THE EFFECTS OF DIFFERENT COOKING TIMES AND TEMPERATURES ON TOMATO SAUCE LYCOPENE CONTENT

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A Thesis
Submitted to the Graduate College of Bowling Green State University in partial fulfillment of the requirements for the degree of

MASTER OF FAMILY CONSUMER SCIENCES

December 2006

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ABSTRACT

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PURPOSE:
The aim of present study was to determine the effects of different cooking times and temperatures on the lycopene content of three brands of commercially processed tomato sauce samples.

METHODS:
The experimentation was conducted on a total of three brands of tomato sauce (n=120) to determine the effects of cooking times (15, 30, 45 minutes) and temperatures (325, 350, 375 degree F) on lycopene content. At each of the constant oven temperatures, the samples were subjected to cooking for different lengths of time. The main effect of time, temperature, and interaction of both was determined by using ANOVA. The lycopene content was measured by using a HPLC (high performance liquid chromatography) technique.

RESULTS:
All three brands were significantly different (P<0.001) in mean lycopene content. For two brands the lycopene content was significantly higher (P<0.05) in the samples cooked for 15 minutes, when compared to 30 and 45 minutes. As a main effect of temperature, two brands showed the highest lycopene content at cooking temperature of 375 °F, but one brand did not show the significant effect of cooking temperature on the lycopene content. All brands showed different results for the interaction effect of time and temperature on...
the lycopene content, and only brand showed the significantly different lycopene content in the sample cooked at 350° F for 30 minutes.

CONCLUSION:

The present study provides the support for the beneficial effect of shorter heat treatment on lycopene availability of tested tomato sauce samples. Heating for a short period of time may help in the better retention of lycopene during home cooking, as well as processing in the industry. The study results could not determine the effects of temperature for the better retention of lycopene into the cooked samples. Further research is needed in this area to determine the multifaceted nature of the lycopene molecule not only at different time-temperature conditions, but also in conjunction with other factors, such as moisture content, solid content, light, oxygen, internal temperature, and type of metal used for processing and storing of tomato products.
ACKNOWLEDGMENTS

I would like to thank all with a deep sense of gratitude who gave me possibility to complete this project on right time. I would like to express my sincere thanks to my advisor Dr. Williford, and committee members, Dr. Coleman and Dr. Hentges, for their continuous help in completing my thesis; without each of their unique expertise, it was not possible to accomplish this project. I specially thank Mr. Jedrzej Romanowicz for helping me all the time to solve endless problems related to HPLC instrument.

I would also like to express my thankfulness to my research assistant, Krystle Zuniga for her help during experimentation. I am thankful to my all friends including my roommates Mita and Ramya for giving me good wishes to achieve my purpose, especially to Mita for her help in editing the paper.

I also want to thank my parents who always want me to go ahead in the life. I do not want to forget my little brother Priyank for his sweet love which always inspires me to make progress in life. I also want to thank my Fiancé - Chirag and his family for their support. Especially, I would like to thank Chirag for his everyday love, understanding, and motivation during long period of thesis completion. Chirag was always a good reminder of timeline and had provided very useful assistance in general formatting of my thesis.

At last, I want to remember god for helping me during all difficult times of my life and giving me stamina to do this hard work. I would like to thanks all that directly or indirectly helped me to complete this project. Thank you.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>CHAPTER I: INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Statement of the Problem</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Significance of the Study</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><strong>CHAPTER II: REVIEW OF LITERATURE</strong></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Lycopene Structure and Chemical Activity</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Lycopene Bioavailability</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Lycopene Bioavailability of Cis and Trans Isomers</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Digestion and Absorption of Lycopene</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Role of Lycopene in Human Health</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Antioxidant Effects</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Gap-junction Communication</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Hypocholesterolemic Effects</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Lycopene and Chronic Diseases</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Lycopene and Prostate Cancer</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Lycopene and Colon Cancer</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Lycopene and Type 2 Diabetes</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Lycopene and Cardiovascular Diseases</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Lycopene and Skin Health</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Lycopene and Male Fertility</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Effects of Cooking Times and Temperatures</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Effects of Drying</td>
<td>17</td>
</tr>
</tbody>
</table>
Effects of Mechanical Homogenization and Heating

Effects of Processing

Effect of Processing On Isomerization

Effects of Cooking

New Findings

Hypotheses of the Study

Objectives of the Study

CHAPTER III: METHODOLOGY

Materials

Sample Design

Moisture Analysis

Heat Treatment

Lycopene Extraction

Filtration

Lycopene Separation

HPLC Analysis

Statistical Analysis

CHAPTER IV: RESULTS AND DISCUSSION

Comparison of Brands

Effects of Moisture Content

Differences in the Internal Temperature of Cooked Tomato Sauce

Samples

CHAPTER V: CONCLUSIONS
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lycopene Content of Various Fruits and Vegetables</td>
<td>2</td>
</tr>
<tr>
<td>2. Lycopene Content of Tomato Sauce at Different Times of Processing</td>
<td>23</td>
</tr>
<tr>
<td>3. Example of Calculation Table Used For Lycopene Determination</td>
<td>30</td>
</tr>
<tr>
<td>4. Mean Lycopene Concentrations of Tested Samples at Different Cooking Times</td>
<td>36</td>
</tr>
<tr>
<td>5. Mean Lycopene Concentrations of Tested Samples at Different Cooking Temperatures</td>
<td>37</td>
</tr>
<tr>
<td>6. Moisture Loss in Tested Samples During Cooking</td>
<td>42</td>
</tr>
<tr>
<td>7. Internal Temperatures of Cooked Tomato Sauce Samples</td>
<td>44</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Molecular Structure of Lycopene</td>
<td>6</td>
</tr>
<tr>
<td>2. Standard Curve for Lycopene</td>
<td>29</td>
</tr>
<tr>
<td>3. Representative Chromatogram From HPLC of Standard (A) and Unknown Sample (B)</td>
<td>31</td>
</tr>
<tr>
<td>4. Interaction Effects of Cooking Time and Temperature on Brand 1</td>
<td>39</td>
</tr>
<tr>
<td>5. Interaction Effects of Cooking Time and Temperature on Brand 2</td>
<td>40</td>
</tr>
<tr>
<td>6. Interaction Effects of Cooking Time and Temperature on Brand 3</td>
<td>40</td>
</tr>
<tr>
<td>7. Interaction Effects of Cooking Time and Temperature From Common Lycopene Analysis of All Three Brands</td>
<td>41</td>
</tr>
</tbody>
</table>
CHAPTER I: INTRODUCTION

Lycopene is a bioactive carotenoid found in many red fruits and vegetables, such as tomatoes, watermelon, pink grapefruit, apricots, and pink guava. Different fruits, vegetables and their products contain different concentrations of lycopene (Table 1). This natural red pigment is synthesized exclusively by plants and microorganisms (fungi, bacteria, and algae). Animals, including human beings, cannot synthesize lycopene; therefore, they obtain lycopene exclusively from their diet (Tapiero and others 2004; Omoni and Aluko 2005).

Although tomatoes contain a lower concentration of nutrients compared to other commercially grown fruit species, they are a major source of lycopene in the American diet because they are consumed in large quantities (Rao and Agarwal 2000; Akanabi and Oludemi 2004). Lycopenes comprise 83% of the total pigments present in tomatoes (Shi and others 1999).

Structurally, lycopene is an acyclic polyene with 11 conjugated double bonds, which increase its' affinity for singlet oxygen and its' free radical scavenging capacity beyond other carotenoids. Lycopene from natural plant sources mainly exists in the all trans configuration, which is the most thermodynamically stable form (Bramley 2000). Lycopene is synthesized in various isomeric forms by microorganisms and higher level plants (Hadley and others 2003). All trans, 5-cis, 9-cis, 13-cis, and 15-cis are the most common isomer forms of lycopene. Lycopene undergoes cis-trans isomerization induced by different factors, such as light, thermal, and chemical reactions, during cooking or processing (Shi and others 1999; Tapiero and others 2004; Goula and others 2006).

The biological activities of lycopene were first observed by Ernster and co-workers in 1959 (Stalh and Sies 1996). In 1996, Stahl and Sies reported that intraperitoneal injection of
lycopene increased the survival rate of irradiated mice, and also gave the mice resistance from bacterial infections. Recently, many epidemiological researchers have reported that lycopene in tomatoes may prevent the onset of chronic types of cancer (Agarwal and Rao 2000; Heber and Lu 2002). Oxidative damage to cellular compounds is a major cause of different kinds of cancer in the biological system (Agarwal and Rao 2000). Therefore, the high free radical scavenging capacity of lycopene may protect body cells against oxidative damage.

