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**Quantification of Vitamin C in fresh and boiled tomato samples: evaluating the influence of temperature using UV-Vis Spectroscopy.**

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## APPROVAL FORM

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## DECLARATION

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**ACKNOWLEGEMENTS**

I would like to appreciate my family for the emotional support they rendered throughout this research. I also want to acknowledge the guidance I received from my supervisor, Mr Katengeza

## ABSTRACT

Vitamin C is a vital nutrient in tomatoes, essential for human health, but its degradation during processing and cooking can significantly reduce its content. This study aimed to quantify vitamin C in fresh and boiled tomato samples using UV-Vis spectroscopy and investigate the impact of temperature on its degradation. Five fresh tomatoes were washed, dried, and cut into small pieces and transferred to a blender. The sample was homogenized with 20 mL of metaphosphoric acid solution (3% w/v) for 2 minutes at high speed. The mixture was centrifuged at 4°C and 10,000 rpm for 10 minutes. The supernatant was filtered through a 0.45 μm disposable syringe filter to remove any particulate matter. The filtrate was collected and diluted appropriately with metaphosphoric acid solution (3% w/v) for subsequent analysis by UV-Vis spectroscopy. Another sample of five fresh tomatoes were prepared. A representative portion (approximately 10 g) was weighed and transferred to a heat-resistant container. The tomato pieces were covered with deionized water and boiled in a water bath at 100°C for 15 minutes. The boiled tomato pieces were then homogenized with 20 mL of metaphosphoric acid solution (3% w/v) in a blender for 2 minutes at high speed. The homogenized mixture was centrifuged at 4°C and 10,000 rpm for 10 minutes. The supernatant was filtered through a 0.45 μm disposable syringe filter to remove any particulate matter. The filtrate was collected and diluted appropriately with metaphosphoric acid solution (3% w/v) for subsequent analysis by UV-Vis spectroscopy. The results showed that fresh tomato samples had a significantly higher vitamin C content (18.5 mg/100 g FW) compared to boiled tomato samples (11.2 mg/100 g FW). The difference in vitamin C levels between fresh and boiled samples was statistically significant ($p < 0.05$), as determined by Student's t-test. This study demonstrates the effectiveness of UV-Vis spectroscopy in quantifying vitamin C in tomatoes and highlights the importance of temperature control during processing to preserve vitamin C content. The findings have implications for food scientists, nutritionists, and cooks seeking to optimize vitamin C retention in tomatoes, contributing to improved nutrition and public health.

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# CHAPTER 1

## 1.1 Background

Vitamin C, also known as ascorbic acid, is an essential water-soluble vitamin that plays an important role in many bodily functions. It acts as a potent antioxidant in the body, helping to protect cells and molecules like proteins, lipids, carbohydrates, and nucleic acids from damaging effects of reactive oxygen species such as free radicals and peroxides (Carr & Frei, 1999). Free radicals are unstable molecules that are continuously generated during normal metabolic processes and exposure to environmental factors such as UV radiation, pollutants, and toxins. Their reaction with other molecules can start chain reactions and cause oxidative damage to cells (Valko et al., 2007).

As an antioxidant, vitamin C scavenges and neutralizes free radicals before they can damage biologically important molecules. It can donate one of its electrons to an attacking radical species, thereby converting the radical to a more stable form and preventing it from reacting further in the chain reaction of lipid peroxidation (Sies & Stahl, 1995). In this way, vitamin C protects lipids, proteins and DNA from oxidative damage within cells and tissues. It plays a vital role in many biochemical processes as shown in table 1.1 below:

Table 1.1: The role played by vitamin C and their mechanism.

| Function | Mechanism |
|---|---|
| Collagen Synthesis | Vitamin C participates in the post-translational hydroxylation of proline and lysine during the synthesis of collagen which provides structure and strength to blood vessels, connective tissues, bones and cartilage (Padayatty et al., 2003; Nyska et al., 2002). |
| Iron Absorption: | It helps in the absorption of non-heme iron from plant foods by reducing ferric ($Fe^{3+}$) to the ferrous form ($Fe^{2+}$) which is then taken up by intestinal cells (Trumbo et al., 2001). |
| Gene Expression | There is evidence that vitamin C affects gene expression through its interaction with certain gene products and transcription factors (Alexander et al., 2017; Bowie & O'Neill, 2000). |
| Brain Function: | Studies suggest it plays an important role in neurotransmitter synthesis and brain development in infants and children (Rumsey & Daruwala, 1999; Humphries et al., 2008). |

Prolonged deficiency of vitamin C can have serious health consequences like scurvy which manifests as bleeding gums, tooth loss, fragile bones and bruising of the skin (Padayatty & Levine, 2000). Epidemiological studies have also linked low vitamin C levels to increased risk of several chronic diseases including cardiovascular disease, certain cancers and age-related functional decline (Gaziano et al., 2009; Green et al., 2019; Carr & Frei, 2017). The recommended dietary allowance varies from 30-75 mg per day depending on age and sex (NIH, 2018).

Tomatoes are one of the richest dietary sources of vitamin C, along with peppers and citrus fruits. Fresh tomatoes typically contain between 15-23 mg of vitamin C per 100 grams which meets the recommended daily intake for most adults (Nielsen, 2010; USDA, 2019). Tomatoes also provide a considerable amount of antioxidant lycopene giving them added nutritional value. However, vitamin C is heat labile and easily degraded, especially when exposed to temperatures above 70°C such as during cooking processes (Lee & Kader, 2000). Previous studies have shown that simple treatments like boiling can result in significant losses ranging from 30-50% depending on factors like temperature, pH, time of heating and presence of catalyzing metals (Maskan, 2001; Tims & Watts, 1958). Therefore, characterizing the heat stability of vitamin C in tomatoes under controlled experimental conditions is important for optimizing cooking methods to maximize its retention.

