IB Higher Level Internal Assessment in Chemistry

Investigating the effect of microwave and stovetop heat exposure on the vitamin C concentration in orange juice

1. Research design

1.1 Research Question

How does increasing the temperature to 20.00 °C (control), 40.00 °C, 60.00 °C, 80.00 °C, and 90.00 °C using microwave and stovetop heating affect the vitamin C concentration in orange juice, in mg/100 cm^3 , as determined by iodometric titration?

1.2 Background Information

Vitamin C, or ascorbic acid, is a water-soluble antioxidant and enzymatic cofactor in food systems; chemically, it is reactive and highly unstable. Literature describes vitamin C as a polar organic acid ($C_6H_8O_6$), molecular mass of 176.12 g mol^{-1} containing a five-member lactone ring and multiple hydroxyl groups responsible for its water-solublility and strong reducing ability (Merck, n.d.). Nevertheless, fruits such as oranges, rich in ascorbic acid remain a crucial dietary source since humans cannot synthesize vitamin C (Rampersaud & Valim, 2017). L-ascorbic acid's reactivity stems from its enediol group ($-\mathrm{C(OH)} = \mathrm{C(OH)}-$), which readily donates electrons to oxidizing agents. This makes the molecule effective in redox reactions, such as iodometric titration, yet also results in poor stability, as the enediol group is easily oxidised during heating or exposure to air. Modern studies affirm that ascorbic acid rapidly degrades in the presence of heat, dissolved oxygen, alkaline pH or catalytic metal ions (Yin et al., 2022). Temperature control as well as oxygen exposure are crucial when measuring vitamin C in heated samples because these degradation pathways are driven by the structural characteristics that allow antioxidant action.

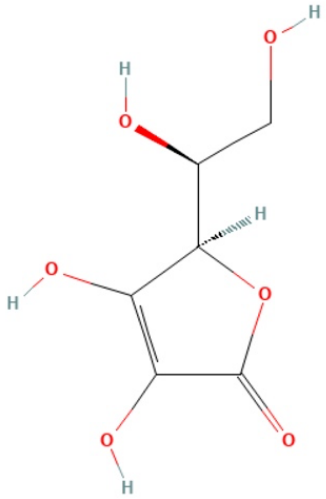


Figure 1. *Molecular structure of L-ascorbic acid, showing the lactone ring and hydroxyl groups (image adapted from PubChem; National Center for Biotechnology Information, n.d.)*

Due to the high thermolability, vitamin C breaks down quickly at high temperatures (Ebrahimi & Dabbagh, 2019). High temperatures decrease the retained concentration and therefore dietery contribution of ascorbic acid by accelerating its conversion to dehydroascorbic acid and other irreversible compounds (Yin et al., 2022). While the rate of loss may vary with temperature and exposure time, short high-heat methods may contain more vitamin C than prolonged low-temperature heating (Amaro & Tadini, 2021). This study examines two common domestic heating methods: stovetop and microwave. Microwave heating uses dielectric heating - electromagnetic waves cause polar molecules (mainly water) to oscillate, generating internal heat through molecular friction (U.S. Food and Drug Administration, n.d.). However, microwaves can create uneven “hot and cold spots” due to variable penetration depth and sample’s geometry (USDA Food Safety and Inspection Service, n.d.). Stovetop heating relies on surface conduction, generally producing more uniform temperature gradients. Such differences in energy transfer may influence vitamin C degradation kinetics in orange juice.

In this study vitamin C concentration was determined via iodometric titration, a common redox method. Where ascorbic acid reduces iodine (I_2) to iodide (I^-) while being oxidized to dehydroascorbic acid:

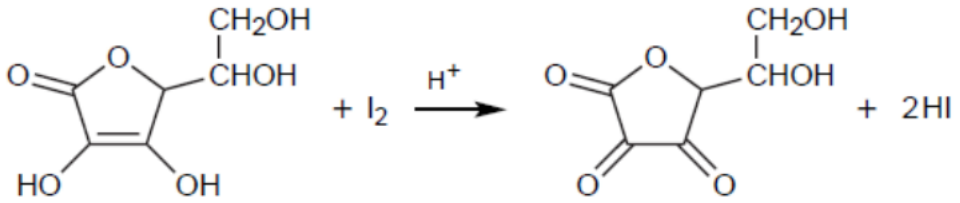


***Figure 2**. Oxidation of L-ascorbic acid by iodine (I_2) during iodometric titration (adapted from Vitamin C Titration to Survive a High Seas Journey (University of Notre Dame, 2013).*

$$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2HI \quad \textbf{Eq. 1}$$