Table 1. Lycopene Content of Various Fruits and Vegetables*

<table>
<thead>
<tr>
<th>Food</th>
<th>Lycopene content (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato foods</td>
<td>0.9-4.2</td>
</tr>
<tr>
<td>Tomatoes, raw</td>
<td>3.7-4.4</td>
</tr>
<tr>
<td>Tomato sauce</td>
<td>7.3-18.0</td>
</tr>
<tr>
<td>Tomato paste</td>
<td>5.4-55.5</td>
</tr>
<tr>
<td>Tomato Soup (condensed)</td>
<td>8.0-10.9</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>5.0-11.6</td>
</tr>
<tr>
<td>Ketchup</td>
<td>9.9-13.4</td>
</tr>
<tr>
<td>Apricots, fresh</td>
<td>0.0005</td>
</tr>
<tr>
<td>Watermelon, fresh</td>
<td>2.3-7.2</td>
</tr>
<tr>
<td>Papaya, fresh</td>
<td>2.0-5.3</td>
</tr>
<tr>
<td>Grapefruit, pink/red</td>
<td>0.2-3.4</td>
</tr>
<tr>
<td>Guava, raw</td>
<td>5.3-5.5</td>
</tr>
<tr>
<td>Vegetable juice</td>
<td>7.3-9.7</td>
</tr>
</tbody>
</table>

*Pohar and others (2003)
Many studies have been published concerning the stability of lycopene during tomato product processing for the manufacturing of tomato paste, tomato juice, and other tomato products (Agarwal and others 2001; Akanabi and Oludemi 2004; Dewanto and others 2002; Goula and others 2006). Heat processing of lycopene-containing plant foods induces “cis” isomerization of lycopene, which has a beneficial effect on the absorption of lycopene. Lycopene in the natural “trans” form is poorly absorbed from fresh tomatoes (Rao and Agarwal 2000). In 2004, Donaldson reported that lycopene is less absorbed from fresh tomatoes than cooked tomatoes. Tomato paste and tomato sauces are the best food sources of lycopene in a bioavailable form for humans; whereas, fresh tomatoes, unheated tomato juices, and other fruits and vegetables are poorer sources of absorbable lycopene.

The scientific literature lacks information on the effects of cooking time and temperature on already processed, purchased for cooking, tomato products. Commonly used cooking times (30-60 minutes) and temperature (300-375°F) ranges for making American recipes, which use tomato sauce were experimental parameters in this study. For example, lasagna is baked at 350°F for 45 minutes; stuffed cabbage rolls at 350°F for 45 minutes; and spaghetti sauce (with tomato sauce and tomato paste), boiled and then simmered for 40-60 minutes. (Betty Crocker, n.d). The aim of this pilot study was to determine the lycopene content of commercially processed tomato sauces heated at commonly used different home cooking times and temperatures, and compare the lycopene content of both the cooked and uncooked samples, so that changes in the initial lycopene content before and after cooking could be determined. The current study expanded the knowledge concerning the effects of cooking times and temperatures on lycopene content in cooked tomato sauce, which is used as an ingredient in many food products.
Statement of the Problem

Many different types of processed tomato products are available in the market to consumers, and lycopene has been shown to vary in concentration in these tomato products. Often, tomatoes are subjected to various processing techniques, such as cooking, freezing, and baking and/or storage before actual human consumption (Agarwal and others 2001). Cooking tomato products can cause oxidation and/or isomerization of lycopene, and thus, increase or decrease the active lycopene content. Hence, investigation of the effects of different cooking times and temperatures on processed tomato sauce is important.

Significance of the Study

Lycopene has an essential role in human health due to its protective role in various kinds of cancers (prostate cancer, breast cancer and lung cancer), as well as cardiovascular diseases (Agarwal and Rao 2000). Tomatoes are a major source of lycopene in daily diets because they are consumed in large quantities by a large sector of the U.S. population (Boileau and others 2002). These tomato products are generally subjected to either heat treatment (cooking) or storage before actual consumption (Sahlin and others 2004). Cooking or storing tomato products may affect lycopene content of tomatoes, either positively or negatively. The purpose of the present study was to determine the effects of different cooking times and temperatures on the lycopene content of processed tomato sauce. Data from this study may enable recommendations for appropriate cooking times and temperatures of tomato sauce to maximize available lycopene.
Lycopene Structure and Chemical Activity

Lycopene belongs to the carotenoid family of plant compounds, and carotenoids are effective antioxidants, which play important roles in delaying or preventing oxidation in human body cells. The properties and functions of a carotenoid molecule are primarily dependent upon the molecule’s structure and chemical activity. The most important characteristic of the carotenoids’ molecule is the conjugated carbon double bond, which is responsible for energy transfer reactions and quenching of oxygen (Young and Lowe 2001).

Carotenoids act by three main mechanisms to prevent oxidative damage in the body: (1) electron transfer, (2) hydrogen abstraction, and (3) addition of a radical species (Young and Lowe 2001). Carotenoids react as either an antioxidant or pro-oxidant depending on various factors. If the carotenoids react with oxygen to produce peroxyl radicals, it can lead to the pro-oxidant effect. High oxygen concentration can lead to the formation of peroxyl radicals, and hence an auto-oxidation or a pro-oxidant effect (Woodall and others 1997). The other factors which may influence the antioxidant or pro-oxidant activities of carotenoids in biological systems are the structure, physical form (cis or trans configuration), cite of action of the carotenoid molecule, potential for interaction with other carotenoids or antioxidant compounds, and the partial pressure of oxygen. Structure involves size, shape of the carotenoid molecule, as well as the nature, position, and number of substituent groups in the carotenoid molecule, which all contribute to molecular reactivity (Woodall and others 1997).

Lycopene is a 40-carbon acyclic open-chain carotenoid (Figure 1). It is a highly unsaturated, straight chain hydrocarbon, containing 11 conjugated and 2 unconjugated double bonds. Lycopene is a lipophilic compound that is insoluble in water, but soluble in organic
solvents, and it has a quenching constant double that of B-carotene and 10 times alpha tocopherol (Hadley and others 2003). The quenching ability is directly related to the position of excited state energy levels, which depend on the length of the conjugated carbon double bone chain (Young and Lowe 2001).

Mevalonate is a precursor of lycopene in plants. Different carotenoids are biosynthesized by a special sequence of the terpenoid pathway in plants. Phytoene, a carotenoid with three conjugated double bonds, is produced first. Phytoene is then followed by an enzymatic desaturation reaction giving rise to lycopene and other carotenoids. These reactions are catalyzed by two membrane bound desaturase enzymes and occur in the plastids of higher plants (Stalh and Sies 1996).

Lycopene Bioavailability

There are approximately 600 known carotenoids in nature, of which only 20 are found in human plasma and tissues of these, lycopene is the most predominant carotenoid in human plasma (Tapiero and others 2004). Human plasma contains a mixture of different isomeric forms of lycopene, of which 50% is the cis isomer form. Lycopene primarily exists in the all-trans isomer in most food sources; however, lycopene found in human and animal tissues is in the cis-isomer form.
There are various factors which affect the bioavailability of lycopene from foods. Lycopene is bonded to the food matrix cell wall. Lycopene is released from the food matrix during the digestion process, as well as during cooking and processing. Thus, the kind of food matrix in which lycopene is bound is an important determinant of the bioavailability (BV) of lycopene (Sahlin and others 2004).

*Lycopene Bioavailability of Cis and Trans Isomers*

Fresh tomatoes are subjected to homogenization and heat treatment for the production of different tomato products, such as tomato puree, tomato sauce, tomato paste and other processed tomato products (Van het Hof and others 2000). Processing and cooking causes isomerization of all-trans lycopene to the cis-isomer form. Hence, processing and cooking are responsible for increasing conversion of the naturally occurring trans form to the cis-isomer form of lycopene, and cis-isomers of lycopene are more bioavailable than the all-trans form. (Omoni and Aluko 2005). Lycopene from tomato paste and tomato puree has been reported to be more readily absorbed than lycopene from raw tomatoes (Boileau and others 2002; Stahl and Sies 1996).

In 1996, Stahl and Sies reported that cis-isomers of lycopene were better absorbed than the all-trans isomers. These researchers analyzed lycopene content in tomato juice by using high pressure liquid chromatography (HPLC), and they reported that tomato juice contained only 20% cis-isomers, while the blood serum was composed of about 50% of cis-isomers after consumption of the same tomato juice. These researchers investigated four different forms of lycopene, of which the all-trans was the predominant form, and the other three were cis-isomers. The researchers concluded that the cis-isomer form was better absorbed in humans than the all-trans form. Cis-isomers have higher solubility in the lipophilic phases. This could enhance their
incorporation into mixed micelles in the intestine, and thus, the cis form would be more bioavailable for absorption than the all-trans form.

In 1997, Gartner and others compared lycopene bioavailability of tomato paste and fresh tomatoes. Healthy human subjects received 23 mg of lycopene from either tomato paste or fresh tomatoes. The carotenoid concentrations of each subject’s plasma were analyzed from the chylomicron fraction. The researchers reported the same lycopene isomer pattern in both fresh tomatoes and tomato paste. However, there was a 2.5-fold higher total (P<0.05) of the all-trans lycopene peak concentrations from subjects’ plasma after ingestion of tomato paste when compared with fresh tomatoes. Thus, lycopene from processed tomato paste was more efficiently absorbed in humans than lycopene from the fresh tomatoes. (Gartner and others1997).

