were constructed for the treatments in Microsoft Excel 2010 version 14.0.6112.5000 for the treatments based an alpha of 0.05, the standard deviation of the mean and the population size.

Results and Discussion

HPLC analysis identified lycopene in the watermelon juice samples and the lipophilic-ORAC assay measured antioxidant activity (see figure 17 and 18). Significantly ($p < 0.05$) higher concentrations of lycopene as well as significantly higher levels of antioxidant activity were identified in the lees samples, as compared to watermelon juice. Additionally, HPLC analysis determined that the lycopene in both of these samples (see figures 19 and 20) was in the all-*trans* formation. This indicates that fermentation of the watermelon juice by *Saccharomyces cerevisiae* did not inhibit its antioxidant activity or affect its isomeric profile. These results are consistent with those reported by Koh and others (2010). They stated that the lycopene content in tomato juice was unaffected by fermentation with bifidobacteria.

An unidentified peak appearing at retention time of approximately 38 minutes was observed in both the watermelon juice and lees samples. However, at the HPLC limit of detection (minimum peak area 0.5"[signal]*min") no lycopene was measured in the wine samples, and no antioxidant activity was measured by the lipophilic-ORAC assay. The absence of lycopene in the wine samples is likely due to its lipophilic nature. After being released from the watermelon tissue during maceration in the vertical chopper and crushing in the wine press, lycopene would

gradually have settled out of the juice over time due to its hydrophobicity. The lycopene would then slowly become part of the lees over the course of fermentation and would have been removed from the wine during racking. This movement of the lycopene was observed by the researchers as a change in color and transparency of the wine samples. The characteristic red color of watermelon was initially present throughout the freshly yeast-inoculated juice; however the color gradually turned from opaque red to pink then translucent as the lycopene settled out of the fermenting juice prior to racking.

As no lycopene was detected in the watermelon wine samples by HPLC, the hydrophilic-ORAC assay was used to quantify the antioxidant activity of these samples. The hydrophilic-ORAC assay measured antioxidant activity in the wine samples (see figure 17) that was significantly ($p< 0.05$) higher than the activity measured by the lipophilic ORAC assay in either the watermelon juice or lees. The source of this activity could be due to the presence of phenolic compounds, which are produced when watermelon plants are exposed to heat or cold stress (Rivero and others, 2001). However, Mattila and others (2006) indicate that watermelon contains only low levels (<10 mg/100 g) of phenolic acids. Therefore, there may be additional water soluble compounds that are also responsible for the observed antioxidant activity. Citrulline is a non-essential amino acid, first identified from watermelon juice, which is also a strong antioxidant that may protect the leaves of the watermelon plant from drought-induced oxidative stress by acting as a hydroxyl radical scavenger (Rimando and Perkins-Veazie, 2005). Citrulline is also water soluble (Curis and others, 2005). Additionally, the potassium metabisulfite that was added as part of the wine-making can have an antioxidant effect (Simon, 1986). Small amounts of SO_2 can also be generated by yeast during fermentation.

Interestingly, the measured average hydrophilic-ORAC value (4.3 µM Trolox Equivalents / gram watermelon tissue) for the watermelon wine is notably higher than the value reported for fresh watermelon tissue (1.23 µM Trolox Equivalents / gram watermelon tissue) by the USDA (USDA Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected

Foods, Release 2, 2010). We cannot say whether this higher value represents normal variation among watermelon samples or whether the wine contained hydrophilic antioxidant compounds not normally found in the juice – perhaps as a product of fermentation or as a result of compounds added to the juice as part of the winemaking process.