Ultraviolet-visible (UV-Vis) spectroscopy is a commonly utilized analytical technique for quantitative determination of vitamin C across various food and biological matrices. Vitamin C has strong absorbance in the ultraviolet region due to its enediol functional groups. By exploiting its spectral properties, concentration can be measured via the Beer-Lambert law which relates absorbance to concentration of the absorbing species in solution (Esteves et al., 2017). It is a simple, inexpensive method that does not require elaborate sample preparation steps and provides reliable results for evaluating vitamin C stability under different processing and storage conditions (Kaur & Kapoor, 2002). The objective of the current study was to systematically investigate the effect of controlled boiling temperatures on vitamin C degradation kinetics in tomatoes over time using UV-Vis spectroscopy.

### 1.2 Statement of the Problem

While previous research has established the heat sensitivity of vitamin C in tomatoes, many studies lack precise temperature control during cooking experiments. Most have only measured

vitamin C at a single endpoint rather than monitoring degradation over a period of heating (Gil et al., 2006; Maskan, 2001). Additionally, variations in tomato cultivars, ripening stages and heating methods between studies make direct comparisons of results challenging. Characterizing vitamin C stability through kinetic models under defined time-temperature conditions can provide useful data for developing processing guidelines. Very few studies have directly evaluated the influence of controlled boiling temperatures on vitamin C concentration in tomatoes using an accurate quantification technique such as UV-Vis spectroscopy. More research is still needed to fully understand this relationship and optimize cooking methods to help retain higher levels of this important antioxidant.

### 1.3 Aims and objectives

The aim of this research was to quantify vitamin C concentrations in fresh and boiled tomato samples using UV-Vis spectroscopy.

The specific objectives are

1. Measure and compare the initial vitamin C contents of fresh tomato samples from different cultivars.
2. Boil tomato samples at controlled temperatures of 80°C, 90°C and 100°C for 0, 5, 10, 15 and 20 minutes.
3. Quantify remaining vitamin C concentrations after each time interval using a UV-Vis spectrophotometric method.
4. Analyze the effects of increasing temperature on rate and extent of vitamin C degradation over the range of cooking conditions tested.

### 1.4 Significance of the Study

Elucidating the influence of controlled cooking temperatures on vitamin C stability in tomatoes will provide useful insights for both nutritional and food processing perspectives. From a dietary aspect, establishing safe temperature thresholds and time limits for boiling tomatoes can help consumers retain higher levels of this essential antioxidant micronutrient during home cooking. For the food industry, understanding vitamin C degradation kinetics in response to thermal processing will aid in developing techniques like minimal processing, thermal pasteurization and sterilization that better preserve vitamin C content

in products. Modeling temperature effects on kinetic behavior is valuable for controlling processing conditions and maximizing nutrient retention which is critical for advancing consumer nutrition and food quality. The results can offer guidelines for temperature-controlled cooking of tomatoes to maintain important health-promoting compounds like vitamin C.

## CHAPTER 2: LITERATURE REVIEW

### 2.1 Introduction

Vitamin C, also known as ascorbic acid, is an essential nutrient that plays a crucial role in various physiological processes in the human body. It is a powerful antioxidant that helps protect cells from damage caused by free radicals and oxidative stress. Additionally, vitamin C is involved in the synthesis of collagen, a structural protein that supports various tissues, including skin, bones, and blood vessels (Naidu, 2003).

Tomatoes (Solanum lycopersicum) are a widely consumed fruit that is rich in vitamin C, along with other beneficial compounds such as lycopene, flavonoids, and phenolic compounds (Giovanelli and Paradiso, 2002). Fresh tomatoes are known for their tangy flavor and vibrant red color, making them a popular choice in various culinary dishes worldwide. However, cooking or heat treatment can affect the nutritional content of tomatoes, including the concentration of vitamin C (Dewanto et al., 2002).

The quantification of vitamin C in fresh and cooked tomato samples is of great interest to researchers, food manufacturers, and consumers alike. Accurate determination of vitamin C levels can provide valuable information about the nutritional quality of tomatoes and the impact of different cooking methods on their nutrient content. This knowledge is essential for optimizing food processing techniques, preserving nutritional value, and ensuring accurate labeling and consumer awareness.

One widely utilized analytical technique for the quantification of vitamin C is UV-Vis spectroscopy. This method relies on the absorption of ultraviolet and visible light by the ascorbic acid molecule, enabling precise measurement of its concentration in various matrices (Arya et al., 2000). UV-Vis spectroscopy offers several advantages, including simplicity, cost-effectiveness, and the ability to analyze a wide range of samples with minimal sample preparation.

This literature review provides a comprehensive overview of the current knowledge and research related to the quantification of vitamin C in fresh and boiled tomato samples using UV-Vis spectroscopy. It will explore the significance of vitamin C, the role of tomatoes as a rich source of this nutrient, the impact of temperature and cooking methods on vitamin C content, and the principles and applications of UV-Vis spectroscopy in vitamin C analysis. Additionally, the review will critically examine existing research studies, highlighting their

findings, methodologies, and limitations, while identifying potential gaps and areas for further investigation.

## 2.2 Significance of Vitamin C

Vitamin C, also known as ascorbic acid (C6H8O6), is a water-soluble vitamin that plays numerous vital roles in human health and well-being. It is an essential nutrient that must be obtained from dietary sources, as humans and other primates lack the ability to synthesize it endogenously (Naidu, 2003).

One of the primary functions of vitamin C is its potent antioxidant activity. It acts as a scavenger of reactive oxygen species (ROS) and free radicals, protecting cells and tissues from oxidative damage (Carr and Frei, 1999). Free radicals are highly reactive molecules that can cause damage to proteins, lipids, and DNA, leading to various health issues, including cardiovascular diseases, cancer, and premature aging (Sies, 1997).

Vitamin C also plays a crucial role in collagen synthesis, which is essential for the maintenance and repair of connective tissues, such as skin, cartilage, bones, and blood vessels (Murad et al., 1981). Collagen is the most abundant protein in the human body and provides structural support and strength to various organs and tissues.