After all vitamin C is consumed, excess iodine reacts with starch to make a blue-green complex that marks the endpoint. To reduce the subjectivity in detecting this color change, the *Colour Picker AR* app was used to match the endpoint colour as a predefined hex/RGB value and titrations ware stopped when the measured colour fell within that value. The aim of this study is to assess how heating temperature and method affect vitamin C concentration in orange juice.

Microwave and stovetop heating were compared at 20.00 °C, 40.00 °C, 60.00 °C, 80.00 °C, and 90.00 °C (± 0.30 °C). For stovetop heating, a thermometer was used to monitor the temperature and microwave heating was controlled using the microwave's built-in infrared sensor, that detects surface temperature by measuring infrared radiation emitted from the sample (Toshiba, n.d.). To minimise pre-treatment oxidation and storage-related vitamin C loss, all oranges were squeezed at once and the juice was used immediately while being protected from direct heat and sunlight. To reduce variation between individual oranges (for instance, ripeness or pulp content), the juice was stored in a single container and thoroughly mixed so that each trial obtained a homogeneous sample. Thus, the findings offer evidence on

vitamin C retention under typical home heating settings and can advice for practical choices regarding temperature and heating method.

1.3 Variables

The independent variable in this investigation was the sample temperature reached during heating set at 20.00 °C, 40.00 °C, 60.00 °C, 80.00 °C, and 90.00 °C (± 0.30 °C). For stovetop heating, a thermometer was used to monitor temperature; likewise, for microwave heating, each target temperature was obtained using the microwave's built-in infrared sensor and for additional accuracy the sample temperature was measured immediately with a thermometer before titration. Therefore, the comparative factor was the heating method (microwave versus stovetop) to compare vitamin C retention at the same target temperatures.

The dependent variable was the vitamin C concentration in orange juice (mg 100 cm^3). Values were calculated from the measured moles of iodine using the 1:1 stoichiometric relationship in *Eq. 1* and the molar mass of ascorbic acid (176.12 g mol^{-1}). The endpoint of the titration was identified as the first blue-green starch-iodine complex, with colour detection standardised using the *Color Picker AR app*.

Controlled variables:

Controlled Variable	Why It Was Controlled	How It Was Controlled
Brand of orange juice	Different brands contain varying levels of ascorbic acid.	Same brand "navel" was used throughout all the trials.
Volume of orange juice	Affects concentration if not consistent.	25.00 cm^3 (± 0.05 cm^3) amount was measured using a pipette for all trials.

Freshness of orange juice	Vitamin C degrades over time or when exposed to air.	All juice was freshly made during the same day, placed in one container, then mixed thoroughly and used right away for all trials. To reduce oxygen exposure and time-based vitamin C loss, the container was kept sealed throughout the samples.
Molarity of iodine solution, 0.00392 mol dm^{-3} (±0.00005 mol dm^{-3})	Inconsistent concentration affects titration results.	Prepared in 1 L (±0.4 cm^3) volumetric flask 1L and standardized with 0.0100 mol dm^{-3} sodium thiosulfate before titration.
Starch indicator volume	Too much or too little amount affects pH and color change visibility.	3 drops of 0.5% starch solution was added to each trial using a pipette.

1.4 Preliminary experiment

A preliminary trial was done to test iodometric titration for analysing vitamin C concentration in orange juice resulting from heat treatment. The aim was to identify procedural errors and increase precision and accuracy.

The main problem was inconsistent blue-black iodine-starch complex, which made endpoint detection challenging. Fresh 0.5% starch solution was added during the orange-yellow stage to improve contrast and reduce subjectivity. The *Colour Picker AR* application

was used to improve endpoint identification: the camera was set up above the flask and the colour was compared to an established reference colour code: hex #755502 - #765709; RGB: R 117-118, G 85-87, B 2-9.