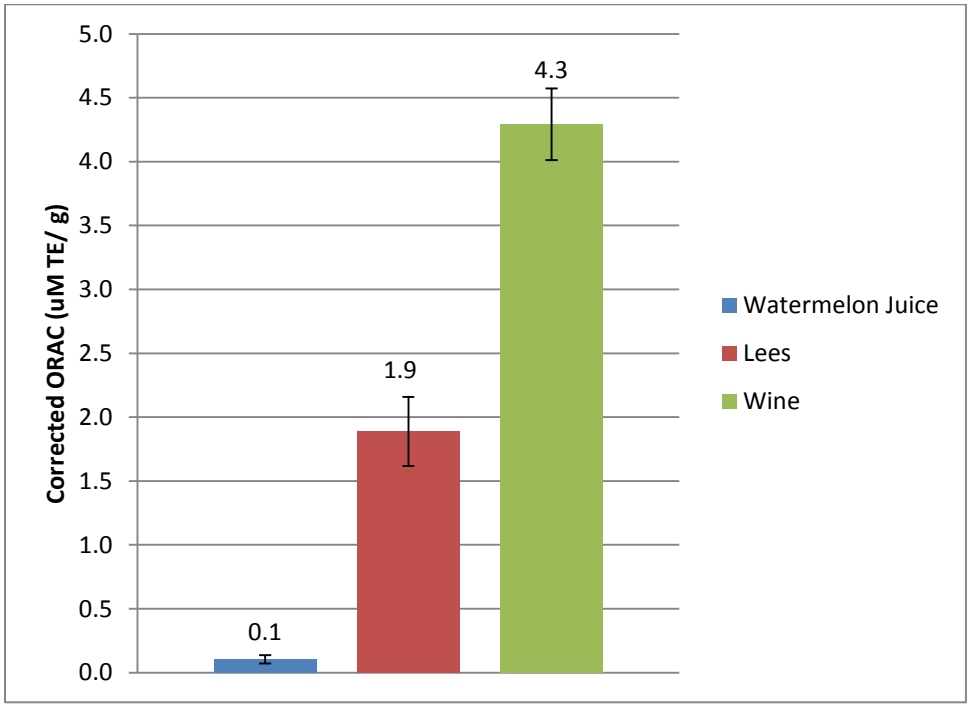

Figure 17. Comparison of antioxidant activity of juice, lees, and wine. Numbers represent an average of duplicate samples for 2 replicates. Watermelon juice and lees values are from lipophilic-ORAC and wine value is from hydrophilic-ORAC.

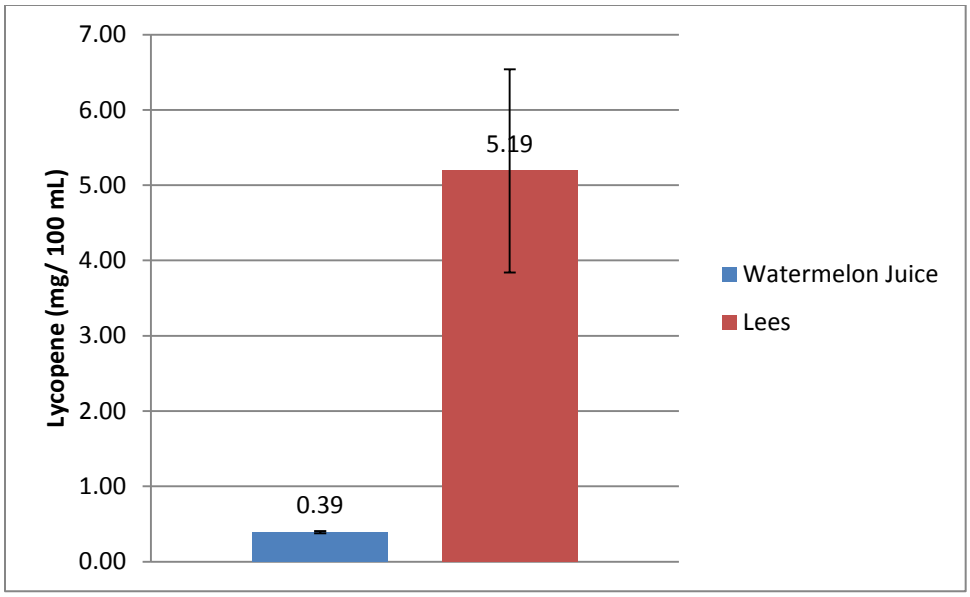

Figure 18. Comparison of lycopene content of juice and lees. Numbers represent an average of duplicate samples for two replicates.

