

Chemistry Investigatory Project: Study of the Presence of Oxalate Ions in Guava Fruit at Different Stages of Ripening

Name:

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Certificate

This is to certify that ****, a student of Class XII, has successfully completed the investigatory project entitled "**Study of the Presence of Oxalate Ions in Guava Fruit at Different Stages of Ripening**" under the guidance and supervision of **** during the academic session 2025-2026. This project is submitted in partial fulfillment of the requirements for the All India Senior School Certificate Examination (AISSCE) in Chemistry, conducted by the Central Board of Secondary Education (CBSE).

The work presented in this project is the result of the student's own investigation and experimentation carried out in the school laboratory. The data, observations, and calculations recorded herein are original and authentic.

Internal Examiner

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Date: _____

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Principal

Signature: _____

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1. Introduction

1.1 Scientific Profile of Guava (*Psidium guajava*)

The guava (*Psidium guajava* L.) is a ubiquitous and economically significant tropical fruit belonging to the family Myrtaceae. Often eulogized as the "apple of the tropics" or the "poor man's apple," it is renowned for its hardness, high yield, and exceptional nutritional value.² The genus *Psidium* comprises approximately 150 species of evergreen shrubs and trees, but *Psidium guajava* is the most commercially important and widely distributed.

Botanical Classification:

- **Kingdom:** Plantae
- **Phylum:** Spermatophyta
- **Class:** Dicotyledonae
- **Order:** Myrtales
- **Family:** Myrtaceae
- **Genus:** *Psidium*
- **Species:** *P. guajava*³

Origin and Distribution:

The guava is native to the Neotropics, specifically the region extending from Mexico through Central America to northern South America (including Peru, Brazil, and Venezuela).⁵ It has an ancient history of cultivation, predating European arrival in the Americas, evidenced by its integration into the traditional diets and pharmacopeia of indigenous populations. The Portuguese and Spanish explorers were instrumental in disseminating the species across the globe, introducing it to the Philippines and India by the 17th century. Today, it is naturalized in virtually all tropical and subtropical regions, including Southeast Asia, Hawaii, the Caribbean, and Africa.⁶

India currently stands as the world's largest producer of guava, contributing significantly to the global supply with an estimated production of 5.42 million tonnes in the 2023-24 agricultural year.⁷ Major producing states include Madhya Pradesh, Uttar Pradesh, and Bihar. The fruit's adaptability to diverse soil types—from heavy clay to light sand—and its tolerance for varying pH levels (4.5 to 8.2) make it a cornerstone of tropical horticulture.²

Morphology:

The guava tree is typically a low-branching shrub or small tree, reaching heights of 3 to 10 meters. It is characterized by smooth, copper-colored bark that peels off in thin flakes, revealing a greenish layer beneath. The leaves are opposite, simple, elliptic to ovate, and possess a distinct aroma when crushed due to the presence of essential oils. The fruit is a berry, varying in shape from spherical (globose) to pear-shaped (pyriform), with a skin that transitions from green to yellow upon maturation. The pulp may be white, pink, yellow, or red, depending on the cultivar, and contains numerous small, hard seeds.⁵

1.2 Nutritional Profile and Phytochemistry

Guava is nutritionally dense, often outperforming more expensive fruits in terms of vitamin and mineral content. Its phytochemical profile is complex, consisting of primary metabolites (sugars, proteins) and secondary metabolites (phenolics, flavonoids, carotenoids).

Vitamin C (Ascorbic Acid):

The most defining nutritional characteristic of guava is its extraordinarily high Vitamin C content. A single fruit can contain between 200 mg to 400 mg of ascorbic acid per 100g of fresh weight.⁸ This is approximately four to five times the concentration found in citrus fruits like oranges (which typically contain ~50 mg/100g). Ascorbic acid is concentrated primarily in the skin and the outer flesh, with levels decreasing towards the central seed cavity. This high antioxidant capacity confers significant free-radical scavenging activity, making guava a potent immunity booster.²

Dietary Fiber (Pectin):