Porrini and others (1998) studied the lycopene bioavailability from tomato puree and raw tomatoes. Ten subjects were used in two different experiments. In the first experiment, subjects (n=9) were assigned randomly to the group consuming the single portions of tomato puree and raw tomato on separate experimental occasions, each containing 16.5 mg of lycopene for 5 days. Blood samples were collected within the first 12 hours, and on each of the following 5 days. In the second experiment, subjects (n=10) were divided into two groups (five in each group) receiving either tomato puree or fresh raw tomato, containing 16.5 mg of lycopene for seven days. Blood samples were collected daily. In the first experiment, various concentrations of plasma total lycopene were reported over time, with a slight increase after 6 hours and a further increase after 12 hours, and plasma lycopene was higher after the consumption of tomato puree than raw tomatoes. In the second experiment, there was a day-by-day increase in the total lycopene concentration in the group consuming tomato puree. The researchers concluded that
plasma total lycopene concentrations were higher after the consumption of processed tomato
puree, rather than after consuming fresh raw tomatoes.

**Digestion and Absorption of Lycopene**

In humans, the ingested carotenoids pass from the stomach to the small intestine, where
the carotenoids are incorporated into micelles formed from dietary lipids and bile acids.
Lycopene is absorbed into the intestinal mucosa cells via passive transport (Stalh and Sies 1996).
The uptake of lycopene into the intestinal mucosa cells is facilitated by the secretion of bile acid
micelles. The intact carotenoids are incorporated into chylomicrons, which are finally released
from the intestinal mucosal cells into the lymphatic system, then to the venous blood, where the
micelles are carried to the liver. However, very little information is available about the intestinal
metabolism of non-provitamin A carotenoids. Whether lycopene is transported intracellularly by
a specific protein or lipid droplets is unclear.

Lycopene has the potential to be absorbed by cells in various tissues, including adrenals,
kidney, adipose, spleen, lung, and reproductive organs, through the action of lipoprotein lipase
enzyme on chylomicrons. Lycopene can accumulate in the liver, or it can be packaged into very
low density lipoprotein (VLDL) and released from the liver back into the blood. Most of the
carotenoids appear initially in the chylomicron and VLDL fraction. Low density lipoprotein
(LDL) from the liver is the major carrier of lycopene. Most stored lycopene in the body has been
found in liver, adrenals, and testes; whereas, other tissues, such as lung and kidney, contained
smaller amounts of lycopene (Stalh and Sies 1996; Boileau and others 2002). The liver is a major
site for accumulation of lycopene. Sometimes, lycopene accumulates in human tissues in the
condition called lycopenemia. Lycopenemia is an excess of lycopene in the blood due to
Roles of Lycopene in Human Health

Lycopene has been shown to act as a potent antioxidant. There are also a number of other potential mechanisms for lycopene, such as gap-junction communication, hypocholesteromic, anti-cancerous, and anti-atherogenic effects, that may reduce the risk for various chronic diseases (Young and Lowe 2001). Thus, lycopene acts by various mechanisms to protect body cells against oxidative damage, and thus, lycopene may play a preventive role in different kinds of chronic cancers and cardiovascular diseases.

Antioxidant Effects

Oxidative stress is widely known to be involved in the causation and progression of several chronic diseases. In the human body, normal metabolic activity, lifestyle activities, and diet are responsible for generating reactive oxygen species [ROS] (Tapiero and others 2004). The singlet oxygen species and related oxidative damage have proven to be responsible for various human chronic diseases. This singlet oxygen is very harmful in nature and can cause damage to DNA, lipids, and enzymes. Carotenoids, in particular lycopene, scavenge these ROS and peroxyl radicals and protect cells against oxidative damage ((Stahl and Sies 1996; Yilmaz and others 2006).

Lycopene quenches singlet oxygen primarily by a physical mechanism in which the excess energy of singlet oxygen is transferred to the lycopene electron-rich structure. The lycopene structure first gets excited by the added energy from singlet oxygen, and then relaxes into its ground state by losing the extra energy as heat. Because of this physical mechanism, the lycopene structure remains unchanged to protect against further singlet reactive oxygen species.
Lycopene can undergo further cycles of singlet oxygen quenching by exciting again and relaxing back to the original position (gaining and losing energy of a singlet oxygen molecule which is responsible for oxidative damage), and thereby, acting like a catalyst in oxidation reactions. (Stahl and Sies 1996).

**Gap-junction Communication**

Gap junctions are cell-to-cell channels, which connect cells with each other for transportation of low-molecular weight compounds, like nutrients. Loss of gap junctional communication (GJC) is one of the features of carcinogenesis. Lycopene, in addition to its antioxidant activity, stimulates gap-junction communication (GJC) between cells (Aust and others 2003, Omoni and Aluko 2005). Oxidation products obtained from lycopene stimulate GJC in WB-F344 cells of rat liver epithelial cells. Lycopene acts as an anticarcinogen by increasing GJC between cells and enhancing the expression of connexin 43 (a gene encoding major gap junction protein connecting the cytosol of neighboring cells), and thus upregulating GJC (Tapiero and others 2004).

Posttranslational modifications (chemical modification of protein structure after its translation), protein trafficking (a rising defect in protein at some stage of biosynthesis which impacts the protein’s normal functional ability) or changes in pH or calcium levels are other mechanisms of carotenoids that may be relevant in the stimulation of GJC (Tapiero and others 2004).

**Hypocholesterolemic Effects**

Lycopene also acts as a hypocholesterolemic agent. Oxidized LDL is highly atherogenic and cytotoxic to cells. This oxidized LDL can cause accumulation of macrophage cholesterol and increase foam cell formation, and also stimulate inflammatory and thrombotic processes in the
blood cells and arterial walls. All the major cells in the arterial wall derived macrophages can oxidize LDL, and eventually, oxidation of LDL can lead to atherosclerosis. LDL is the major cholesterol carrier in humans. LDL contains cholesteryl ester with polyunsaturated fatty acids, such as linoleic and arachidonic acids, which are prone to oxidation. Lycopene is a lipid soluble antioxidant that is located in the cell membrane, which might have an inhibitory effect on LDL oxidation. Lycopene has been shown to augment the activity of the macrophage LDL receptor, and thus, can reduce plasma LDL cholesterol concentrations which may, thereby, decrease cardiovascular diseases (Heber and Lu 2002; Visioli and others 2003).

In 2003, Visioli and others studied tomato products and lipid oxidation. Lipid oxidation is mainly oxidation of low-density lipoproteins (LDL). LDL is considered bad cholesterol, which contributes to various kinds of cardiovascular diseases, atherosclerosis, and cancers. Free radicals are responsible for the oxidative damage, and thus, can lead to an increased risk of cancers and CHD. These researchers demonstrated that after a week on a controlled diet, there was a decrease in the subjects’ (N=12) concentration of blood lycopene, which was followed by a significant (P<0.05) increase in LDL oxidation. Afterwards, the same subjects received tomato products, which produced significant increases in plasma lycopene concentration. The researchers concluded that there was a protective role of tomato carotenoids against lipid peroxidation (Visioli and others 2003).

Lycopene and Chronic Diseases

Lycopene gained the attention of the scientific community in human health because of its antioxidant activity. Studies have shown that lycopene intake was associated with a decreased risk of various chronic cancers, such as prostate, lung, breast, colon, pancreas, stomach, rectum, esophagus, oral cavity, and cervix (Giovannucci 1999; Agarwal and Rao 2000; Heber and Lu
2002; Weisburger 2002; Omoni and Aluko 2005). Weisburger (2002) has stated that there is a lower incidence of chronic diseases in populations with regular intakes of tomato products that are rich in lycopene. Lycopene has been shown to protect important biomolecules, such as lipids, low-density lipoproteins, proteins, and DNA against oxidative damage, which is how lycopene contributes to the prevention of cancers, atherogenesis, and cell proliferation (Agarwal and Rao 2000; Weisburger 2002; Stalh and others 2001; Arab and others 2002).

In 1999, Giovannucci conducted an observational study concerning the relationships between tomato lycopene consumption and various cancers in a total of 72 patient cases. Giovannucci reported that 57 cases had inverse associations between tomato intake, or level of blood lycopene, and the risk of cancer. Additionally, 37 cases had statistically significant inverse associations, and no study was found that indicated higher tomato intake was associated with an increased risk of cancer.

Weisburger (2002) collected data on the incidence of chronic diseases and food traditions in people from the Mediterranean region. Mediterranean traditional foods are rich in vegetables, fruits and cooked tomatoes, along with olive oil. Weisburger reported a lower risk of various chronic diseases in individuals from this region of the world that appeared to be diet related.

In 2004, Rao evaluated the effects of long term consumption of various processed tomato products on a total 17 healthy human subjects, 10 men and 7 non-pregnant women. Foods containing lycopene were excluded from their diets for a two-week washout period. After that, all subjects received test tomato products including tomato juice, tomato sauce, tomato paste, ketchup, spaghetti sauce, and ready-to-serve tomato soup with 30 mg of lycopene/day for four weeks. Significant increases (p<0.05) in the level of serum lycopene, from 181.79 +/-31.25 to 684.7 +/-113.91 nmol/L, and significant (P<0.05), increases in total antioxidant potential from
2.26 +/-0.015 to 2.38 +/- 0.17 mmol/L were reported, with a reduction in lipid and protein oxidation \([p<0.05]\) (Rao 2004).

**Lycopene and Prostate Cancer**

Prostate cancer has become a major health problem and has received wide research attention over the last decade. Prostate cancer prevalence is 198,000 new cases per year in the U.S. and causes 31,500 deaths each year (Hadley and others 2002). Prostate cancer rates are high in specific regions of the world like North America and northern Europe, while low in most of Asia. These data created recent interest in the study of environmental factors that are responsible for increased risks of prostate cancer. Several studies investigated the role of tomato lycopene as a chemo preventive agent for prostate cancer (Pohar and others 2003; Takeoka and others 2001; Giovannucci and others 2002).