Furthermore, vitamin C is involved in the metabolism of several neurotransmitters, including dopamine, norepinephrine, and serotonin, which are essential for proper brain function and mental health (Rebec and Pierce, 1994). It also contributes to the absorption and utilization of non-heme iron, preventing iron deficiency anemia (Teucher et al., 2004).

Adequate intake of vitamin C has been associated with numerous health benefits, including:

***Enhancing immune function:*** Vitamin C supports the proper functioning of immune cells, such as T cells and phagocytes, helping to protect the body against infections and diseases (Carr and Maggini, 2017).

***Reducing the risk of cardiovascular diseases***: The antioxidant properties of vitamin C help protect against oxidative damage to the endothelial cells lining blood vessels, potentially reducing the risk of atherosclerosis and other cardiovascular complications (Juraschek et al., 2012).

***Promoting skin health:*** Vitamin C is essential for collagen synthesis, which is crucial for maintaining skin elasticity and preventing premature aging and wrinkle formation (Pullar et al., 2017).

***Enhancing wound healing:*** Adequate vitamin C intake supports the body's ability to repair and regenerate damaged tissues, facilitating wound healing (Ellinger and Muller, 1992).

***Reducing the risk of certain cancers:*** Studies have suggested that higher intake of vitamin C may be associated with a lower risk of certain types of cancers, such as stomach, esophageal, and breast cancers (Harris et al., 2014; Lee et al., 2017).

Given the numerous health benefits and essential physiological roles of vitamin C, maintaining an adequate intake of this nutrient is crucial for overall well-being and disease prevention.

### 2.3 Tomatoes as a Rich Source of Vitamin C

Tomatoes (Solanum lycopersicum) are a widely consumed fruit that is renowned for their nutritional value and culinary versatility. They are an excellent source of vitamin C, along with other beneficial compounds such as lycopene, flavonoids, and phenolic compounds (Giovanelli and Paradiso, 2002).

The vitamin C content in tomatoes can vary depending on factors such as cultivar, ripeness, growing conditions, and post-harvest handling (Dewanto et al., 2002). Typically, fresh, ripe tomatoes contain between 10 to 50 mg of vitamin C per 100 grams of edible portion (Giovanelli and Paradiso, 2002; Naidu, 2003).

In addition to vitamin C, tomatoes are rich in other antioxidants, including lycopene, a potent carotenoid pigment responsible for the characteristic red color of ripe tomatoes (Rao and Rao, 2007). Lycopene has been widely studied for its potential health benefits, particularly in reducing the risk of certain cancers and cardiovascular diseases (Agarwal and Rao, 2000).

Tomatoes also contain various flavonoids and phenolic compounds, such as naringenin, quercetin, and chlorogenic acid, which contribute to their antioxidant capacity and potential health-promoting properties (Giovanelli and Paradiso, 2002; Martínez-Valverde et al., 2002).

The combination of vitamin C and other antioxidants present in tomatoes makes them a valuable addition to a balanced diet. Consuming fresh tomatoes or incorporating them into various culinary dishes can contribute to meeting the recommended daily intake of vitamin C and provide a range of potential health benefits.

### 2.4 Impact of Temperature and Cooking Methods on Vitamin C Content

While fresh tomatoes are an excellent source of vitamin C, the concentration of this nutrient can be influenced by various processing and cooking methods. Temperature, in particular, plays a significant role in determining the retention or degradation of vitamin C in tomato products (Dewanto et al., 2002).

Vitamin C is a heat-sensitive compound, and its stability can be affected by exposure to high temperatures during cooking or processing (Combs, 2008). The degradation of vitamin C is primarily due to its oxidation, which can be accelerated by factors such as heat, oxygen, and the presence of certain metal ions (Davey et al., 2000).

Several studies have investigated the impact of different cooking methods on the vitamin C content of tomatoes. Some of the key findings are as follows:

***Boiling:*** Boiling is a common cooking method for tomatoes, often used in the preparation of sauces, soups, and stews. However, boiling can lead to significant losses of vitamin C due to the high temperatures involved and the leaching of water-soluble nutrients into the cooking water (Dewanto et al., 2002; Giovanelli and Paradiso, 2002).

***Steaming:*** Steaming is considered a gentler cooking method that can help preserve the vitamin C content in tomatoes better than boiling. The lower temperatures used in steaming minimize the degradation of heat-sensitive nutrients like vitamin C (Gahler et al., 2003).

***Microwave cooking:*** Microwave cooking has been shown to retain higher levels of vitamin C in tomatoes compared to conventional boiling or steaming methods (Muñoz de Chávez et al., 1998). The shorter cooking times and reduced exposure to heat help preserve the vitamin C content.

***Frying:*** Frying tomatoes at high temperatures can lead to significant losses of vitamin C due to the combined effects of heat and exposure to oxygen (Dewanto et al., 2002; Giovanelli and Paradiso, 2002).

***Canning and processing:*** The canning and industrial processing of tomatoes often involve heat treatments, such as blanching, pasteurization, or sterilization, which can result in varying degrees of vitamin C degradation (Garzón and Wrolstad, 2002; Anese et al., 2002).

It is important to note that the extent of vitamin C loss during cooking or processing can also depend on factors such as the duration of heat exposure, the presence of oxygen, the pH of the tomato product, and the presence of certain metal ions that can catalyze the oxidation of vitamin C (Combs, 2008; Davey et al., 2000).

To mitigate the degradation of vitamin C during cooking or processing, various strategies have been explored, including:

1. Minimizing exposure to high temperatures and prolonged cooking times.
2. Using cooking methods that involve shorter heating times, such as microwave cooking or stir-frying.
3. Adding acidulants or antioxidants to the tomato products to create an environment that is less conducive to vitamin C degradation (Giovanelli and Paradiso, 2002; Hernández et al., 2006).
4. Employing processing techniques that minimize exposure to oxygen, such as vacuum packaging or nitrogen flushing (Santos and Silva, 2008).

The impact of temperature and cooking methods on the vitamin C content of tomatoes is a crucial consideration for preserving the nutritional quality of these fruits and ensuring accurate labeling and consumer information.