Guava is an excellent source of dietary fiber, particularly pectin, a structural heteropolysaccharide found in the primary cell walls and middle lamella of terrestrial plants. Pectin content in guava ranges from 0.5% to 1.9% of fresh weight.¹⁰ It plays a critical role in the fruit's texture (firmness) and is widely used in the food industry as a gelling agent for jams and jellies. During digestion, pectin helps regulate bowel movements and can lower blood cholesterol levels.¹¹

Antioxidants and Phenolics:

The fruit is rich in phenolic compounds, including myricetin, apigenin, ellagic acid, and anthocyanins. Pink and red-fleshed varieties are particularly high in lycopene, a carotenoid pigment responsible for the red color. Lycopene is a powerful antioxidant known to protect cells from photodamage and reduce the risk of prostate cancer.¹² The total phenolic content is highest in the skin and unripe fruit, decreasing slightly as the fruit ripens, although the bioavailability of certain compounds may increase.¹³

Minerals:

In terms of mineral content, guava is a robust source of potassium (417 mg/100g), comparable to bananas, which aids in regulating blood pressure. It

also contains appreciable amounts of magnesium, phosphorus, and small amounts of calcium and iron.¹⁰

Organic Acids:

The organoleptic profile (taste) of guava is governed by the sugar-acid ratio. The predominant organic acids are citric acid and malic acid, which provide the tartness. However, a significant anti-nutrient present in guava is oxalic acid ($\text{H}_2\text{C}_2\text{O}_4$). While essential for the plant's calcium regulation and defense mechanisms, oxalic acid has profound implications for human health, which is the central focus of this investigatory project.¹⁴

1.3 Medical Implications: The Chemistry of Oxalates and Nephrolithiasis

The study of oxalate ions in food sources is not merely of academic interest; it has direct clinical relevance to urology and nephrology, specifically concerning **Hyperoxaluria** and **Nephrolithiasis** (kidney stone disease).

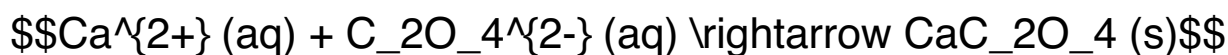
Chemical Nature of Oxalate:

Oxalate ($\text{C}_2\text{O}_4^{2-}$) is the dianion of oxalic acid, a dicarboxylic acid. In the human body, it is a metabolic end-product, meaning it cannot be further metabolized and must be excreted via urine. It is derived from two sources:

1. **Exogenous (Dietary):** Consumption of oxalate-rich foods like spinach, rhubarb, beets, nuts, and guava.
2. **Endogenous (Metabolic):** Synthesis within the liver from precursors like ascorbic acid, glyoxylate, and hydroxyproline.¹⁵

Pathophysiology of Kidney Stones:

Under normal physiological conditions, oxalate is excreted harmlessly. However, oxalate has a very high affinity for divalent cations, particularly Calcium (Ca^{2+}). When the concentration of urinary oxalate exceeds its solubility product, it precipitates with calcium to form Calcium Oxalate (CaC_2O_4).



Calcium oxalate crystals (typically in the form of monohydrate or dihydrate) are highly insoluble. These crystals can aggregate in the renal tubules, forming calculi (stones). Calcium oxalate stones account for approximately 75-80% of all kidney stone cases globally.¹⁶

Hyperoxaluria:

This condition is defined by excessive urinary excretion of oxalate (>40-45 mg/day). It can be:

- **Primary Hyperoxaluria:** A rare genetic disorder where hepatic enzymes are deficient, leading to massive overproduction of oxalate.

- **Enteric Hyperoxaluria:** Caused by intestinal malabsorption disorders (e.g., Crohn's disease), where free fatty acids bind calcium in the gut, leaving oxalate free to be absorbed into the bloodstream.
- **Dietary Hyperoxaluria:** Caused by excessive consumption of high-oxalate foods.¹⁷

The Ascorbic Acid Connection:

Crucially, ascorbic acid (Vitamin C) can be metabolized into oxalate in the human body. High-dose supplementation of Vitamin C (>1000 mg/day) has been linked to increased risk of stone formation. Since guava is exceptionally rich in Vitamin C, and also contains direct oxalate, its consumption impacts the "oxalate load" through both direct ingestion and metabolic conversion.¹⁵ Understanding the oxalate content at different ripening stages helps in formulating dietary guidelines for stone-formers.