A study in Greece compared the effect of dietary habits of men with \((n=320)\) and without \((n=246)\) prostate cancer. These researchers reported that the men with prostate cancer had a significantly lower intake of cooked tomatoes \((P<0.005)\) versus raw tomatoes \((P<0.12)\), and they concluded that tomato lycopene consumption from 8 to 16 servings per month was associated with a 15 % reduction in the incidence of prostate cancer (Pohar and others 2003). Similarly, Takeoka and others (2001) also reported that processed tomato products were related to lower levels of prostate cancer.

In 2002, Giovannucci and others conducted a large scale study of tomato products, lycopene, and prostate cancer risk. The study consisted of an ongoing prospective cohort of 47,365 U.S. males. The researchers reported that frequent intake of tomato products or lycopene was associated with a reduced risk of prostate cancer. In addition, the researchers reported that
the consumption of a primary source of bioavailable lycopene, tomato sauce, was associated with an even greater reduction in prostate cancer risk.

**Lycopene and Colon Cancer**

Colon-rectal cancer is a primary cause of cancer deaths in the United States. Martinez-Ferrer and co-workers (2006) conducted a study on colon cancer and dietary lycopene. The researchers analyzed Glutathione-S-transferase (GST) enzyme in the liver. They found that lycopene is related to an increase in the levels of detoxifying enzymes in the liver. These enzymes are involved in the detoxification of cancerous metabolites in the body. Further, they also found that lycopene suppresses colon cancer by preventing the initiation and promotion stages of cancer in male rats. This study suggested a beneficial role of dietary lycopene against colon cancer.

Lycopene is also hypothesized to increase carotenoid concentration in plasma and lymphocytes. This increase would be related to an improvement in lymphocyte resistance to oxidative stress. Thus, lycopene has an important role during inflammation and immune responses (Heber and others 2002). Furthermore, studies have also supported the roles of lycopene in reducing the risk of lung cancer among human beings (Michaud and others 2000; Arab and others 2002).

**Lycopene and Type 2 Diabetes**

There is a skyrocketing increase in the incidence of type 2 diabetes mellitus (DM) around the world. Wang and others (2006) published the results of their type 2 DM and lycopene study, which was conducted from 1992 to 2003 on men and women in the US. The subjects were free from cardiovascular disease and cancer, but had type 2 DM. The study reported a significant decrease in type 2 DM risk with increased consumption of lycopene in the diet.
Lycopene and Cardiovascular Diseases

In 2003, Sesso and others studied the relationship between dietary lycopene and cardiovascular disease (CVD) in a large scale study of 39,876 female subjects. These researchers stated that dietary lycopene was not strongly related to reduction in CVD risk; however, there was an inverse relationship between higher levels of tomato-based products’ consumption and the risk of CVD.

Recently, Wood and Johnson (2004) reported on the monthly consumption of tomatoes, serum lycopene level, and self-reported history of congestive heart failure (CHF) from data collected on adult participants in the Third National Health and Nutrition Examination survey (NHANES III). Significant (P<0.05) inverse relationships were found between lycopene and C-reactive protein (the protein responsible for cell proliferation). Furthermore, the monthly consumption of tomatoes was inversely related to white blood cell count in CHF patients. In 2002, Khachik and others indicated that tomato-based diets containing lycopene could play an important role as a chemopreventive agent, from their review of epidemiological studies.

Recently, Yilmaz and others (2006) conducted a study on the efficiency of Adriamycin (ADR), antibiotics – anticancer agent with and without lycopene on cardiac toxicity and nephro toxicity in rat models. The study clearly indicted that ADR treatment along with lycopene might prevent this toxicity in rats. ADR with lycopene was effective in diminishing lipid peroxidation in both heart and kidney cells.

Lycopene and Skin Health

Lycopene has been shown to protect skin against UV-induced erythema (Stalh and others 2001; Porrini and Riso 2000; Mayer-Miebach and others 2005). Stalh and others (2001) conducted an experimental study on 19 human subjects. Skin was exposed to a solar simulator
to induce erythema. The researchers reported that a tomato-based diet was efficient in scavenging reactive oxygen species generated under photooxidative stress.

**Lycopene and Male Infertility**

In 2002, Gupta and Kumar conducted a study on lycopene and male infertility in New Delhi, India. They studied 30 male subjects from 2000 to 2003. All subjects had received 2000 mcg of oral lycopene, two times a day for three months. After three months, semen samples were analyzed for fertility characteristics. The scientists reported improvement in overall quality of semen with improvement in mortality and morphology of sperm in 20 of the 30 subjects.

**Effects of Cooking Times and Temperatures**

There are several studies available in the literature concerning the effects of various cooking times and temperatures on the lycopene content during processing of tomatoes. Some studies supported the beneficial role of heat treatment in releasing lycopene and making it available, but some studies reported contradictory findings on the positive effects of heat treatment on tomato lycopene content. The results of various studies are complicated in terms of reaching one general conclusion, and often the results conflict. Most studies have been done on the time and temperature effect during processing; only limited studies have found effects of cooking time and temperature on already commercially processed tomato products (Sahlin and others 2004).

**Effects of Drying**

Zanoni and others (1999) studied the effects of drying on weight loss and lycopene content of tomatoes. The temperature causes a loss of moisture or water from the tomatoes and results in loss of weight. The study results showed that lycopene has a higher stability during
drying because lycopene content did not change during drying at 80 °C. However, there was a decrease in lycopene content up to 10% while drying at 110 °C.

*Effects of Mechanical Homogenization and Heating*

Van het Hot and others (2000) did a systematic study on the effects of mechanical homogenization and heating on the bioavailability of carotenoids in canned tomatoes. The plasma carotenoid concentration was measured after four days of daily consumption by healthy human subjects. The human subjects were divided into two groups. Group one (N=17) received tomatoes with minimal heating (80 °C), and the second group (N=16) received tomatoes with extensive heating (1 h at 100 °C). Homogenization (P<0.05) and heat treatment tended to enhance the lycopene concentration in both triglyceride-rich lipoproteins and plasma. The researchers concluded that the intactness of the tomato cellular matrix was an important factor in determining the bioavailability of lycopene. Matrix disruption by mechanical homogenization and/or heat treatment enhances lycopene bioavailability.

*Effects of Processing*

Abushita and others (2000) studied the effects of processing on the carotenoids and antioxidant vitamins contents of tomatoes. Tomatoes, which undergo washing, chopping, hot-break extraction, sieving, vacuum evaporation, filling, sterilization, and storage as a part of normal food processing operations, were analyzed. Hot-break extraction was carried out at 90 °C for 5-10 minutes. The sieving temperature was 70-80 °C, and the vacuum evaporation temperature was 60-70 °C for four hours. The final sterilization step was at 100 °C for 30 minutes. Significant increases in lycopene content during food processing of raw tomatoes to tomato paste were reported. The increases in both all-trans and cis-lycopene were measured. The all-trans lycopene increased from 1189 mcg/g in raw tomatoes to 1628 mcg/g in the final tomato paste.
paste dry matter of tomatoes. However, there was not a significant increase in cis-lycopene. Cis-lycopene increased from 20 mcg/g in raw tomatoes to 25 mcg/g in the final tomato paste (dry matter analysis). Therefore, lycopene underwent only slight isomerization to form cis-isomer during processing.

Dewanto and others (2002) studied the effects of thermal processing on the nutritive value of tomatoes and total antioxidant activity. Raw tomato samples were subjected to heat treatments of 88 °C for 2, 5, and 30 minutes. The raw tomatoes had lycopene contents of 2.01+/-.04 mg of trans-lycopene/g of tomato. After heating for 2, 5, and 30 min, the trans-lycopene content had increased to 3.11+/-.04, 5.45+/-.02, and 5.32+/-.05 mg/g of tomato (P<0.01), respectively. An increase in the total antioxidant from 5.3 to 6.8 mc mol was reported. Percent change in lycopene was very significant (P<0.01), compared to raw tomatoes, 54.4, 171.1, and 164.3 mg/g after heating for 2, 5, and 30 minutes, respectively. Thus, the results of this study clearly demonstrated that lycopene content significantly increased with an increased amount of heat treatment time. The increase in lycopene concentration might be related to the dehydration loss of water or release of lycopene from the cell wall.

In contrast, Takeola and others (2001) reported conflicting results on the effects of processing on lycopene content of tomato samples. The researchers conducted a study on the tomato samples from two processing plants at three different points (raw tomatoes, juice after hot break scald, and final paste). Statistically significant (P<0.05) decreases were reported in lycopene levels of 9-28 %, as the fresh tomatoes were processed into paste. Lycopene is very sensitive to oxidative and thermal degradation at the high temperatures and prolong time periods of processing. Results indicated that the greatest loss of lycopene during processing was caused by the extensive processing time and the condition required in achieving specific Brix level of
tomato paste. Brix level is an approximate percent of water-soluble solids, mostly percent sugar, in the finished product.