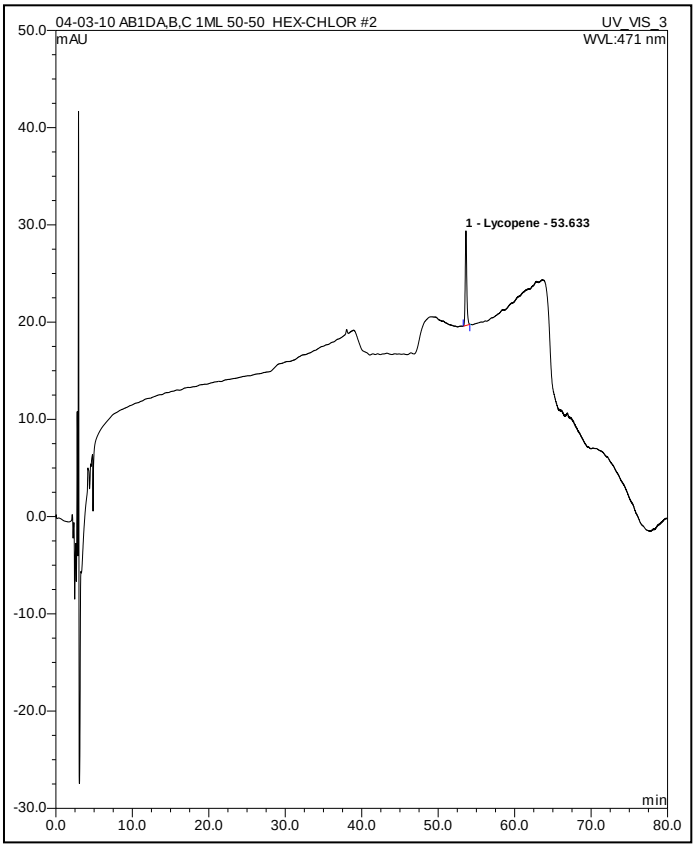

Figure 19. Typical HPLC chromatogram for juice.

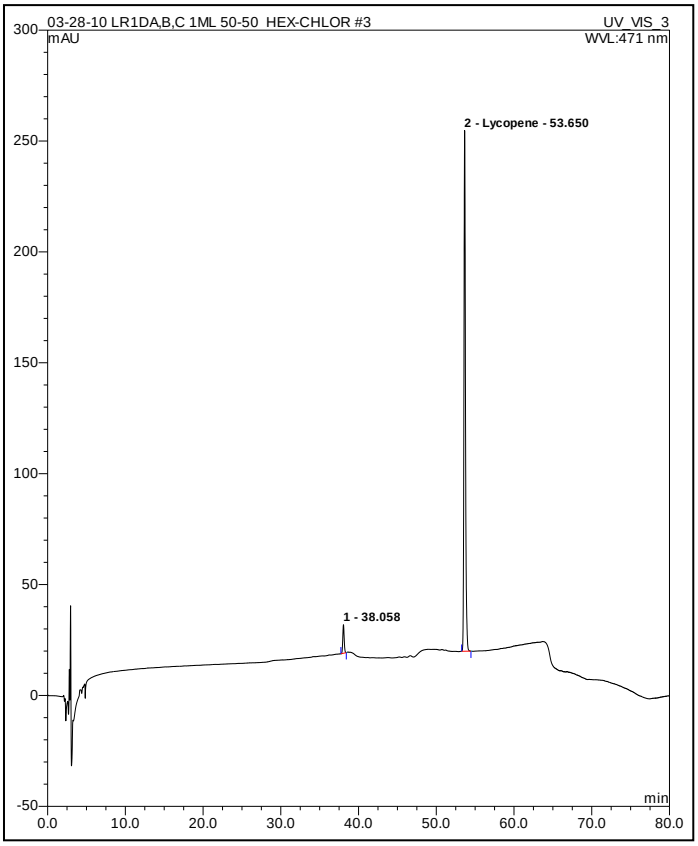

Figure 20. Typical HPLC chromatogram for lees.

Conclusions

Neither the basic wine-making techniques utilized nor the fermentation of the watermelon juice by *Saccharomyces cerevisiae* affected the profile of geometric lycopene isomers found in the lees of wine made from watermelon juice. While antioxidant activity was measured by the lipophilic ORAC assay in both the juice and lees, the activity in the lees was significantly higher. No lycopene was detected in the finished wine and no antioxidant activity was detected in the wine by the lipophilic ORAC assay. However, the hydrophilic ORAC assay measured antioxidant activity in the wine that was higher than the antioxidant activity measured by the lipophilic ORAC assay in both the juice and the lees. Some portion of this activity may be

due to phenolic compounds in the watermelon juice; however, these compounds are typically found in low concentrations. It is also possible that citrulline, an amino acid found in watermelon rind, may have played a role. Also, the potassium metabisulfite the was added as part of wine-making process, as well as the SO2 generated by the yeast as a by-product of fermentation, may have influenced the antioxidant activity. However, no analysis was performed to measure these compounds. This may be an area for future research.