1.4 Physiology of Fruit Ripening: A Biochemical Perspective

Ripening is a complex, genetically programmed developmental phase that transforms a physiologically mature but inedible plant organ into a palatable and nutritious fruit. In guava, this process is **climacteric**, characterized by a dramatic surge in respiration rate and ethylene production at the onset of ripening.¹⁸

The Climacteric Rise:

As the guava matures, it enters the climacteric phase where cellular respiration spikes. This oxidative phosphorylation generates the ATP required for the synthesis of ripening-related enzymes. Ethylene (C_2H_4), a gaseous plant hormone, acts as the master switch, triggering the expression of genes responsible for:

1. **Chlorophyll Degradation:** The enzyme chlorophyllase breaks down the green pigment, unmasking the yellow carotenoids and anthocyanins.¹⁹
2. **Starch Hydrolysis:** Amylase enzymes hydrolyze stored starch into simpler sugars like sucrose, fructose, and glucose. This causes the total soluble solids (TSS) to rise, increasing sweetness.²⁰
3. **Cell Wall Softening:** This is the most significant textural change. The rigid cell wall, composed of cellulose, hemicellulose, and pectin, undergoes disassembly. Enzymes such as **Polygalacturonase (PG)**, **Pectin Methylesterase (PME)**, and **β -Galactosidase** solubilize the protopectin in the middle lamella. This "ungluing" of cells turns the hard, crisp flesh into soft, creamy pulp.²¹

Metabolic Shift of Organic Acids:

During this metabolic overhaul, the concentrations of organic acids fluctuate. While citric and malic acid levels often decrease (reducing tartness), the metabolism of ascorbic acid is of particular interest. In many fruit tissues, the degradation of ascorbic acid (via the oxidization to dehydroascorbic acid and subsequent hydrolysis) serves as a biosynthetic pathway for oxalic acid.²³

Consequently, as the fruit ripens and ascorbic acid turnover increases, an accumulation of oxalate ions is theoretically expected. This biochemical pathway forms the hypothesis of our investigation.

2. Objective of the Study

The primary scientific objective of this investigatory project is **to conduct a comparative volumetric analysis of the oxalate ion content in *Psidium guajava* (guava) fruit at three distinct stages of ripening.**

The specific aims are:

1. **To extract** free oxalate ions from the pulp of Raw (Green), Semi-ripe (Yellowish-Green), and Fully Ripe (Yellow) guava fruits using acid digestion.
2. **To estimate** the concentration of oxalate ions in each extract using Potassium Permanganate (KMnO_4) redox titration.
3. **To quantify** the strength of oxalate ions (in g/L) and determine the trend of oxalate accumulation relative to the ripening process.
4. **To correlate** the experimental findings with the biochemical theory of ascorbate-oxalate conversion during plant senescence.

3. Theoretical Framework

3.1 Principles of Volumetric Analysis

Volumetric analysis, or titrimetry, is a quantitative chemical analysis method used to determine the unknown concentration of an identified analyte by measuring the volume of a solution of known concentration (the titrant) required to react completely with the analyte. The point of stoichiometric equivalence is known as the **equivalence point**, while the visual completion of the reaction (often signaled by a color change) is the **endpoint**.²⁴

3.2 Redox Titration: The Mechanism of Permanganometry

This experiment relies on **Permanganometry**, a type of redox titration where Potassium Permanganate (KMnO_4) is used as the oxidizing agent. The analyte, oxalate ion ($\text{C}_2\text{O}_4^{2-}$), acts as the reducing agent.

The Role of the Oxidizing Agent (KMnO_4):

Potassium permanganate is a powerful oxidizing agent, particularly in an acidic medium. The manganese atom in the permanganate ion (MnO_4^-) is in the +7 oxidation state. During the reaction, it gains 5 electrons and is reduced to the manganous ion (Mn^{2+}), where it exists in the +2 oxidation state.



The Role of the Reducing Agent ($\text{C}_2\text{O}_4^{2-}$):

The oxalate ion consists of two carbon atoms, each in the +3 oxidation state. During the titration, the oxalate is oxidized to carbon dioxide (CO_2), where carbon enters the +4 oxidation state. Each oxalate ion loses 2 electrons.