Sahlin and others (2004) also reported the negative effects of heat treatment on tomato lycopene content. Sahlin and others (2004) studied the effects of boiling, baking, and frying on tomatoes. Cooking resulted in a loss of moisture from all the samples. Results showed that baking, boiling, and frying of tomatoes resulted in a significant decrease (P<0.01) in lycopene contents when compared to raw tomatoes. Frying caused the largest loss of nutrients, including lycopene content. The researchers finally concluded that cooking does have harmful effects on overall nutritional value, lycopene content, and antioxidant activity of tomatoes.

Effect of Processing On Isomerization

Nguyen and others (2001) conducted studies on thermal isomerization susceptibility of carotenoids in different tomato varieties. Five varieties of tomatoes with distinctively different carotenoid distributions were used. The tomatoes were subjected to heat treatments and operations similar to those in the tomato processing industries, such as boiling, addition of fat (cooking oil), chopping, and agitation. The physical structure of tomato tissues were examined by electron microscopy, which showed that heat treatment imparted changes to the ultra structure of the tomato tissue, such as cell wall and organelle deformation. As a result, lycopene was released more efficiently from the bound cell matrix and could be more bioavailable from processed and cooked tomato products. Thus, thermal processing enhanced the bioavailability of lycopene; but heat had limited effect on isomerization of various forms of lycopene and did not alter the chemical nature of the molecule itself (Nguyen and others 2001).

Nguyen and Schwartz (1998) stated that lycopene remained relatively resistant to heat processing during processing of different tomato products. Lycopene did not subject to
geometrical conversion (isomerization) during normal thermal treatment, but it did change during elevated heat treatment, which is not used commonly during either cooking or processing in the food industries. Similarly, Boileau and others (2002) found that heating tomato products for up to three hours can only minimally increase the concentration of cis-isomers. The lycopene isomers, all-trans and cis isomers were relatively stable to heat treatment. The researchers concluded that normal food processing and cooking did not change the isomer distribution of lycopene to a great extent, and additionally, the concentration of cis-isomers in these tomato products was far below the high levels of cis-isomers in human blood (hemoglobin).

On the other hand, Shi and others (1999) reported contradictory results on the effects of thermal processing on the isomerization of lycopene. They studied the effects of dehydration on lycopene degradation and isomerization. Experiments were conducted to compare the effect of osmotic treatment, vacuum-drying, air drying, and various combinations on lycopene content. Tomatoes were subjected to osmotic treatment at 25 °C for 4 hours, vacuum-drying at 55°C for 4-8 hours, and/or air-drying at 95 °C for 6-10 hours. A significant increase in the cis-isomer was observed, with a simultaneous decrease in the all-trans isomers using the different dehydration methods. There was also an increase in cis-isomers with increased dehydration time and temperature. In addition, the researchers stated that heat treatment disintegrated tomato tissue and exposed lycopene to oxygen and light, which resulted in the destruction of some lycopene.

Effects of Cooking

Recently, Mayeaux and others (2006) conducted a study on the effects of various cooking conditions (baking, microwaving, and frying) on tomato lycopene content. The researchers found that the percent of remaining lycopene was decreased with increased time and temperature of cooking. They further reported that lycopene was very unstable when it was exposed to cooking
temperature about 100 °C. Microwaving and baking caused less loss of lycopene compared to frying, because the presence of high temperature conditions during frying.

New Findings

In 2004, Seybold and others confirmed a new finding on the effects of heat treatment on lycopene. The researchers observed a decrease in lycopene content beyond 90 minutes of heat treatment. Seybold and others (2004) designed a study to determine the effects of various cooking times and temperatures on different tomato products. Samples of tomato sauce were subjected to medium heat treatment (temperature not given) for 210 minutes and collected treated samples after 0, 5, 25, 30, 50, 60, 120, 150, 180, and 210 minutes of cooking. Study results showed that lycopene content increased during thermal processing until 90 minutes, due to release of lycopene from the cell wall as an effect of the heat treatment, but beyond 90 minutes, there was a decrease. Therefore, excessive heat might have negative effects on lycopene content. Dry weight basis (dwb) analysis has shown that lycopene content of tomato sauce increased with increased amount of cooking time, until 90 minutes (Table 2) The increase or decrease in lycopene content could be related to the variety of the tomatoes, loss of water in cooking, or release of lycopene during heat treatment.

Mayer-Miebach and co-workers reported similar results on the deleterious effects of increased processing time on lycopene content. They studied the effects of drying and thermal treatment on carrot lycopene. Carrots underwent different time and temperature treatment during drying (50, 60, 70, 90 °C), and heating (25, 70, 90, 100, 120, 130, 140 °C) from 0.5 to 5 hours. There was no change in lycopene content at 70 °C. Lycopene started to decrease at 100 °C for 2 hours. This suggested that lycopene had higher temperature thermal stability. Heating carrots above 100 °C caused isomerization of the all trans forms to the cis form.
However, cis isomer levels increased only during the first hour, and started to decrease after two hours cooking time.

Table 2. Lycopene Content of Tomato Sauce at Different Times of Processing*

<table>
<thead>
<tr>
<th>Heating time (min)</th>
<th>Lycopene (mg/100g) dwb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>99.7+/-4.5</td>
</tr>
<tr>
<td>5</td>
<td>98.5+/- 8.9</td>
</tr>
<tr>
<td>30</td>
<td>120.5+/12.0</td>
</tr>
<tr>
<td>60</td>
<td>79.5+/-1.2</td>
</tr>
<tr>
<td>90</td>
<td>101.6+/9.1</td>
</tr>
<tr>
<td>120</td>
<td>93.9+/1.0</td>
</tr>
<tr>
<td>150</td>
<td>99.7+/3.1</td>
</tr>
<tr>
<td>180</td>
<td>87.4+/2.4</td>
</tr>
<tr>
<td>210</td>
<td>91.7+/3.4</td>
</tr>
</tbody>
</table>

* Seybold and others (2004)

The goal of the current pilot study was to determine the effects of cooking time and temperature on lycopene content of commercially processed tomato sauces used as ingredients in food dishes. Based on the review of the existing literature, the following hypotheses were tested:

Hypotheses of the Study

Ho$_1$: The mean lycopene content of tomato sauce samples will be greater at successive time intervals at constant oven temperatures.

Ho$_2$: The mean lycopene content of tomato sauce samples will be higher with increased cooking temperatures at constant cooking time.
H₀₃: The mean lycopene content of tomato sauce samples will be higher with increased cooking time and temperatures.

Objectives of the Study

1. Determine the effects of different cooking times on the lycopene content of commercially processed tomato sauces.

2. Determine the effects of different cooking temperatures on the lycopene content of commercially processed tomato sauces.

3. Determine the combined effects of different cooking time and temperature on the lycopene content of commercially processed tomato sauce.

4. Determine and compare the moisture content of uncooked and cooked commercially processed tomato sauces.
CHAPTER III: METHODOLOGY

Materials

All organic solvents used for the separation of lycopene were purchased from Fisher Scientific. The lycopene standard (1.454 mg/L) was received from Chromatodex research lab.

Tomato sauce cans (1000 gm can) of three different brands (Kroger, Dei Fratelli, and Hunt’s) were purchased from a local market for the experimental samples. The containers had sealed content, were rust free and undamaged when purchased, and were in the same desirable state (not expired) during the time of experimentation. Representative samples of each brand were from the same lot (same manufacture date or batch number) to maintain experimental consistency. Each brand of tomato sauce was purchased in enough quantity to minimize chances of variation and to maintain experimental homogeneity in sample selection.

Study Design

This study was conducted on three different brands of tomato sauces which were heated at different oven temperatures (325, 350, and 375°F) for different amounts of time (15, 30 and 45 minutes). There were 10 samples (three cooking times x three temperatures = nine samples, plus one uncooked sample), for each brand. The study was conducted in duplicate. Therefore, there were a total of 10 x 2 = 20 samples from one brand, and each extracted sample was analyzed in triplicate for lycopene by HPLC analysis. The two closest values of peak area were taken from the triplicates sample runs and averaged for the final lycopene content calculation. There were a total of 120 HPLC samples analyzed for lycopene content. All experimental procedures were conducted under dim light, and containers were also wrapped in aluminum foil to diminish light exposure and potential lycopene destruction in the samples.
Moisture Analysis

Tomato sauce samples were weighed on aluminum plates (each containing 15 gm) in duplicate, on a Mettler Balance Toledo scale to two decimal places. A representative sample from each brand of tomato sauce was tested for moisture content using the following procedure: Set Infrared moisture analyzer (IR – 30) to auto zero and than adjust the temperature to 90 °C and set the time for 35 min, which was the optimum time-temperature for tomato sauce samples because it is very important that the sample should not be burned during the moisture analysis. Sample weights were recorded before and after moisture analysis. Percent moisture content was noted at the end of analysis. Thus, moisture content of uncooked tomato sauce was conducted in duplicate by heating 15 g samples at 90 °C for 35 minutes on an Infrared moisture analyzer (IR – 30).

Pre and post sample weight was measured and recorded before and after desired heat treatment for cooked samples. Moisture loss during cooking was calculated using the equation below.

Moisture loss = initial weight – final weight/ initial weight x 100.