Colour measurements were taken at 90° above the marked spot within a 15-minute span, so day-light change and interference were minimal. All samples were also placed in the same marked spot. For each titration, five measurements were taken from different random points on the solution surface and then averaged; the titration was completed when the mean value fell within that specified range.

A second limitation was uneven exposure to heat. Heating samples separately caused small temperature differences. This was fixed by heating each of the five trials for every temperature group in a single beaker. After stirring and keeping an eye on it with a calibrated thermometer, the mixture was then divided equally. Right after heating, the beaker was cooled in an ice-water bath to rapidly cool down and limit further heat driven vitamin C loss (Thuy et al., 2020). Titration was carried out once the juice had returned to room temperature, ensuring comparable reaction conditions across trials.

For microwave heating, target temperatures were achieved using the microwave's built-in infrared sensor to reach each set temperature. The effective power output of the Toshiba microwave used in the experiment was additionally tested in a separate trial by heating a known mass of water and applying $Q = mc\Delta T$ and $P = Q/t$ (see *Determination of Microwave Power Output*) to double-check its accuracy and not fully rely on manufacturer specifications.

1.5 Procedure

Equipment
Oranges

Thermometer (± 0.30 °C)
0.250 g (± 0.001 g) soluble starch
Volumetric pipette 25.00 cm^3 (± 0.05 cm^3)
250.00 cm^3 conical flasks
250 cm^3 beaker
Toshiba ND300C microwave over (Toshiba Corporation) and single burner electric laboratory hot plate (Home Science Tools)
25.00 cm^3 (± 0.25 cm^3) graduated cylinder
10.0 cm^3 (± 0.05 cm^3) graduated cylinder
100.0 cm^3 (± 0.5 cm^3) graduated cylinder
Potassium Iodide
1.000 dm^3 (± 0.0004 dm^3) volumetric flask
10.00 cm^3 (± 0.02 cm^3) pipette
Analytical balance scale (± 0.001 g, BA-W Series, Infitek)
Burette 25.00 cm^3 (± 0.030 cm^3) and retort stand

Preparation of starch indicator solution (0.5%) (University of Canterbury, n.d.):

A 0.5% starch indicator solution was made by weighing 0.250 g of soluble starch using an analytical balance (± 0.001 g) then adding it to a 100 cm^3 conical flask containing 50 cm^3 of near-boiling distilled water. The mixture was constantly stirred until the starch fully dissolved. Then the solution was allowed to cool to room temperature before use.

Iodine-potassium iodide solution 1000.0 cm^3 (± 0.4 cm^3) (University of Canterbury, n.d.):

An iodine-potassium iodide solution was prepared by accurately weighing 2.000 g of iodine (I_2) and 3.000 g of potassium iodide (KI) on an analytical balance (± 0.001 g). In a clean beaker, potassium iodide was dissolved in about 75 cm^3 of distilled water then afterwards the iodine crystals were gradually added and stirred until a dark-brown triiodide (I_3^-) solution formed. The solution was poured into a 1000 cm^3 volumetric flask and thoroughly mixed.

Standardization of iodine solution *(University of California, Davis, 2023)*:

The prepared iodide solution was standardized using sodium thiosulfate ($Na_2S_2O_3$) as the titrant. A 25.000 cm^3 portion of 0.010 mol dm^{-3} $Na_2S_2O_3$ solution was placed in a burette (± 0.030 cm^3). 25.000 cm^3 of the prepared iodine solution was transferred into a conical flask using a volumetric pipette (± 0.05 cm^{-3}). Three drops of a 0.5% starch solution were added to the flask as an indicator. The iodine solution was titrated with sodium thiosulfate while the mixture was continually swirled, until the brown-red colour disappeared completely, indicating that all iodine had been reduced to iodide.

Reaction Equation:

$$I_3^-(aq) + 2S_2O_3^{2-}(aq) \rightarrow 3I^-(aq) + S_4O_6^{2-}(aq)$$

Eq. 2 *Iodine reduced to iodide; thiosulfate oxidized to tetrathionate.*

Standardization calculations:

Measured titre volume of $Na_2S_2O_3$ is 98.000 cm^3 (±0.200 cm^3).