Self-Indicator Action:

One of the distinct advantages of permanganometry is that no external indicator is required. The permanganate ion (MnO_4^-) is a deep, intense purple color. The reduced product, the manganous ion (Mn^{2+}), is essentially colorless in dilute solutions.

- **Before Endpoint:** As KMnO_4 is added from the burette, it is immediately reduced by the oxalate, and the purple color disappears.
- **At Endpoint:** Once all the oxalate ions have been consumed, the very next drop of KMnO_4 added has no reducing agent to react with. It remains unreduced, imparting a **permanent faint pink color** to the solution. This sharp color transition signals the completion of the titration.²⁵

3.3 Chemical Kinetics and Thermodynamics of the Reaction

Temperature Dependence:

The reaction between oxalate ions and permanganate ions is kinetically slow at room temperature. This is due to the high activation energy required to rupture the covalent carbon-carbon bond in the oxalate ion. If titrated cold, the reaction would be sluggish, and the endpoint would be indistinct (fading pink color).

To overcome this energy barrier, the oxalic acid solution (or fruit extract) is heated to 60°C - 70°C . This thermal energy increases the molecular collision frequency and the fraction of molecules possessing energy greater than the activation energy, allowing the reaction to proceed instantaneously.²⁷

Autocatalysis:

The reaction is an example of autocatalysis.

Initially, the reaction is slow even with heating. However, as the reaction proceeds, Manganous ions (Mn^{2+}) are produced. These Mn^{2+} ions act as a homogenous catalyst for the reaction. They lower the activation energy for the reduction of subsequent permanganate ions. Consequently, the first few drops of permanganate take time to decolorize, but subsequent additions decolorize rapidly as the concentration of the autocatalyst (Mn^{2+}) builds up.²⁸

Acidity Requirements:

The titration must be performed in the presence of dilute Sulfuric Acid (H_2SO_4).

- Why not Hydrochloric Acid (HCl)? KMnO_4 is a strong enough oxidizing agent to oxidize chloride ions (Cl^-) to chlorine gas (Cl_2). This would consume permanganate in a side reaction, leading to an erroneously high reading.

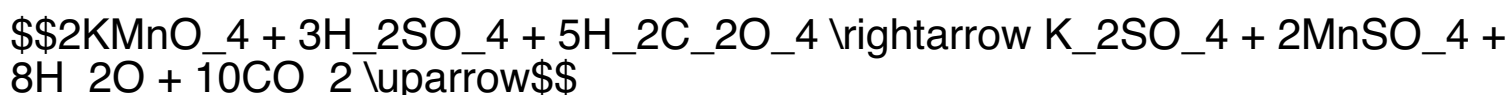


- **Why not Nitric Acid (HNO_3)?** Nitric acid is itself a strong oxidizing agent. It would oxidize the oxalate alongside the permanganate, leading to an erroneously low reading.
- Sulfuric acid is stable and non-volatile, providing the necessary H^+ ions without interfering in the redox transfer.²⁸

3.4 Reaction Stoichiometry: Molecular and Ionic Equations

The quantitative relationship between the reactants is governed by the following balanced chemical equations.

Molecular Equation:



Ionic Equations:

- Reduction Half-Reaction:



(Gain of 5 electrons)

- Oxidation Half-Reaction:



(Loss of 2 electrons)

To balance the electrons transferred (Lowest Common Multiple of 2 and 5 is 10):

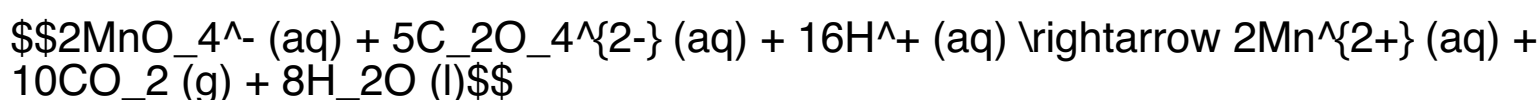
Multiply the Reduction half-reaction by 2:



Multiply the Oxidation half-reaction by 5:



Overall Balanced Ionic Equation:



Stoichiometric Ratio:

$$\frac{\text{Moles of } \text{KMnO}_4}{\text{Moles of Oxalate}} = \frac{2}{5}$$

4. Materials and Apparatus

To ensure precise measurements and safe experimentation, the following apparatus and chemicals were utilized.