Heat Treatment

Twenty grams of tomato sauce was used as standard weight for the different heat treatments. Each sample was labeled according to related time and temperature. The oven was preset to the required temperature and checked with a thermometer to match with the desired test temperature. The labeled Erlenmeyer flask (125 ml) containing 20 gm of sample was placed in the oven set at the required temperature and time. The temperature was measured and recorded immediately upon removal of the samples. The samples were cooled to room temperature before chemical analysis.
Lycopene Extraction

Lycopene was extracted using a mixture of hexane-acetone-methanol (50:25:25), according to the procedure described by Sadler and others (1990) and Shi and others (1999). As mentioned, all experimental procedures were completed under minimal light, and containers were wrapped in aluminum foil. One hundred milliliters of the solvent mixture was added to the flask and agitated for 15 minutes by a wrist action shaker. Fifteen ml of water was then added into the flask followed by another 5 minutes of agitation to separate the solution fractions.

Filtration

The sample was then filtered through a Buchner funnel, using Whatman paper (No.2) and water generated vacuum. The Whatman paper was saturated with methanol before starting filtration to seal to the funnel. The filtrate was collected and used in the separation step.

Lycopene Separation

For the final step of sample preparation, the filtrate was poured into a 500 ml separatory funnel covered with aluminum foil, and allowed to sit for five minutes to separate into two distinct layers: polar and nonpolar phases. The lower layer was separated carefully into a waste beaker without disturbing the upper layer. Then, the upper layer containing lycopene was collected into a labeled clean beaker and covered with aluminum foil. The sample volume was measured. Samples were refrigerated at 40 °F for 24 +/- 4 hours and analyzed by HPLC (high pressure liquid chromatography) for lycopene content. Extracts was analyzed in triplicate of which the two closest values of peak area were used for lycopene analysis.

HPLC Analysis

Lycopene concentration of the tomato sauce was measured using a HPLC system described by Sadler and others (1990) and Shi and others (1999). A Hitachi series liquid
chromatogram instrument was used, consisting of a solvent delivery pump, controller, and
UV/visible detector for the determination of lycopene. Separation was achieved by using an
analytical polymeric C30 column (4.6mm x 25 cm). A guard column packed with C 30
stationary phase was used in-line for all separations. The mobile phase consisted of acetone:
butyric acid: ethanol (50:25:50). A 60 mcl sample was injected into the detector via a micro
syringe. The flow rate was adjusted to 1 ml/min, and peak areas were measured at 450 nm. The
lycopene calibration curve was generated from peak heights of standards run on HPLC by a
Window Excel application (Figure 2).

The peaks of lycopene were identified by comparing their retention times and peak area
with that of a pure standard (Figure 3). Detector signals were acquired and integrated on a chart
printer. Chart speed was set to 2.50 mm/min with an attenuation of 3 to get a properly visible
lycopene peak. Lycopene concentrations of unknown samples were calculated by extrapolation
on the calibration curve. Each sample was diluted to fit on the standard calibration curve because
peak areas of direct samples were too high. Therefore, samples were diluted in 1:20, 1:25, 1:30,
or 1:40, depending on their peak areas after their first run on HPLC. Dilution was started from
low dilution factor to high, e.g. 21 to 41. The dilution factor used for each sample was recorded
for later application in calculating the lycopene concentration.

The linear regression equation $y = mx + b$ was used to determine the lycopene
concentration. Where $m = 6146.20$, slope of regression line,

$b = 587.43$, intercept of regression line

$r^2 = 0.9995$ correlation coefficient

$y =$ area under peak from HPLC of unknown samples

$x =$ concentration of unknown samples
Figure 2 – Standard Curve For Lycopene

Explanation of Lycopene Calculation

E.g. for R1 (Table 3), Y = MX + B. Use the values from the Table 3 for Y = 8385, M = 6146.2, and B = 587.43; X will be 1.26.

To get milligram in liter, multiply it by dilution factor 41 (1:40), 1.26 x 41 = 51.66 Mg/L

To get milligram in sample volume, use sample volume recorded. E.g. 41 ml

Therefore, 51.66 x 0.041 = 2.11 mg of lycopene was there in R1 raw sample.
Table 3. Example of Calculation Table Used For Lycopene Determination

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Peak area</th>
<th>Lycopene (mg/L)</th>
<th>Extraction vol (ml)</th>
<th>Lycopene (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>1:40</td>
<td>8057</td>
<td></td>
<td>41</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8385</td>
<td>1.26</td>
<td>51.66</td>
<td>2.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8742</td>
<td>1.32</td>
<td>54.12</td>
<td>2.21</td>
</tr>
<tr>
<td>R2</td>
<td>1:40</td>
<td>9645</td>
<td>1.47</td>
<td>60.27</td>
<td>2.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9547</td>
<td>1.45</td>
<td>59.45</td>
<td>2.49</td>
</tr>
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<td>2.31</td>
<td>71.61</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>73.78</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
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<td>71.61</td>
<td>3.15</td>
</tr>
<tr>
<td>B1</td>
<td>1:30</td>
<td>15448</td>
<td></td>
<td>42</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td>2.21</td>
<td>68.51</td>
<td>2.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14857</td>
<td>2.32</td>
<td>71.92</td>
<td>3.02</td>
</tr>
<tr>
<td>B2</td>
<td>1:30</td>
<td>13764</td>
<td></td>
<td>43</td>
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<td></td>
<td>12782</td>
<td>1.98</td>
<td>61.38</td>
<td>2.63</td>
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<td></td>
<td>12878</td>
<td>1.99</td>
<td>61.69</td>
<td>2.65</td>
</tr>
<tr>
<td>C1</td>
<td>1:30</td>
<td>15028</td>
<td></td>
<td>41</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>16935</td>
<td>2.65</td>
<td>82.15</td>
<td>3.36</td>
</tr>
</tbody>
</table>

*Values were recorded for each sample for all three brands after calculating lycopene concentration.
Figure 3 – Representative Chromatogram From HPLC of Standard (A) and Unknown Sample (B)

Statistical Analysis

Data was analyzed by a SPSS computer software program for factorial ANOVA. The study design had two independent variables with three different conditions each, i.e., three temperatures and three cooking time, for a 3 x 3 factorial design. Two-way ANOVA was used to determine the effect of each independent variable and their interaction. Uncooked tomato sauce was used as control. The Scheffe test was run to determine the differences between particular groups.
CHAPTER IV: RESULTS AND DISCUSSION

Lycopene possesses a unique chemical structure which makes it very sensitive to oxidation and isomerization reactions during the cooking and processing of tomato products in the food industry, as well as normal home cooking (Shi and others 1999). Lycopene is bound to the cell wall matrix of food products (Sahlin and others 2004; Ven het Hof and others 2004). Lycopene is embedded in protein complexes or lipid droplets of the tomato products. Lycopene exists in a crystalline embedded form which gives it resistance to heat destruction (Shi and others 1999; Rao and others 2000; Dewanto and others 2002; Sahlin and others 2004; Ven het Hof and others 2004). Heat treatment results in the release of oxy and hydroxy enzymes which can destroy lycopene; however, lycopene does not get destroyed easily in mild conditions of heat exposure (Shi and others 1999; Sahlin and others 2004).

There are many factors other than heat, such as oxygen, light exposure, product moisture content, solid content, presence of pro-oxidants and lipids, and type of metal used, e.g. aluminum, steel, glass, copper in the product container, that affect the lycopene content of tomato products. These factors interact in conjunction with time and temperature to make lycopene either heat resistant or heat sensitive (Shi and others 1999; Akanbi and others 2004; Goula and others 2006). Further, the linear structure of lycopene forms multilayered or aggregated structure as an effect of various technological factors, e.g. thermal and mechanical processing. The multilayered or aggregated form makes lycopene resistant under mild time and temperature conditions. However, extensive heat treatment (high time and temperature conditions) may result in disruption of the structure and release of lycopene (Shi and others 1999; Nguyen and others 2001).
The objectives of the present pilot study was to determine the effects of cooking time and temperature on the lycopene content of already processed, commercially available tomato sauces. Three different brands of tomato sauce were tested to determine the effects of both cooking time and temperature on commercially processed tomato sauces. The exploratory analyses results demonstrated that both times and temperatures during home cooking effectively enhanced the lycopene content from the processed tomato sauces.

Comparison of Brands

In the present study, the researcher took 20 gms sample of tomato sauce from each of the brands to analyze the lycopene content. The lycopene content of different brands ranged from 2.35 – 17 mg/100 gm of tomato sauce. This level is similar to the 7.3 – 18 mg of lycopene/100 gm tomato sauce estimated by Pohar and others (2003).

The main effects of time, temperature, and the interaction effect on the lycopene content were examined for each brand separately, along with the common analyses. The brands were significantly different in mean lycopene content, F (1, 117) = 119.66, P<0.0001. The Scheffe test revealed significant differences (P<0.001) between the brands. The average of mean lycopene contents were 2.53 ± 0.44, 1.43 ± 0.57, and 0.97 ± 0.34 for Brands 1, 2 and 3, respectively. The results for the main effect of time and temperature for separate brand analyses are provided in Tables 4 and table 5.