Molarity of $Na_2S_2O_3$:

$$C = \frac{n}{V}$$

$$(n)Na_2S_2O_3 = c \cdot V = 0.00200\ mol\ dm^{-3} \cdot 0.098000\ dm^3 = 1.96 \cdot 10^{-4} mol$$

By using stoichiometry 1 mole of I_3^- reacts with 2 moles of $S_2O_3^{2-}$:

$$n(I_2) = \frac{1.96 \cdot 10^{-4}}{2} = 9.80 \cdot 10^{-5}\ mol$$

The concentration of iodine was then determined as:

$$c(I_2\ solution) = \frac{n}{V} = \frac{9.80 \cdot 10^{-5}}{0.0250\ dm^3} = 0.00392\ \ mol\ dm^{-3}$$

Uncertainty calculations:

$$PU\ (of\ I_2) = \frac{\pm 0.001\ g}{2.000\ g} \cdot 100\% = \pm 0.05\%$$

$$PU(of\ Kl) = \frac{\pm 0.001\ g}{3.000\ g} \cdot 100\% = \pm 0.03\%$$

$$PU(of\ volume) = \frac{\pm 0.4\ cm^3}{1000\ cm^3} \cdot 100\% = \pm 0.04\%$$

$$PU(total\ of\ solution) = 0.05 + 0.03 + 0.04 = \pm 0.12\%$$

$$\%U(Na_2S_2O_3) = \frac{\pm 0.200}{98.000} \cdot 100\% = \pm 0.2\%$$

$$\%U(I_2\ solution) = \frac{0.05\ cm^3}{25.00\ cm^3} \cdot 100\% = \pm 0.2\%$$

$$\%U(total) = 0.12 + 0.2 + 0.2 = \pm 0.52\%$$

$$AU(I_2\ solution\ conc.) = \frac{0.52\% \cdot 0.00392\ mol\ dm^{-3}}{100\%} = 0.00002\ mol\ dm^{-3}$$

$$c(I_2\ solution) = 0.00392\ mol\ dm^{-3}\ (\pm\ 0.00002\ mol\ dm^{-3})$$

Iodine solution concentration with uncertainty is:

$$0.00392\ mol\ dm^{-3}\ (\pm\ 0.00002\ mol\ dm^{-3}).$$

Heating of orange juice samples

Using a volumetric pipette, the sample was evenly divided into five 25.00 cm^3 parts. This ensured consistency throughout trials. A digital timer was used to record time. Prior to titration, heated samples were cooled to room temperature 20 °C (± 0.03 °C), measured with a thermometer and with the aim to stop additional ascorbic acid thermal degradation during analysis.

Determination of Microwave Power Output

To estimate the energy delivered by the microwave during heating, a sample of 100.0 cm^3 of water measured using graduated cylinder was heated over 60 seconds. The initial temperature (Ti) of the water was 24.0°C, and the final temperature (Tf) after heating was 51.0°C. The temperature change (ΔT) was calculated as:

$$\Delta T = Tf - Ti = 51.0\,℃ - 24.0\,℃ = 27.0\,℃$$

The energy absorbed by the water was then determined using the specific heat capacity formula:

$$q = mc\Delta T$$

Where:

- $m = 100.0$ cm^3;
- $c = 4.18\,J\,g^{-1}\,K^{-1} = 4.18\,J\,g^{-1}\,K^{-1}$ (specific heat capacity of water);
- $\Delta T = 27.0$ °C

$$q = 100.0 \cdot 4.18 \cdot 27.0 = 11286\,J$$

The power output of the microwave was calculated as the energy per unit time:

$$Power = q/t = 11268J/60s = 188.1J\ s^{-1} = 188.1\ W$$

This value represents the effective heating power of the microwave during the experiment.

Uncertainty calculations:

$$PU(volume) = \frac{\pm 0.5\ cm^3}{100.0\ cm^3} \cdot 100\% = 0.5\%$$

Temperature change: $\Delta T = 51.0\ ℃ - 24.0\ ℃ = 27.0\ ℃$

$$PU(temp) = \frac{\pm 0.60\ ℃}{27.00\ ℃} \cdot 100\% = 2.2\%$$

$$\%U(total) = 0.5\% + 2.2\% = 2.7\%$$

$$AU(micro.power\ output) = 2.7 \cdot 188.1\ W \div 100 = \pm 5\ W$$

Microwave power output with uncertainty is: $188\ W\ (\pm\ 5\ W)$.