Apparatus:

1. **Burette (50 mL):** Calibrated glass burette with a glass stopcock (avoid rubber due to KMnO_4 attack). Used for dispensing the titrant.
2. **Pipette (10 mL or 20 mL):** For accurately measuring the aliquot of guava extract.
3. **Conical Flask (Titration Flask, 250 mL):** The vessel where the reaction takes place.
4. **Volumetric Flasks (100 mL, 250 mL):** For preparing standard solutions and fruit extracts of fixed volume.
5. **Beakers (250 mL):** For boiling the fruit pulp.
6. **Pestle and Mortar:** Ceramic, for mechanically crushing the fruit tissue to release cellular contents.
7. **Funnel and Filter Paper:** (Whatman No. 1 or equivalent) for separating the insoluble pulp from the oxalate-containing filtrate.
8. **Bunsen Burner / Hot Plate:** Heat source for the reaction.
9. **Tripod Stand and Wire Gauze:** To support the glassware during heating.
10. **Thermometer (0-110°C):** To monitor the reaction temperature (60-70°C).
11. **Digital Analytical Balance:** For weighing chemicals and fruit samples with high precision ($\pm 0.001\text{g}$).
12. **Glazed White Tile:** Placed under the flask to enhance the visibility of the pink endpoint.

Chemicals:

1. **Guava Fruits:** Three distinct samples:
 - Sample A: Raw (Green, hard).
 - Sample B: Semi-ripe (Greenish-yellow, firm).
 - Sample C: Fully Ripe (Yellow, soft).
2. **Potassium Permanganate (KMnO_4):** Crystalline solid (or pre-prepared 0.1 N solution).
3. **Oxalic Acid Dihydrate ($\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$):** Primary standard grade.
4. **Sulfuric Acid (H_2SO_4):** Concentrated acid diluted to approximately 1.0 M (Dilute H_2SO_4).
5. **Distilled Water:** Free from mineral ions to prevent side reactions.

5. Experimental Procedure

The experiment was conducted in three distinct phases: Standardization, Extraction, and Estimation.

5.1 Preparation of Standard Solutions

A. Preparation of 0.1 N Standard Oxalic Acid Solution:

Since KMnO_4 is a secondary standard (it is difficult to obtain in 100% pure form and its solution concentration changes over time due to light sensitivity), its exact normality must be determined by titrating it against a primary standard like oxalic acid.²⁹

1. Calculation:

- Molar Mass of Oxalic Acid ($\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$) = 126.07 g/mol .
- Basicity = 2 (Releases 2 H^+ ions).
- Equivalent Mass = $\frac{\text{Molar Mass}}{\text{Basicity}} = \frac{126.07}{2} \approx 63 \text{ g/eq}$.
- To prepare 250 mL of 0.1 N solution:

$$\text{Mass} = \text{Normality} \times \text{Eq. Mass} \times \text{Volume (L)}$$

$$\text{Mass} = 0.1 \times 63 \times 0.25 = 1.575 \text{ g}$$

- Weighing:** Exactly 1.575 g of pure oxalic acid crystals were weighed on a watch glass.
- Dissolution:** The crystals were transferred to a 250 mL volumetric flask, dissolved in a small quantity of distilled water, and then diluted to the 250 mL mark. The flask was shaken thoroughly to ensure homogeneity.

B. Standardization of Potassium Permanganate Solution:

- The burette was rinsed and filled with the KMnO_4 solution. Air bubbles were removed from the nozzle.
- 10 mL of the standard 0.1 N oxalic acid solution was pipetted into a clean conical flask.
- Approximately 20 mL (one test tube) of dilute H_2SO_4 was added to the flask.
- The flask was heated to about 60°C - 70°C .
- The hot solution was titrated against KMnO_4 with constant swirling.
- The titration was stopped immediately upon the appearance of a **permanent pale pink color**.
- The readings were recorded, and the procedure was repeated to obtain concordant values.