Different types of tomatoes have different lycopene content depending on their ripening stage, firmness, genotype, differences in the structure, solid content, moisture content, processing techniques involved, and the presence of other ingredients (e.g. lipid, protein). These variables might have been related to the different lycopene contents found in the commercial brands studied, as similarly reported. Mature tomatoes have more lycopene content than immature or
early picked tomatoes. Some varieties of tomatoes have less moisture content and more solids content, and thus, they may be more concentrated in lycopene than others. Different companies use different processing time and temperature conditions for manufacture of tomato products. The lycopene content is also depend on the ingredients added during processing to deliver taste, flavor, or for preserving the tomato products. Tomatoes are stored for a different time period before manufacturing. Afterwards, tomato products are stored for variable time under different storage conditions. Even though all samples in the current study were from the same batch for an individual brand, batch variations between brands may account for much of the lycopene variation between samples. The presence of one or more variable that can affect the lycopene content before, during, and after processing of tomatoes/tomato products might be related to the observed differences in the lycopene content among the three tested brands (Akanbi and others 2004; Seybold and others 2004).

Although the common data analysis for all three tested brands in the current study were statistically significant for the main effect of time on the lycopene content, they were not in the hypothesized direction, \( F(2,110) = 3.65, P<0.03 \) (Table 4). \( H_0 \): The mean lycopene content of tomato sauce samples will be greater at successive time intervals at constant oven temperatures. This expectation of time affect on lycopene content was specifically tested by examining the main effect of cooking time on the three different brands of commercially processed tomato sauces. Similarly, the Scheffe test for common analysis of all three brands did not show significant differences in lycopene content at different cooking times. Therefore, the current study’s results rejected the first hypothesis about the positive effects of increased cooking time on the lycopene content of tomato sauce samples.
For Brands 1 and 2, the effect of time on lycopene content demonstrated that the sample cooked for 15 minutes had significantly higher ($P \leq 0.05$) lycopene content when compared to the samples cooked for either 30 and 45 minutes at any of the constant oven test temperatures. Moreover, only Brands 1 and 2 showed the highest lycopene content ($P \leq 0.05$) in the samples cooked for 15 minutes, when compared with the uncooked and samples cooked for 30 and 45 minutes, at any constant oven temperature. Brand 3 did not show significant differences between particular cooking times with the Scheffe test. Although not significant, the cooked samples had higher lycopene contents than the uncooked samples for all three brands (Table 4).

This current study result is consistent with the findings reported by Dewanto and others (2002), Seybold and others (2004), and Mayeaux and others 2006. Dewanto and co-workers reported an increase in the lycopene content of tomato sauce for the first 15 minutes of cooking, after which the lycopene started degrading. Similarly, Seybold and others (2004) found an increase in the lycopene content for the first 30 minutes of heat treatment. Longer heating time resulted in the loss of lycopene from the tested tomato sauces in both of these studies. Mayeaux and others (2006) observed that only 64.1% and 51.3% of lycopene was retained when a tomato slurry was baked at 350 and 420°F ($^\circ$C was converted to $^\circ$F) for 15 minutes, respectively. More lycopene was retained during the shorter cooking time.

Bound lycopene is released from the cell matrix when raw tomatoes undergo various steps of commercial processing of tomato products. However, there may be some lycopene still bound to the matrix in processed tomato products. Increases in the lycopene content in the 15 min. cooked samples may contribute to the release of more bound lycopene from the commercially processed tomato sauce, allowing for more efficient chemical separation.
Table 4 - Mean Lycopene Concentrations of Tested Samples at Different Cooking Times

<table>
<thead>
<tr>
<th>Brand</th>
<th>Univariate F</th>
<th>Mean Lycopene (mg) (Std. Error)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uncooked</td>
</tr>
<tr>
<td>Common</td>
<td>F (2, 110) = 3.66, P&lt;0.03</td>
<td>1.36 (0.23)</td>
</tr>
<tr>
<td>1</td>
<td>F (2, 30) = 11.77, P &lt; 0.001</td>
<td>2.34(^a) (0.18)</td>
</tr>
<tr>
<td>2</td>
<td>F (2, 30) = 10.84, P &lt; 0.001</td>
<td>0.90 (0.19)</td>
</tr>
<tr>
<td>3</td>
<td>F (2, 30) = 0.45, P &gt; 0.05, Not Sig.</td>
<td>0.85 (0.13)</td>
</tr>
</tbody>
</table>

Different letter superscripts within a row indicate significant differences at p < 0.05.

There was not a significant difference in the lycopene content between the 30 and 45 minutes cooked tomato sauce samples from common analysis and separate analysis for Brand 3. This finding is in agreement with a study reported by Dewanto and others (2002), which might be explained by the complete release of lycopene from the cell matrix being reached at 15 minutes of cooking. Therefore, there may be no further bound lycopene available for release after 15 minutes of cooking. Also, the additional heating might have resulted in some destruction of already released lycopene, and thus, the lycopene content started to decline after 15 minutes of cooking the tomato sauce samples.

The common lycopene analysis for all three brands supported the hypothesis for the main effect of temperature. The main effect of temperature was significant, \( F_{(2,110)} = 3.30, P<0.05 \), and the differences in the means as cooking temperatures were as hypothesized. The second hypothesis predicted: \( Ho_2 \): The mean lycopene content of tomato sauce samples will be higher with increased cooking temperatures at constant cooking time. This hypothesis was specifically tested by examining the main effect of cooking temperatures on the three different
brands of tomato sauce. These data support the present study hypothesis about the beneficial effects of increased cooking temperature on the tomato lycopene content. However, the Scheffe test for common analysis of all three brands indicated no significant differences in lycopene content at different cooking temperatures (Table 5).

Table 5 – Mean Lycopene contents of Tested Samples at Different Cooking Temperatures

<table>
<thead>
<tr>
<th>Brand</th>
<th>Univariate F</th>
<th>Mean Lycopene (mg) (Std. Error)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uncooked</td>
</tr>
<tr>
<td>Common</td>
<td>F (2, 110) =3.34, P&lt;0.04</td>
<td>1.36 (0.23)</td>
</tr>
<tr>
<td>1</td>
<td>F (2, 30) = 0.27, P &gt; 0.05</td>
<td>2.33 (0.18)</td>
</tr>
<tr>
<td>2</td>
<td>F (2, 30) =10.08, P &lt; 0.0001</td>
<td>0.90&lt;sup&gt;a&lt;/sup&gt; (0.19)</td>
</tr>
<tr>
<td>3</td>
<td>F (2, 30) = 19.98, P&lt;0.0001</td>
<td>0.85&lt;sup&gt;e&lt;/sup&gt; (0.13)</td>
</tr>
</tbody>
</table>

Different letter superscripts within a row indicate significant differences at p ≤ 0.05.

Brands 2&3 showed a significant main effect (P<0.005) of cooking temperatures on the tested tomato sauce samples (Table 5). Brand 1 showed a higher lycopene content at cooking temperature of 375 °F, but it was not significant. The Scheffe test did not show a significance difference in the lycopene contents with Brand 1. With Brand 2, the lycopene content was significantly higher (P<0.05) at the cooking temperature of 375 °F, when compared with both the uncooked, and the samples cooked at 325 and 350 ° F. With Brand 3, the lycopene content was significantly higher at cooking temperature of 375 ° F, when compared with uncooked sample and samples cooked at 325 and 350 ° F. (Table 5).
The results for the effects of cooking temperatures are not consistent with the findings of Takeoka and others (2001), Sahlin and others (2004), Miebach and others (2005), Mayeaux and others (2006) and Goula and others (2006), who found greater losses of lycopene at higher temperature heat treatment. Miebach and others (2005) and Mayeaux and others (2006) found that lycopene started degrading at a cooking temperatures of greater than 212°F, and also found losses of lycopene between tested cooking temperatures in ranges from 212 – 420°F.

In contrast, the current study results were in agreement with Abushita and others (2000), Nguyen and others (2001), and Dewanto and others (2002). These researchers found an increase in the lycopene content of tomato sauce during heat treatment at temperatures from 190 – 220°F. However, these studies were conducted to determine the effects of processing time and temperature on tomato lycopene, therefore, the temperatures ranged from 150 - 250°F, and these studies did not measure the effect of cooking temperatures between 300 - 400°F on the tomato products’ lycopene content (Abushita and others 2000; Nguyen and others 2001; Dewanto and others 2002).

The interaction effect of time and temperature was not consistent with all three tested brands. Brand 1 had the higher lycopene content for the sample cooked at 325°F for 15 minutes (Figure 4), but it was not significantly different from the other samples from the same brand. Brand 2 had a significantly (P<0.02) higher lycopene content for the sample cooked at 350°F for 15 minutes (Figure 5). While Brand 3 showed slightly higher lycopene content for the sample cooked at 375°F for 30 minutes which was not significantly different than either the other cooked or uncooked samples at different times and temperatures from the same brand (Figure 6). The combined statistical analysis of the lycopene contents from all brands showed a slightly higher lycopene content in samples cooked at 375°F for 15 minutes versus other samples cooked
at different cooking time and temperatures (Figure 7). The results from all three tested brands favored the beneficial effects of the shorter length of cooking on the lycopene content of the processed tomato sauces, but the present study could not determine the optimal cooking temperatures on the processed tomato sauce for retention or increase in lycopene content.

![Figure 4. Interaction Effects of Cooking Time and Temperature on Brand 1](image)

No significant differences were found.