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CHAPTER VI

Conclusions

Watermelon is the leading U. S. melon crop in terms of acreage, production, and per capita consumption. However, large portions of the crop are left behind in the field as culls. The objective of our research was twofold: to create further processed products from watermelon that could be made from these culls, and to determine the effect of the food processing techniques used on the total lycopene content, its isomeric forms, and the impact on antioxidant activity.

The pressing, maceration, and heating techniques that were used to extract the watermelon had differential impact on extractable lycopene content, and antioxidant activity, but no impact on isomeric form. The results suggest that maceration may be a more effective technique for preparing watermelon tissue for lycopene extraction than pressing alone, however more research is needed to make a definitive observation.

The thermal processing temperatures utilized to create the watermelon –based tomato sauce analogue caused a significant reduction in total lycopene content as compared to unheated controls, and an increase in *cis*-isomers of lycopene was observed.

However, no effect on antioxidant activity was observed. These results indicate that the processing temperatures utilized had a detrimental impact on lycopene; therefore, we may conclude that value-added products from culled watermelon likely could not be made using this method without negatively impacting lycopene content.

Wine made from watermelon had no measureable lycopene content; this was likely due to the hydrophobic nature of the lycopene and not from the wine-making techniques utilized. However, antioxidant activity was still measured in the wine using the hydrophilic ORAC assay. This antioxidant activity may have been due to the presence of phenolic compounds, citrulline, or the potassium metabisulfite that was used as a processing aid. No analysis was performed to measure these compounds. No changes in isomeric form were observed in the lees of the watermelon wine. Our results indicate that our process is viable for making wine from culled watermelon.

APPPENDICES

Moisture Analysis Experiment 1

Treatment	Pan (grams)	Pan+sample (g)	Pan+dried sample (g)	%Moisture
1	1.0032	3.1492	1.2152	90.1212
1	0.9907	3.2198	1.2170	89.8479
1	1.0035	3.0746	1.2181	89.6384
2	1.0021	3.1699	1.2016	90.7971
2	1.033	3.3000	1.2164	90.7215
2	0.9952	3.1616	1.2091	90.1265
3	1.0049	3.1811	1.1689	92.4639
3	0.9957	3.1976	1.1650	92.3112
3	1.0034	3.3165	1.1820	92.2788
4	0.9891	3.0101	1.1597	91.5585
4	0.9904	3.1365	1.1776	91.2772
4	0.9959	3.4657	1.2132	91.2017

Moisture Analysis Experiment 2

Treatment	Pan (grams)	Pan+sample (grams)	Pan+dried sample (grams)	%Moisture
Uncooked	0.9685	3.2314	1.1974	89.8847
Uncooked	0.9736	3.1121	1.1906	89.8527
Uncooked	0.9649	3.1150	1.1784	90.0202
Uncooked	0.9725	3.0978	1.1839	90.0532
Uncooked	0.9732	3.0734	1.1353	92.2817
Uncooked	0.9659	3.0049	1.1234	92.2756

Experiment 2

Reduction in watermelon weight by heating

	Watermelon 1	Watermelon 2	Watermelon 3
Start weight (g)	759.9	758.2	743.6
End weight (g)	249.0	221.4	203.7
% Weight	32.7675	29.2007	27.3938
% Reduction	67.2325	70.7993	72.6062

Reduction in watermelon solvent weight by vacuum drying

	Watermelon 1		Watermelon 2		Watermelon 3	
	A	B	A	B	A	B
Start weight(g)	33.5235	32.6658	31.9477	34.1312	33.4559	33.7122
End weight(g)	9.91199	9.6956	9.7988	10.5108	10.2209	10.2031
% Weight	29.5670	29.6814	30.6713	30.7954	30.5503	30.2654
% Reduction	70.4330	70.3186	69.3287	69.2046	69.4497	69.7346