5.2 Extraction of Oxalate Ions from Guava Pulp

To ensure a fair comparison, a uniform extraction method was applied to all three fruit samples.

- Sample Selection:** 50.0 g of the fresh Raw (Green) guava was weighed.
- Maceration:** The fruit flesh was cut into small pieces and crushed thoroughly in a mortar and pestle to break down the cell walls and release the cytoplasm containing the oxalate ions.
- Acid Digestion:** The crushed pulp was transferred to a 250 mL beaker.

Approximately 50 mL of dilute sulfuric acid was added.

- *Scientific Rationale:* The acid helps to solubilize the calcium oxalate crystals (raphides) present in the plant tissue, converting insoluble calcium oxalate into soluble oxalic acid and calcium sulfate ($\text{CaC}_2\text{O}_4 + \text{H}_2\text{SO}_4 \rightarrow \text{H}_2\text{C}_2\text{O}_4 + \text{CaSO}_4$). Boiling aids this dissolution and denatures enzymes that might degrade oxalate.³⁰

4. **Boiling:** The mixture was boiled for 15 minutes with stirring.
5. **Filtration:** The contents were cooled and filtered through Whatman filter paper into a 100 mL volumetric flask. The residue was washed with small amounts of hot distilled water to ensure quantitative transfer of all oxalate ions.
6. **Dilution:** The filtrate was made up to the 100 mL mark with distilled water. This solution served as the "Raw Guava Extract."
7. **Repetition:** The exact same procedure was repeated for the Semi-ripe and Fully Ripe guava samples.

5.3 Titration Methodology

1. The burette was refilled with the standardized KMnO_4 solution.
2. 10 mL of the prepared **Raw Guava Extract** was pipetted into a conical flask.
3. 10 mL of dilute H_2SO_4 was added to maintain the acidic medium.
4. The flask was heated to 60°C .
5. Titration was performed against KMnO_4 until the permanent pink endpoint was achieved.
6. The volume of KMnO_4 consumed was recorded.
7. The titration was repeated three times for the Raw sample to ensure accuracy.
8. The entire titration process was repeated for the **Semi-ripe** and **Fully Ripe** extracts.

6. Observations and Data Recording

6.1 Standardization of Potassium Permanganate (KMnO_4)

- **Normality of Standard Oxalic Acid (N_{ox}):** 0.1 N
- **Volume of Oxalic Acid taken (V_{ox}):** 10.0 mL
- **Indicator:** Self-indicator (KMnO_4)
- **Endpoint:** Colorless to Permanent Pink

S. No.	Initial Burette Reading (mL)	Final Burette Reading (mL)	Volume of KMnO_4 Used (V_1) (mL)
1	0.0	9.8	9.8
2	9.8	19.6	9.8
3	19.6	29.4	9.8

Concordant Volume of KMnO_4 (V_1) = 9.8 mL

6.2 Titration of Raw (Green) Guava Extract

- Volume of Extract taken (V_2): 10.0 mL

S. No.	Initial Burette Reading (mL)	Final Burette Reading (mL)	Volume of KMnO_4 Used (mL)
1	0.0	13.2	13.2
2	13.2	26.5	13.3
3	26.5	39.7	13.2

Concordant Volume (V_{raw}) = 13.2 mL

6.3 Titration of Semi-Ripe (Yellowish-Green) Guava Extract

- Volume of Extract taken (V_2): 10.0 mL

S. No.	Initial Burette Reading (mL)	Final Burette Reading (mL)	Volume of KMnO_4 Used (mL)
1	0.0	13.6	13.6
2	13.6	27.3	13.7
3	27.3	41.0	13.7

Concordant Volume (V_{semi}) = 13.7 mL

6.4 Titration of Fully Ripe (Yellow) Guava Extract

- Volume of Extract taken (V_2): 10.0 mL

S. No.	Initial Burette Reading (mL)	Final Burette Reading (mL)	Volume of KMnO_4 Used (mL)
1	0.0	13.9	13.9
2	13.9	27.8	13.9
3	27.8	41.7	13.9

Concordant Volume (V_{ripe}) = 13.9 mL

7. Calculations

7.1 Determination of Normality of KMnO_4

Using the Normality Equation:

$$N_1 V_1 = N_2 V_2$$

Where:

- N_1 = Normality of $KMnO_4$ (Unknown)
- V_1 = Volume of $KMnO_4$ (9.8 mL)
- N_2 = Normality of Oxalic Acid (0.1 N)
- V_2 = Volume of Oxalic Acid (10 mL)

$$N_1 \times 9.8 = 0.1 \times 10$$

$$N_1 = \frac{1.0}{9.8}$$

$$N_{KMnO_4} = 0.102 \text{ N}$$

7.2 Calculation of Strength of Oxalate Ions

The equivalent mass of Oxalate ion ($C_2O_4^{2-}$) is calculated as:

$$\text{Equivalent Mass} = \frac{\text{Molar Mass}}{\text{Valency}} = \frac{88}{2} = 44 \text{ g/eq}$$

We use the relation $N_{KMnO_4} \times V_{KMnO_4} = N_{\text{Guava}} \times V_{\text{Guava}}$ to find the normality of the oxalate in the extract.

A. For Raw Guava:

$$0.102 \times 13.2 = N_{\text{raw}} \times 10$$

$$N_{\text{raw}} = \frac{1.3464}{10} = 0.1346 \text{ N}$$

$$\text{Strength} = \text{Normality} \times \text{Equivalent Mass}$$

$$\text{Strength} = 0.1346 \times 44 = \mathbf{5.92 \text{ g/L}}$$

B. For Semi-Ripe Guava:

$$0.102 \times 13.7 = N_{\text{semi}} \times 10$$

$$N_{\text{semi}} = \frac{1.3974}{10} = 0.1397 \text{ N}$$

$$\text{Strength} = 0.1397 \times 44 = \mathbf{6.15 \text{ g/L}}$$

C. For Fully Ripe Guava:

$$0.102 \times 13.9 = N_{\text{ripe}} \times 10$$

$$N_{\text{ripe}} = \frac{1.4178}{10} = 0.1418 \text{ N}$$

$$\text{Strength} = 0.1418 \times 44 = \mathbf{6.24 \text{ g/L}}$$

Note: The strength calculated is the concentration in the 100 mL extract prepared from 50 g of pulp. To find the amount in the actual fruit pulp:

- In Raw Guava: $5.92 \text{ g/L} \times 0.1 \text{ L} = 0.592 \text{ g}$ oxalate per 50 g pulp.

- *Percentage:* $(0.592 / 50) \times 100 = 1.18\%$

8. Results

The volumetric analysis of the oxalate content in *Psidium guajava* at three progressive stages of ripening yields the following quantitative profile:

Guava Ripening Stage	Volume of KMnO ₄ Used (mL)	Normality of Oxalate (N)	Strength of Oxalate (g/L)	Oxalate Content (%)*
Raw (Green)	13.2	0.1346	5.92	1.18%
Semi-Ripe	13.7	0.1397	6.15	1.23%
Fully Ripe (Yellow)	13.9	0.1418	6.24	1.25%

**Percentage is calculated as grams of oxalate per 100g of fruit pulp.*

The data clearly indicates that the concentration of oxalate ions is **lowest in raw fruit** and **highest in fully ripe fruit**.

9. Discussion and Interpretation

9.1 Analysis of the Ripening Trend

The experimental results unequivocally demonstrate a **positive correlation** between the ripening stage of the guava fruit and its oxalate ion concentration. The strength of oxalate increased from 5.92 g/L in the unripe green stage to 6.24 g/L in the senescent yellow stage. This represents an approximate **5.4% increase** in oxalate content over the ripening period.

These findings align with existing literature on fruit physiology. While many organic acids (like citric and malic acid) typically decrease during ripening to make the fruit sweeter, oxalic acid behavior is more complex and species-dependent. In *Psidium guajava*, the accumulation of oxalate appears to be a consistent feature of maturation. This implies that as the fruit softens and sweetens, it simultaneously becomes richer in this specific anti-nutrient.