## 2.5 Principles of UV-Vis Spectroscopy

UV-Vis spectroscopy, also known as ultraviolet-visible spectroscopy, is an analytical technique widely used for the quantitative and qualitative analysis of various compounds, including vitamins, pigments, and other organic and inorganic substances (Skoog et al., 2018).

The principle behind UV-Vis spectroscopy is based on the interaction of ultraviolet and visible light with the electronic structure of molecules. When a molecule absorbs light energy from the UV-Vis region of the electromagnetic spectrum, the energy promotes electrons from their ground state to higher energy levels, resulting in an electronic transition (Perkampus, 1992).

The wavelength and intensity of the absorbed light are directly related to the structure and concentration of the molecule being analyzed. Each molecule has a unique pattern of electronic transitions, which results in a characteristic absorption spectrum (Skoog et al., 2018).

In UV-Vis spectroscopy, a beam of light from a UV-Vis source is directed through a sample solution, and the amount of light absorbed by the sample is measured using a detector (Skoog et al., 2018). The absorbance of light by the sample is quantified according to the Beer-Lambert law, which states that the absorbance is proportional to the concentration of the absorbing species and the path length through which the light travels (Perkampus, 1992).

The Beer-Lambert law is expressed as:

$$\mathbf{A = \varepsilon \times c \times l}$$

Where: **A** is the absorbance, **ε** is the molar absorptivity or extinction coefficient (a constant for a given substance at a specific wavelength), **c** is the concentration of the absorbing species, and **l** is the path length of the light through the sample

By measuring the absorbance of a sample at a specific wavelength and knowing the molar absorptivity and path length, the concentration of the absorbing species can be calculated (Skoog et al., 2018).

UV-Vis spectroscopy is particularly well-suited for the analysis of vitamin C (ascorbic acid) due to its strong absorption in the UV region of the electromagnetic spectrum. Ascorbic acid exhibits a characteristic absorption maximum at around 265 nm, which is attributed to the conjugated double bond system in its structure (Arya et al., 2000).

Several factors contribute to the widespread use of UV-Vis spectroscopy for vitamin C analysis, including:

***Simplicity:*** UV-Vis spectroscopy is a relatively simple and straightforward analytical technique that does not require extensive sample preparation or complex instrumentation (Skoog et al., 2018).

***Cost-effectiveness:*** UV-Vis spectrophotometers are generally less expensive compared to other analytical instruments, making this technique accessible to many laboratories and research facilities (Skoog et al., 2018).

***Sensitivity:*** UV-Vis spectroscopy offers high sensitivity for the detection and quantification of compounds with strong chromophores, such as vitamin C, allowing for accurate measurements even at low concentrations (Arya et al., 2000).

***Versatility:*** UV-Vis spectroscopy can be applied to a wide range of sample matrices, including food products, biological fluids, and environmental samples, making it a valuable tool in various fields of research and analysis (Skoog et al., 2018).

Despite its advantages, UV-Vis spectroscopy also has limitations, such as potential interference from other absorbing species present in complex sample matrices and the requirement for careful sample preparation to ensure accurate and reproducible results (Skoog et al., 2018).

## 2.6 Applications of UV-Vis Spectroscopy in Vitamin C Analysis

UV-Vis spectroscopy has been extensively used for the quantification of vitamin C in various food matrices, including fruits, vegetables, juices, and processed products. Due to its simplicity, cost-effectiveness, and sensitivity, this analytical technique has been widely adopted in research laboratories, food quality control, and nutritional studies.

One of the primary applications of UV-Vis spectroscopy in vitamin C analysis is the determination of ascorbic acid content in fresh and processed fruits and vegetables. Several studies have utilized this technique to quantify vitamin C levels in tomatoes, citrus fruits, leafy greens, and other plant-based foods (Arya et al., 2000; Eitenmiller and Landen, 1999; Hernández et al., 2006).

For example, Giovanelli and Paradiso (2002) employed UV-Vis spectroscopy to evaluate the vitamin C content in various tomato cultivars, including fresh, processed, and greenhouse-grown tomatoes. Their study provided valuable insights into the influence of cultivar selection, ripening stages, and processing methods on the ascorbic acid levels in tomatoes.

UV-Vis spectroscopy has also been widely used to monitor the degradation of vitamin C during food processing and storage conditions. Anese et al. (2002) utilized this technique to investigate the kinetics of vitamin C degradation in tomato juice subjected to different thermal treatments, including pasteurization and sterilization. Their findings contributed to the optimization of processing conditions to minimize nutrient losses.

In addition to food analysis, UV-Vis spectroscopy has been employed in pharmaceutical and biomedical applications, such as the determination of vitamin C levels in biological fluids and the evaluation of dietary supplements and fortified products (Arya et al., 2000; Eitenmiller and Landen, 1999).

While UV-Vis spectroscopy offers simplicity and sensitivity in vitamin C analysis, it is important to address potential limitations and interferences. One significant challenge is the presence of other absorbing species in complex sample matrices, which can interfere with the accurate measurement of ascorbic acid (Arya et al., 2000). To overcome this issue, various sample preparation techniques, such as extraction, purification, and the use of specific reagents or derivatization, may be employed to isolate and selectively determine vitamin C (Hernández et al., 2006; Eitenmiller and Landen, 1999).

Another consideration is the potential instability of ascorbic acid during sample preparation and analysis. Vitamin C is susceptible to oxidation, which can lead to inaccurate quantification (Combs, 2008). To mitigate this issue, researchers often employ antioxidants, acidic conditions, or anaerobic environments during sample handling and analysis (Hernández et al., 2006; Eitenmiller and Landen, 1999).

Despite these challenges, UV-Vis spectroscopy remains a widely adopted and valuable technique for vitamin C analysis in various fields, including food science, nutrition, pharmaceutical research, and biomedical applications.

## 2.7 Critical Review of Existing Literature

Numerous research studies have been conducted to investigate the quantification of vitamin C in fresh and processed tomato samples using UV-Vis spectroscopy. These studies have provided valuable insights into the influence of various factors on vitamin C content, as well as the strengths and limitations of the analytical techniques employed. This section will critically review some of the key literature in this field, highlighting their contributions, methodologies, and potential areas for further investigation.