Original from Perkins-Veazie and others 2006

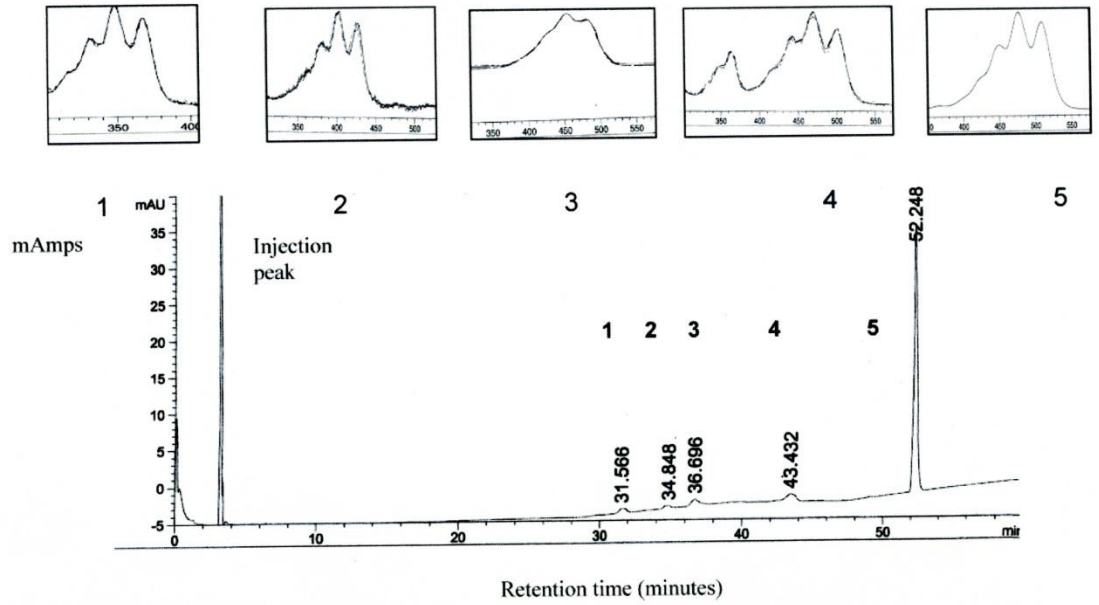
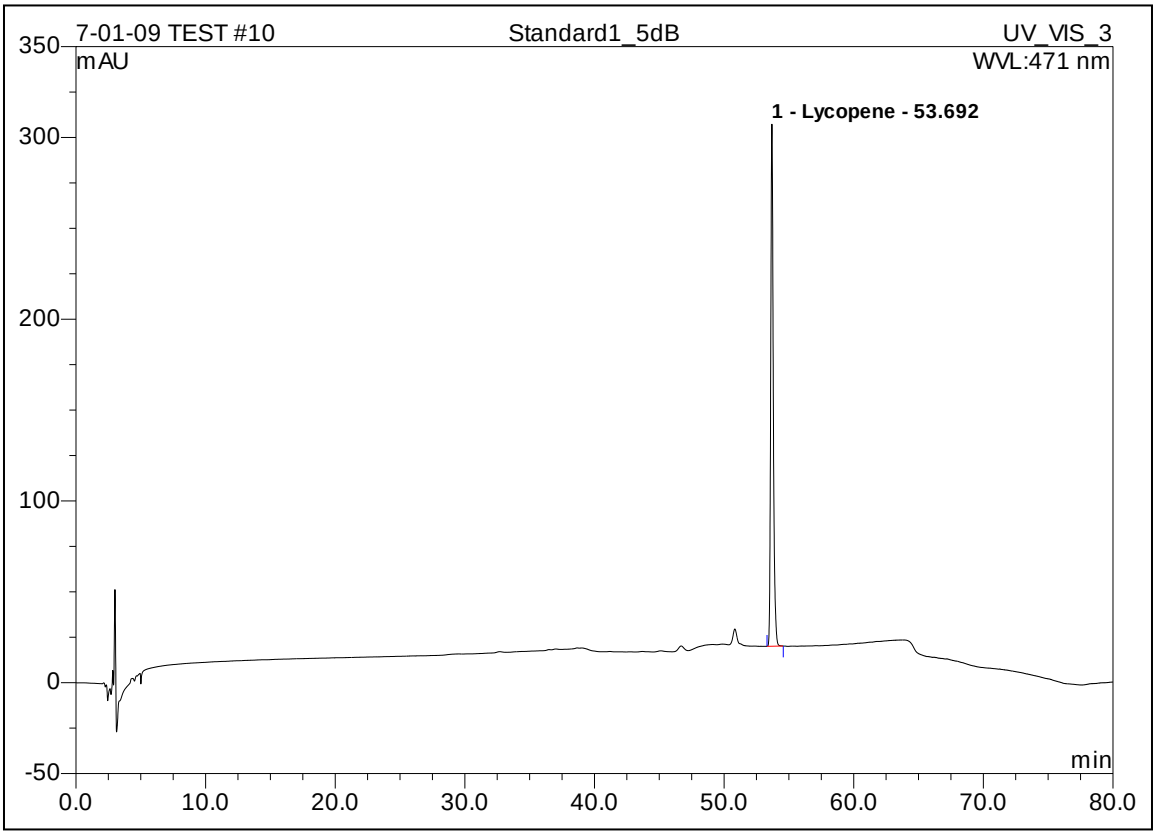