Ho$_3$ : The mean lycopene content of tomato sauce samples will be higher with increased cooking time and temperatures. The results for the interaction effect of cooking time and temperature on lycopene content were in the hypothesized higher direction, but were not significant. Therefore, the finding of the present study did not support the positive effects of increased cooking time and temperature on the lycopene content of commercially processed tomato sauces, and Ho$_3$ was rejected. More extensive testing data is needed to determine the interaction effects of cooking time and temperature on the lycopene content of processed tomato sauces.
Figure 5. Interaction Effects of Cooking Time and Temperature on Brand 2
Significant differences were found at P<0.02.

Figure 6. Interaction Effects of Cooking Time and Temperature On Brand 3
No Significant differences were found.
Effects of Moisture Content

The moisture contents of the uncooked samples from Brand 1 (68.35%) and Brand 3 (73.58%) were higher when compared with Brand 2 (65.02%). The mean lycopene contents of the uncooked samples were 2.33 ± 0.18, 0.89 ± 0.19, and 0.84 ± 0.12 mg/20g of tomato sauce sample for Brands 1, 2 and 3, respectively. Goula and others (2006) stated that the presence of moisture or water can facilitate enzymatic activity, and thus, contribute toward the loss of lycopene through enzymatic reactions in the tomato sauce. Brand 2 had the lowest moisture content, and the highest yield of lycopene during cooking compared to when compared to Brands 1&3. Cooking and processing causes the loss of moisture from the tomato products due to heat dehydration. Therefore, the cooked tomato sauce samples in the current study had higher lycopene, when compared with uncooked (raw) tomato sauce samples. The loss of moisture increased with the length of cooking, and was similar among all three Brands at any constant cooking time (Table 6). At any give temperature, moisture loss was the highest in the samples.
cooked for 45 minutes. According to Goula and others (2006), the lycopene content should have increased with the loss of moisture from the sample; however, this was not consistently true with the current study.

In contrast, Mayeaux and others (2006) stated that the presence of moisture or water may help in slowing down the heat transfer to the tomato product, and thus, may lessen the heat damage to the product. The average of mean lycopene contents were 2.52 ± 0.05, 1.42 ± 0.05, and 0.97 ± 0.03 mg/20g of tomato sauce sample for Brands 1, 2 and 3, respectively. The increase in the lycopene content was 8, 59, and 15 % for Brands 1, 2 and 3, respectively. Brand 2 had the lowest moisture content as purchased (65.02%), and it showed the highest yield of lycopene (59%), when compared with the other two brands. This finding is not consistent with the results observed by Mayeaux and others (2006).

Table 6 - Moisture Loss in Tested Samples During Cooking

<table>
<thead>
<tr>
<th>Temp (°F)</th>
<th>Time (min)</th>
<th>Brand 1</th>
<th>Brand 2</th>
<th>Brand 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>325</td>
<td>15</td>
<td>52.08</td>
<td>50.53</td>
<td>52.16</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>74.63</td>
<td>68.48</td>
<td>77.84</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>92.17</td>
<td>88.73</td>
<td>92.56</td>
</tr>
<tr>
<td>350</td>
<td>15</td>
<td>52.55</td>
<td>51.44</td>
<td>52.63</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>74.88</td>
<td>68.04</td>
<td>77.7</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>92.29</td>
<td>89.1</td>
<td>92.23</td>
</tr>
<tr>
<td>375</td>
<td>15</td>
<td>52.79</td>
<td>51.09</td>
<td>52.79</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>74.88</td>
<td>68.45</td>
<td>78.09</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>92.62</td>
<td>88.1</td>
<td>92.7</td>
</tr>
</tbody>
</table>
Differences in the Internal Temperature of Cooked Tomato Sauce Samples

The internal temperatures of the cooked tomato sauce samples were measured immediately after each heat treatment (Table 7). No reviewed studies measured the effects of internal temperatures on the lycopene content of tomatoes or tomato products; therefore, the researcher did not predict changes in the internal temperature with changes in cooking time and temperature conditions. There were no differences found with ANOVA and the Scheffe test between tested cooked samples. The possible reason might be related to the selection of narrow ranges of cooking time (15, 30, 45 minutes) and temperature (325, 350, 375°F) in the current study design. More data are needed to determine the differences in the internal temperature of tomato sauce samples cooked at various time-temperature conditions, and to determine the effects of internal temperature on tomato sauce lycopene content.
Table 7. Internal Temperatures of Cooked Tomato Sauce Samples

<table>
<thead>
<tr>
<th>Temp °F</th>
<th>Time (min)</th>
<th>Brand 1</th>
<th>Brand 2</th>
<th>Brand 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>325</td>
<td>15</td>
<td>85</td>
<td>87</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>78</td>
<td>88</td>
<td>91</td>
</tr>
<tr>
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<td>45</td>
<td>87</td>
<td>96</td>
<td>88</td>
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<tr>
<td>350</td>
<td>15</td>
<td>89</td>
<td>93</td>
<td>91</td>
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<td>30</td>
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<td></td>
<td>45</td>
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<td>86</td>
<td>86</td>
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<tr>
<td>375</td>
<td>15</td>
<td>89</td>
<td>88</td>
<td>88</td>
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<td></td>
<td>30</td>
<td>86</td>
<td>89</td>
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<td></td>
<td>45</td>
<td>84</td>
<td>83</td>
<td>87</td>
</tr>
</tbody>
</table>
CHAPTER V: CONCLUSIONS

Summary

All three tested brands of commercial tomato sauces provided different results for the effects of cooking time and temperature on the lycopene content. The lycopene content of the tested tomato sauce samples increased with an increase in cooking temperature; however, the differences were not significant between various cooking temperatures. All three brands demonstrated significant effects of cooking time on the lycopene content of the processed tomato sauces. Lycopene content increased significantly ($P<0.005$) in samples cooked from all three Brands during the first 15 minutes of heat treatment, but then the lycopene content began to start degrading during cooking times beyond 15 minutes. This is supported by the possible continued release of lycopene from the commercially processed tomato sauces during the first 15 minutes of cooking time with the decrease in moisture content. The lycopene content was higher in the samples cooked at 375°F for 15 minutes, but it was not significantly different from the other samples cooked at various times and temperatures. Thus, the present study supported a shorter time heat treatment as an ideal home cooking method for recipes which use tomato sauce as an ingredient. Cooking for a shorter period of time may help to retain more lycopene in the home prepared food.

More research is recommended in this area to investigate the complicated nature of the lycopene molecule, not only in relation to time and temperature effects, but also how do moisture content, oxygen, light exposure, solid content, presence of oxidant or pro-oxidants, lipids, types of metal used in preserving the tomato product, e.g. sodium, aluminum, glass, plastic, steel, and other variables.
Limitations

There were many limitations to the present study which were barriers in achieving ideal experimental conditions.

- The major limitation was the budget to conduct the study. Because of the limited budget, experiments could not be conducted with more current technologically advanced equipment.
- The HPLC instrument used for data collection for the lycopene analysis of the tomato sauce samples was very old. Therefore, this study might not show the accuracy related to newer models of standard dual pump double column HPLC instruments.
- This HPLC instrument was unable to measure the different isomers of lycopene due to analytical column limitations. It is possible that the lycopene was not actually degraded, but only isomerized which was undetected by the column used.
- The laboratory temperature was not able to be regulated well enough in the area where data collection occurred. Temperature stability is an essential part of research that uses HPLC instruments, since HPLC columns are very sensitive to temperature fluctuation. This could have affected peak areas and retention time of analyzed samples, and thus, the accuracy of the lycopene concentration calculation of the tested samples.
- The current study was conducted on limited parameters of tested times and temperatures, and the ranges for time and temperature parameters may have been too close to each other. Therefore, the present study did not show the significant differences in lycopene concentrations at different levels of time and temperature condition.
- In addition, the current study was conducted on a small quantity of tomato sauce (20 grams) in a laboratory setting, without the addition of any other cooking ingredients,
which would not be the case with actual home cooking. Therefore, the results can expect to be different during ordinary cooking operations because of changes in tomato quantity used, ingredients, and interaction with other variables.

- Since lycopene is sensitive toward light, the whole experimentation was conducted under filtered indirect natural light to control the negative effects of the light variable on the lycopene in the tomato sauce samples. Natural light was used as the source of light to carry out the experimentation; still, the natural light was not consistent throughout the experimentation because of the difference between sunny versus cloudy days.

Future Recommendations

- Use of a HPLC column which can separate cis and trans isomers is recommended to determine if the change in the lycopene content was related to the actual degradation or it was isomerization changes only.

- The changes in lycopene concentrations in the samples might be more different if a wide range of cooking temperatures are used, e.g. 300, 350 and 400 °F. Again, this possibility applies similarly to the different cooking time choices. Study design should test more different time parameters, e.g. 15, 25, 35, 45, 55, 65, 75 and 90 minutes, etc. Identification of the exact time that lycopene starts to degrade due to the temperature.

- Actual recipe formation and checking the lycopene content of some original recipes containing tomato sauce after normal cooking operations is recommended.

- Use of dim light or alternative light sources (dark room) to conduct the experimentation procedure will help to control the light destruction variable.
- The lycopene standard is very expensive and highly unstable; this is one of the major barriers to conduct scientific studies on lycopene. There should be a reasonable substitute available to conducting studies on lycopene content.

- The study results favored the short time cooking treatment for the optimum yield of lycopene. Therefore, it is recommended to add tomatoes or tomato products towards the end of a cooking operation, and then allow that foodstuff to be in dry heat for shorter periods of time to preserve the lycopene.
REFERENCES