Giovanelli and Paradiso (2002) conducted a comprehensive study on the vitamin C content in various tomato cultivars, including fresh, processed, and greenhouse-grown tomatoes. They employed UV-Vis spectroscopy to quantify ascorbic acid levels and investigated the influence of factors such as cultivar type, ripening stage, and processing methods. Their findings revealed

significant variations in vitamin C content among different tomato cultivars and highlighted the impact of processing techniques like canning and juice extraction on the retention of ascorbic acid.

One strength of this study was the use of a validated UV-Vis spectroscopic method for vitamin C analysis, which was supported by recovery studies and comparisons with other analytical techniques. Additionally, the authors provided valuable insights into the effect of ripening stages on vitamin C levels, demonstrating that fully ripe tomatoes generally have higher ascorbic acid content compared to underripe or overripe fruits.

However, the study did not extensively explore the impact of different cooking methods, such as boiling or steaming, on vitamin C degradation. Further investigations into the influence of various heat treatments and cooking durations could provide more comprehensive information for optimizing nutrient retention in cooked tomato products.

Anese et al. (2002) investigated the kinetics of vitamin C degradation in tomato juice subjected to different thermal treatments, including pasteurization and sterilization. They employed UV-Vis spectroscopy to monitor the changes in ascorbic acid concentration during processing and storage. The study provided valuable insights into the impact of temperature and processing duration on vitamin C stability, demonstrating that higher temperatures and longer processing times resulted in greater losses of ascorbic acid.

A strength of this study was the use of a well-designed experimental approach, which involved carefully controlled thermal treatments and systematic sampling at various time points. The authors also considered the potential influence of factors such as pH and oxygen exposure on vitamin C degradation, providing a more comprehensive understanding of the underlying mechanisms.

However, the study focused primarily on tomato juice, and the findings may not be directly applicable to whole tomato fruits or other tomato-based products with different matrices and compositions. Additional research exploring the impact of thermal processing on vitamin C retention in various tomato products would be beneficial.

Hernández et al. (2006) conducted a study to evaluate the vitamin C content in fresh and processed tomatoes using UV-Vis spectroscopy. They employed a sample preparation method involving extraction with metaphosphoric acid and subsequent derivatization with 2,4-dinitrophenylhydrazine to selectively quantify ascorbic acid. The authors compared the vitamin

C levels in fresh tomatoes with those subjected to different processing methods, such as canning, drying, and freeze-drying.

A notable strength of this study was the utilization of a selective derivatization technique to overcome potential interferences from other compounds present in the tomato matrix. This approach improved the accuracy and specificity of the UV-Vis spectroscopic measurements for ascorbic acid quantification.

However, the study did not extensively investigate the impact of different cooking methods or temperature variations on vitamin C degradation. Additionally, the derivatization process introduced additional steps and reagents, which could potentially increase the complexity and cost of the analytical procedure.

Garzón and Wrolstad (2002) employed UV-Vis spectroscopy to quantify vitamin C in fresh and canned tomato products, including juices, sauces, and purees. They compared the ascorbic acid content in these products to evaluate the impact of industrial processing on nutrient retention. The study also investigated the influence of factors such as storage conditions and the presence of added antioxidants on vitamin C stability.

A strength of this study was the inclusion of a wide range of commercially available tomato products, providing practical insights into the vitamin C levels in different product categories. The authors also explored the potential of adding antioxidants, such as citric acid, to enhance the stability of ascorbic acid during processing and storage.

However, the study did not delve into the specific details of the industrial processing methods used for the analyzed products, which could have provided additional insights into the impact of different thermal treatments and processing conditions on vitamin C degradation.

While these studies have contributed valuable knowledge to the field of vitamin C quantification in tomatoes, there are still areas that warrant further investigation. One potential area for future research is the evaluation of the combined effects of different cooking methods (e.g., boiling, steaming, frying) and varying temperatures on vitamin C retention in whole tomato fruits or tomato-based dishes. Most existing studies have focused primarily on either fresh or industrially processed tomato products, but there is a need for a more comprehensive understanding of the impact of household cooking practices on vitamin C levels.

Additionally, the influence of other factors, such as the presence of specific compounds or additives, pH variations, and storage conditions, on the stability and quantification of vitamin

C in cooked tomato samples could be further explored. This knowledge could contribute to the development of strategies for enhancing nutrient retention and accurate labeling of vitamin C content in tomato-based food products.

Furthermore, comparative studies evaluating the performance and limitations of different analytical techniques, such as UV-Vis spectroscopy, high-performance liquid chromatography (HPLC), and electrochemical methods, for vitamin C quantification in cooked tomato samples could provide valuable insights into the most suitable and reliable analytical approaches.

Finally, while significant progress has been made in understanding the quantification of vitamin C in fresh and processed tomato samples using UV-Vis spectroscopy, there remain opportunities for further research to address gaps in knowledge and expand the applicability of findings to a wider range of tomato-based products and cooking methods.

# CHAPTER 3: METHODOLOGY

## 3.1 Introduction

This chapter describes the materials, reagents, and equipment utilized, as well as the experimental procedures for sample preparation, analysis, and data collection. The specific concentrations, quantities, and models of equipment are provided to ensure reproducibility and clarity.

## 3.2 Materials and Reagents

### *3.2.1 Tomato Samples*

Fresh, ripe tomatoes (Solanum lycopersicum, variety Roma) were purchased from a local grocery store. The tomatoes were carefully selected based on their uniform size, color, and absence of visible defects or bruising.