Figure 1. HPLC chromatogram of the watermelon cultivar Xite (Hazera 6007). Peaks 1–5 represent the following: 1, phytofluene; 2, unknown; 3, β-carotene; 4, *cis*-lycopene; and 5, *trans*-lycopene.

Dionix HPLC Chromatogram of Lycopene standard at 471 nm



HPLC Standard used to identify lycopene (Dionix system). 20 µL injection volume.

Peak	Compound	Retention Time(min)	Amount(µg)	λ used (nm)
1	Lycopene	53.692	1.727	471

VITA

Darren Dante Scott

Candidate for the Degree of

Doctor of Philosophy

Thesis: DETERMINATION OF LYCOPENE ISOMERS IN MODEL FOOD SYSTEMS AND THEIR EFFECTIVENESS AS ANTIOXIDANTS

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Sensory analysis in support of product development

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Name: Darren Dante Scott Date of Degree: May, 2012

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: DETERMINATION OF LYCOPENE ISOMERS IN MODEL FOOD SYSTEMS AND THEIR EFFECTIVENESS AS ANTIOXIDANTS

Pages in Study: 84 Candidate for the Degree of Doctor of Philosophy

Major Field: Food Science

Scope and Method of Study: We compared methods of extraction and processing that could be used to develop potential value-added products from watermelon culls and determined the effect of these extraction and processing methods on lycopene concentration, lycopene isomeric form, and final-product antioxidant capacity.

Findings and Conclusions: Unfiltered, macerated watermelon tissue had higher concentrations of lycopene than pressed/ filtered watermelon juice. This was true for both heated and unheated juice and macerate samples. When heated and unheated samples were averaged within treatments, unfiltered, macerated treatments possessed about 109% more lycopene than press/filtered juice treatments. It seems likely that this difference was due to some portion of lycopene being retained in the material used to filter the pressed juice samples. However, additional research is required to quantify this. Treatments alone or in combination had no effect on lycopene isomeric form. Overall, within the parameters tested, maceration appeared to be a better method for maximizing lycopene recovery from culled watermelons than pressing/filtering.

Thermal processing of watermelon by boiling watermelon puree until sufficient water was removed by evaporation to render it the consistency of tomato sauce had a measurable effect on lycopene concentration but not on antioxidant activity as measured by the lipophilic Oxygen Radical Absorbance Capacity (ORAC) assay. It also caused the formation of detectable low levels of *cis*-isomers of lycopene that were not quantified.

Neither basic wine-making techniques nor fermentation by *Saccharomyces cerevisiae* caused a change in the isomeric form of lycopene found in the lees (settled residues) of the finished wine. No antioxidant activity was detected by the lipophilic ORAC assay in the wine. However, antioxidant activity was detected by the hydrophilic ORAC assay. This may have been due to the presence of phenolic compounds, citrulline, or sulfur dioxide in the finished wine. However, no analysis was performed to measure these compounds. Our results indicate that our process is viable for making wine from culled watermelon.

ADVISER'S APPROVAL: William McGlynn