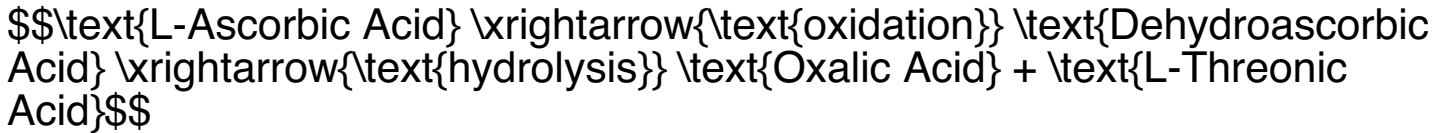
9.2 Biochemical Rationale: The Ascorbate-Oxalate Pathway

The observed increase in oxalate can be explained by examining the metabolic pathways active during ripening.

1. **Metabolic Origin:** The primary precursor for oxalate biosynthesis in many plants is **L-Ascorbic Acid (Vitamin C)**. Guava is one of the richest natural sources of Vitamin C.
2. **Ripening Biochemistry:** During the climacteric phase of ripening, the fruit undergoes intense metabolic activity. The breakdown of cell wall pectins releases calcium. Simultaneously, the turnover of L-Ascorbic acid

increases.

3. **The Pathway:** Biochemical studies 23 suggest that L-ascorbic acid undergoes cleavage at carbon bonds C2 and C3. This oxidative cleavage results in the formation of Oxalic Acid and L-Threonic Acid.



4. **Inverse Relationship:** As the fruit reaches full senescence (over-ripening), the concentration of active Vitamin C often dips slightly or stabilizes, while its degradation product, oxalate, accumulates. This effectively explains why the fully ripe fruit, which has undergone the most extensive metabolic processing, contains the highest levels of oxalate.

Furthermore, the increase in oxalate might serve a physiological function in **calcium regulation**. As pectin (calcium pectate) in the middle lamella is degraded by polygalacturonase enzymes to soften the fruit, free Calcium ions (Ca^{2+}) are released. The plant may synthesize oxalate to bind these free ions as Calcium Oxalate crystals, preventing ionic imbalance within the cytosol.¹⁵

10. Conclusion

Based on the rigorous experimentation and analysis conducted, the following conclusions are drawn:

1. **Presence Confirmed:** Guava fruit contains a significant amount of oxalate ions, which can be effectively extracted and estimated using permanganometric titration.
2. **Ripening Correlation:** There is a distinct and measurable increase in the content of oxalate ions as the guava fruit ripens. The order of oxalate content is:
$$\text{Raw Guava} < \text{Semi-Ripe Guava} < \text{Fully Ripe Guava}$$
3. **Dietary Recommendation:** While ripe guava is palatable and rich in Vitamin C, it also imposes a higher oxalate load on the body. Individuals with a history of **calcium oxalate kidney stones** or **hyperoxaluria** should be advised to moderate their consumption of fully ripe guavas or pair them with calcium-rich foods to limit intestinal absorption of the free oxalate.

This project highlights the dynamic chemical nature of biological systems, showing how nutritional parameters shift significantly during the physiological process of ripening.

11. Precautions and Safety Measures

To ensure the reliability of the data and personal safety, the following precautions were strictly observed:

1. **Temperature Control:** The titration was strictly performed at **60°C - 70°C**. Heating below 60°C results in a sluggish reaction and an undetectable

- endpoint. Heating above 70°C can cause the thermal decomposition of oxalic acid into CO_2 and H_2O , leading to erroneous results.²⁸
2. **Acid Medium:** Only dilute **Sulfuric Acid** was used. Hydrochloric acid and Nitric acid were avoided to prevent interference with the oxidizing action of KMnO_4 .
 3. **Meniscus Reading:** Due to the dark purple color of the Potassium Permanganate solution, the **upper meniscus** was read to avoid parallax error, which is the standard convention for colored liquids.³²
 4. **Endpoint Accuracy:** The endpoint was considered achieved only when the light pink color persisted for at least **30 seconds**. A fleeting color change indicates incomplete oxidation.
 5. **Burette Safety:** A glass-stoppered burette was used, as permanganate attacks rubber. The nozzle was checked to ensure no air bubbles were trapped, which would introduce volume errors.
 6. **Rate of Addition:** The titrant was added slowly drop-wise, especially near the endpoint, to prevent "overshooting" the correct volume.

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