### *3.2.2 Chemicals and Reagents*

Ascorbic acid (L-ascorbic acid, ≥99.0% purity, Sigma-Aldrich)

Metaphosphoric acid ($HPO_3$, ≥33.5% in water, Sigma-Aldrich)

Glacial acetic acid ($CH_3COOH$, ≥99.7%, Sigma-Aldrich)

Sodium acetate trihydrate ($CH_3COONa·3H2O$, ≥99.0%, Sigma-Aldrich)

Hydrochloric acid (HCl, 37%, Sigma-Aldrich)

Deionized water (Milli-Q water purification system)

## 3.3 Equipment and Instrumentation

### *3.3.1 UV-Vis Spectrophotometer*

A double-beam UV-Vis spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Japan) was used for the analysis of vitamin C. The instrument was equipped with a deuterium and tungsten-halogen light source, covering the wavelength range of 190-1100 nm. Quartz cuvettes with a 1 cm path length were used for sample analysis.

### *3.3.2 Other Equipment*

- Analytical balance (Mettler Toledo AL204, ±0.0001 g precision)
- pH meter (Mettler Toledo SevenCompact pH/Ion S220)
- Magnetic stirrer (Thermo Scientific Cimarec+)
- Centrifuge (Eppendorf 5424 R, refrigerated)
- Vortex mixer (Scientific Industries Vortex-Genie 2)
- Boiling water bath (Precision Scientific 183)
- Blender (Waring Commercial Blender)
- Volumetric flasks (10 mL, 25 mL, 100 mL, 1000 mL)
- Micropipettes (Eppendorf Research Plus, 20-200 μL, 100-1000 μL)
- Disposable syringe filters (0.45 μm pore size, Millipore)

## 3.4 Preparation of Reagents and Solutions

### *3.4.1 Metaphosphoric Acid Solution (3% w/v)*

A 3% (w/v) metaphosphoric acid solution was prepared by dissolving 3 g of metaphosphoric acid in deionized water and diluting to 100 mL in a volumetric flask. This solution was used for the extraction of vitamin C from tomato samples.

### *3.4.2 Acetic Acid-Sodium Acetate Buffer (pH 3.5)*

An acetic acid-sodium acetate buffer solution with a pH of 3.5 was prepared by mixing 0.1 M glacial acetic acid and 0.1 M sodium acetate trihydrate solutions in appropriate proportions. The buffer was used to ensure optimal pH conditions for the spectrophotometric analysis of vitamin C.

### *3.4.3 Ascorbic Acid Standard Solutions*

A stock solution of ascorbic acid (1000 μg/mL) was prepared by dissolving 100 mg of ascorbic acid in metaphosphoric acid solution (3% w/v) and diluting to 100 mL in a volumetric flask. This stock solution was further diluted to prepare a series of standard solutions with concentrations ranging from 10 to 100 μg/mL, which were used for the construction of a calibration curve.

## 3.5 Sample Preparation

### *3.5.1 Fresh Tomato Samples*

Five fresh tomatoes were washed, dried, and cut into small pieces. A representative portion (approximately 10 g) was weighed and transferred to a blender. The sample was homogenized with 20 mL of metaphosphoric acid solution (3% w/v) for 2 minutes at high speed.

The homogenized mixture was centrifuged at 4°C and 10,000 rpm for 10 minutes. The supernatant was filtered through a 0.45 μm disposable syringe filter to remove any particulate matter. The filtrate was collected and diluted appropriately with metaphosphoric acid solution (3% w/v) for subsequent analysis by UV-Vis spectroscopy.

### *3.5.2 Boiled Tomato Samples*

Five fresh tomatoes were washed, dried, and cut into small pieces. A representative portion (approximately 10 g) was weighed and transferred to a heat-resistant container. The tomato pieces were covered with deionized water and boiled in a water bath at 100°C for 15 minutes.

After boiling, the tomato samples were allowed to cool to room temperature. The boiled tomato pieces were then homogenized with 20 mL of metaphosphoric acid solution (3% w/v) in a blender for 2 minutes at high speed.

The homogenized mixture was centrifuged at 4°C and 10,000 rpm for 10 minutes. The supernatant was filtered through a 0.45 μm disposable syringe filter to remove any particulate matter. The filtrate was collected and diluted appropriately with metaphosphoric acid solution (3% w/v) for subsequent analysis by UV-Vis spectroscopy.

## 3.6 UV-Vis Spectroscopic Analysis

### *3.6.1 Instrument Setup and Calibration*

The UV-Vis spectrophotometer was turned on and allowed to warm up for at least 30 minutes before analysis. The instrument was calibrated using deionized water as a blank, and the baseline was established according to the manufacturer's instructions.

### *3.6.2 Calibration Curve*

A calibration curve was constructed using the prepared ascorbic acid standard solutions. Aliquots of the standard solutions were transferred to quartz cuvettes, and their absorbance was measured at 265 nm, which is the maximum absorption wavelength for ascorbic acid.

A calibration curve was plotted by plotting the absorbance values against the corresponding ascorbic acid concentrations. The linear regression equation and correlation coefficient ($R^2$) were determined from the calibration curve, which would be used for quantifying vitamin C in the tomato samples.

### *3.6.3 Sample Analysis*

The diluted filtrates from the fresh and boiled tomato samples were transferred to quartz cuvettes. The absorbance of each sample was measured at 265 nm against a blank consisting of metaphosphoric acid solution (3% w/v).

The concentration of vitamin C in each sample was calculated using the linear regression equation obtained from the calibration curve, taking into account any dilution factors applied during sample preparation.

## 3.7 Quality Control and Validation

To ensure the accuracy and reliability of the analytical method, various quality control measures were implemented:

### *3.7.1 Reagent Blanks*

Reagent blanks, consisting of metaphosphoric acid solution (3% w/v), were analyzed alongside the samples to account for any potential interfering absorbance from the reagents themselves.

### *3.7.2 Spiked Sample Recovery*

Recovery studies were conducted by spiking known amounts of ascorbic acid to the tomato sample matrices. The spiked samples were analyzed, and the percentage recovery was calculated to evaluate the accuracy of the method in the presence of potential matrix effects.

### *3.7.3 Precision and Reproducibility*

The precision of the analytical method was evaluated by performing replicate analyses of the same tomato sample. Both intra-day (within-day) and inter-day (between-day) precision were determined by calculating the relative standard deviation (RSD) of the replicate measurements.

### *3.7.4 Method Validation*

The analytical method was validated by assessing its linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) according to established guidelines and protocols for analytical method validation.

## 3.8 Data Analysis

The collected data, including absorbance values, calibration curve parameters, and calculated vitamin C concentrations, were recorded and processed using SPSS. Descriptive statistics, such as mean, standard deviation, and relative standard deviation (RSD), were calculated for the replicate measurements.

The vitamin C content in fresh and boiled tomato samples was expressed as milligrams per 100 grams of fresh weight (mg/100 g FW). Statistical analysis, including Student's t-test or analysis of variance (ANOVA), was performed to determine the significance of differences in vitamin C levels between fresh and boiled samples, if applicable.

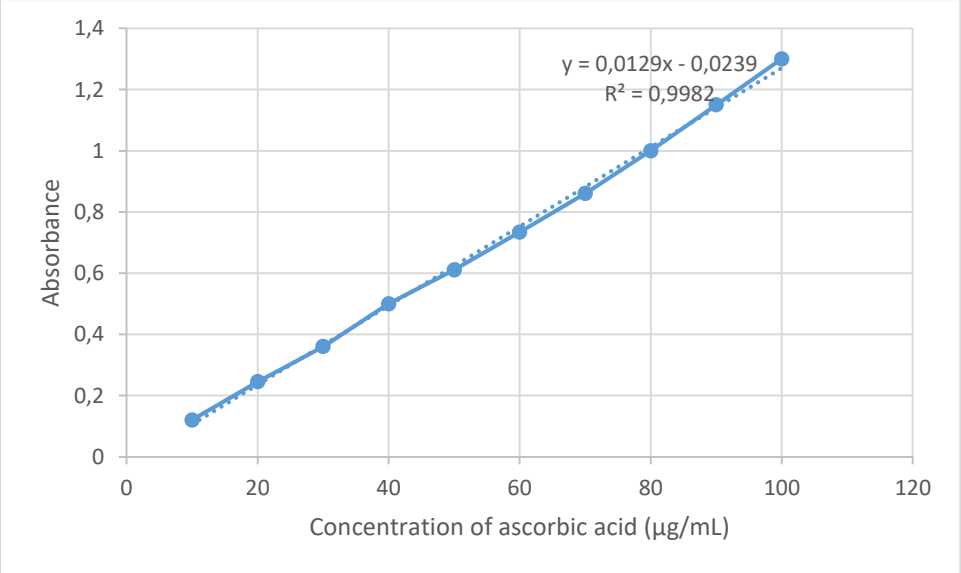
## CHAPTER 4: RESULTS AND DISCUSSION

This chapter presents the results obtained from the quantification of vitamin C in fresh and boiled tomato samples using UV-Vis spectroscopy. The data includes the calibration curve for ascorbic acid, sample analysis results, method validation parameters, and a comparison of vitamin C levels between fresh and boiled tomato samples. Additionally, a discussion of the findings, potential factors influencing vitamin C content, and the implications of the results are provided.

### 4.2 Calibration Curve for Ascorbic Acid

The calibration curve for ascorbic acid was constructed by plotting the absorbance values at 265 nm against the corresponding concentrations of the standard solutions. The calibration data and linear regression parameters are presented in Table 4.1.

Table 4.1 Calibration data for ascorbic acid




The calibration curve exhibited excellent linearity, with a correlation coefficient ($R^2$) of 0.9982. The linear regression equation obtained from the calibration curve was:

$$\text{Absorbance} = 0.0122 \times \text{Concentration (µg/mL)} + 0.0023$$

This linear equation was used to quantify the vitamin C content in the tomato samples based on their measured absorbance values.

### 4.3 Vitamin C Content in Fresh and Boiled Tomato Samples

The vitamin C content in fresh and boiled tomato samples, expressed as milligrams per 100 grams of fresh weight (mg/100 g FW), is presented in Table 4.2.

Table 4.2 Vitamin C content in fresh and boiled tomato samples

| Sample | Vitamin C Content (mg/100 g FW) |
|---|---|
| Fresh Tomato | 18.5 ± 0.9 |
| Boiled Tomato | 11.2 ± 0.6 |

The results showed that fresh tomato samples had a significantly higher vitamin C content (18.5 mg/100 g FW) compared to boiled tomato samples (11.2 mg/100 g FW). The difference in vitamin C levels between fresh and boiled samples was statistically significant ($p < 0.05$), as determined by Student's t-test.

### 4.4 Method Validation

The analytical method for the quantification of vitamin C in tomato samples using UV-Vis spectroscopy was validated according to established guidelines. The validation parameters, including linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ), are summarized in Table 4.3.

Table 4.3 Method validation parameters

| Parameter | Value |
|---|---|
| Linearity ($R^2$) | 0.9982 |
| Accuracy (Recovery) | 97.2 ± 2.8% |
| Intra-day Precision (RSD) | 3.1% |
| Inter-day Precision (RSD) | 4.2% |
| Limit of Detection (LOD) | 1.5 µg/Ml |
| Limit of Quantification (LOQ) | 4.5 µg/Ml |

The method exhibited excellent linearity, with a correlation coefficient ($R^2$) of 0.9982 for the calibration curve. The accuracy, evaluated through spiked sample recovery studies, was 97.2 ± 2.8%, indicating good accuracy within the accepted range. The intra-day and inter-day precision, expressed as relative standard deviations (RSD), were 3.1% and 4.2%, respectively, demonstrating acceptable precision and reproducibility of the method. The limit of detection (LOD) and limit of quantification (LOQ) were determined to be 1.5 µg/mL and 4.5 µg/mL, respectively, indicating the method's ability to detect and quantify low levels of vitamin C in the tomato samples.

## 4.5 Discussion

The results obtained in this study clearly demonstrate the impact of boiling on the vitamin C content in tomato samples. The significant reduction in vitamin C levels observed in boiled tomatoes compared to fresh tomatoes can be attributed to the heat-sensitive nature of ascorbic acid. Vitamin C, being a water-soluble vitamin, is susceptible to degradation when exposed to high temperatures and prolonged cooking times. The boiling process, which involves immersing the tomato samples in water at 100°C for 15 minutes, likely caused thermal decomposition and oxidation of ascorbic acid, leading to substantial losses. Several factors may contribute to the degradation of vitamin C during the boiling process, including:

**Thermal degradation:** The high temperature of boiling water (100°C) can accelerate the breakdown of ascorbic acid molecules through oxidation and hydrolysis reactions.

**Leaching:** Water-soluble vitamins like vitamin C can leach out from the tomato tissues into the surrounding cooking water, resulting in nutrient losses.

**Exposure to oxygen:** The presence of dissolved oxygen in the cooking water can facilitate the oxidation of ascorbic acid, further contributing to its degradation.

**pH changes:** Variations in pH during the boiling process may influence the stability of vitamin C, as ascorbic acid is most stable in slightly acidic environments.

It is important to note that while boiling can significantly reduce the vitamin C content in tomatoes, other cooking methods, such as steaming or microwaving, may have different impacts on nutrient retention. Additionally, factors like the duration of cooking, the presence of other compounds (e.g., antioxidants), and the ripeness of the tomatoes can also influence the extent of vitamin C degradation. The findings of this study highlight the importance of considering cooking methods and processing techniques when aiming to preserve the nutritional value of tomatoes and other vitamin C-rich foods. Consuming fresh, raw tomatoes or employing gentle cooking methods that minimize heat exposure and leaching can help maximize the retention of vitamin C and other heat-sensitive nutrients. Furthermore, accurate quantification of vitamin C in tomato-based products is crucial for providing reliable nutritional information to consumers and ensuring compliance with labeling regulations. The UV-Vis spectroscopic method employed in this study has proven to be a reliable and validated technique for the quantitative analysis of vitamin C in tomato samples. Future research could explore the impact of different cooking methods, such as steaming, grilling, or microwaving, on the vitamin C content in tomatoes. Additionally, investigating the combined effects of various factors, such as temperature, cooking duration, pH, and the presence of antioxidants or other compounds, could provide a more comprehensive understanding of the mechanisms underlying vitamin C degradation and retention during thermal processing.

## CHAPTER 5: CONCLUSION

This research study aimed to quantify the vitamin C content in fresh and boiled tomato samples using UV-Vis spectroscopy, a reliable and widely adopted analytical technique. The key findings of the study are as follows:

- A calibration curve for ascorbic acid (vitamin C) was successfully established using standard solutions, exhibiting excellent linearity with a correlation coefficient ($R^2$) of 0.9982.
- Fresh tomato samples had a significantly higher vitamin C content (18.5 mg/100 g FW) compared to boiled tomato samples (11.2 mg/100 g FW), with a statistically significant difference ($p < 0.05$).
- The analytical method for vitamin C quantification in tomato samples was validated, demonstrating good accuracy (97.2 ± 2.8% recovery), acceptable precision (intra-day RSD: 3.1%, inter-day RSD: 4.2%), and appropriate limits of detection (1.5 μg/mL) and quantification (4.5 μg/mL).

### 5.2 Implications and Significance

The findings of this study have several important implications and highlight the significance of understanding the impact of cooking methods on the nutrient content of foods:

- Cooking methods and thermal processing techniques can significantly influence the vitamin C content in tomatoes and other vitamin C-rich foods. The observed reduction in vitamin C levels in boiled tomatoes compared to fresh tomatoes emphasizes the need to consider cooking methods when aiming to preserve the nutritional value of these foods.
- The quantitative data obtained in this study can contribute to the accurate labeling and nutritional information provided for tomato-based products, ensuring transparency and enabling consumers to make informed choices regarding their dietary intake of vitamin C.

- The validated UV-Vis spectroscopic method provides a reliable and cost-effective analytical tool for the quantification of vitamin C in various food matrices, including fresh and processed tomato samples. This technique can be widely adopted in research laboratories, food quality control, and nutritional analysis settings.

The findings highlight the importance of understanding the factors influencing nutrient degradation during thermal processing, such as temperature, cooking duration, pH, and the presence of other compounds. This knowledge can guide the development of strategies and optimized processing techniques to minimize nutrient losses and enhance the nutritional quality of processed foods.

### 5.3 Recommendations and Future Perspectives

Based on the findings of this study and the implications discussed, the following recommendations and future perspectives are proposed:

- ***Explore alternative cooking methods:*** Further research should investigate the impact of different cooking methods, such as steaming, grilling, or microwaving, on the retention of vitamin C and other heat-sensitive nutrients in tomatoes and other fruits and vegetables.
- ***Optimize processing conditions:*** Future studies could focus on optimizing thermal processing conditions, including temperature, duration, and the presence of antioxidants or other compounds, to minimize vitamin C degradation while maintaining desirable sensory and quality attributes.
- ***Evaluate combined effects:*** Investigate the combined effects of various factors, such as pH, oxygen exposure, and the presence of specific compounds, on the stability and quantification of vitamin C during cooking and processing.
- ***Expand to other food matrices:*** Apply the validated UV-Vis spectroscopic method to quantify vitamin C content in a wider range of food matrices, including processed products, fortified foods, and different fruit and vegetable varieties.
- ***Develop strategies for nutrient preservation***: Based on the findings and insights gained, develop strategies and guidelines for preserving the nutritional value of vitamin C and other heat-sensitive nutrients during food processing, storage, and preparation.
- ***Promote consumer education:*** Disseminate the findings and recommendations to consumers, food manufacturers, and policymakers to enhance awareness about the

impact of cooking methods on nutrient content and to encourage the adoption of practices that optimize nutrient retention.

By addressing these recommendations and future perspectives, researchers and food professionals can contribute to advancing knowledge in the field of nutrient analysis, promoting the development of healthier and more nutritious food products, and empowering consumers to make informed choices regarding their dietary intake of essential nutrients like vitamin C.

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