Titration of Orange Juice Samples

A 25.000 cm³ (± 0.030 cm^3) burette was filled with the prepared iodine solution. 25.00 cm³ of orange juice was transferred into a 250 cm³ Erlenmeyer flask using a volumetric pipette (± 0.05 cm^3). Three drops of 0.5% starch solution were added to the flask. Samples were titrated with iodine solution from the burette under magnetic stirring to the first greenish-blue endpoint, standardised using *Colour Picker AR* app by matching the solution colour to a pre-selected reference RBG value (hex #755502 - #765709; RGB: R 117-118, G 85-87, B 2-9).



Figure 3. *Prepared orange juice samples in conical flasks with standardized iodine-potassium iodide (I_2) solution prior to iodometric titration.*

1.6 Risk assessment

Material name and Chemical formula	**Associated risks**	**Protective measures taken**
Iodine, I_2	Harmful in contact with skin or if inhaled. Very toxic to aquatic life. May cause eye and skin irritation. 	Handle with gloves, goggles, and lab coat. Use in a well-ventilated area or fume hood. Avoid inhalation and direct contact with skin. Dispose of properly to prevent environmental harm.

Potassium iodide, KI	Harmful if swallowed. Causes serious eye and skin irritation. May cause allergic reactions and is toxic to aquatic life. 	Wear gloves, goggles, and a lab coat. Use in a ventilated area. Avoid ingestion and dispose of properly to prevent environmental harm.
Sodium thiosulfate, $Na_2S_2O_3$	Low toxicity but may cause eye and skin irritation, Dust may cause respiratory irritation.	Use with gloves, goggles and lab coat Avoid inhalation and contact with skin and eyes. Use in a well-ventilated area. Dispose of properly to prevent environmental harm

Table 1. *The risk assessment of all chemicals employed in the experiment, listing the associated risks and protective measures taken. Based on: PubChem; National Center for Biotechnology Information, n.d.*

Environmental risks: The reagents used could pose significant harm to the environment if not managed properly after the experiment. To prevent this, all solutions were discarded into appropriate waste

containers for later utilization.

Ethical risks: There were no notable ethical risks associated with the experiment.

2.1 Raw data

2.1.1 Quantitative data

Temperature (± 0.30 °C)	1st Trial (± 0.060)	2nd Trial (± 0.060)	3rd Trial (± 0.060)	4th Trial (± 0.060)	5th Trial (± 0.060)	Average (± 0.060)

20.00 (control)	18.250	18.200	18.300	18.100	18.150	18.200
40.00	17.950	18.100	18.020	18.010	18.040	17.932
60.00	17.800	17.750	17.650	17.750	17.750	17.740
80.00	17.600	17.550	17.500	17.650	17.550	17.600
90.00 (boiling point)	17.250	17.200	17.300	17.150	17.250	17.280

Table 2. *Volume of 0.00392M Iodine Solution Used to Titrate 25.00 cm³ of Orange Juice (Stovetop Heating)*

Temperature (±0.30 °C)	1st Trial (± 0.060)	2nd Trial (± 0.060)	3rd Trial (± 0.060)	4th Trial (± 0.060)	5th Trial (± 0.060)	Average (± 0.060)
20.00 °C (Control)	18.750	18.700	18.650	18.750	18.800	18.730
40.00 °C	18.300	18.250	18.350	18.200	18.250	18.230
60.00 °C	18.150	18.050	18.100	18.050	18.100	18.100
80.00 °C	17.600	17.700	17.750	17.650	17.700	17.680
90.00 °C (boiling point)	17.300	17.350	17.400	17.200	17.250	17.300

Table 3. *Volume of 0.00392M Iodine Solution Used to Titrate 25.00 cm³ of Orange Juice (Microwave Heating)*

2.1.2 Qualitative data

At the beginning of the titration, the orange juice appeared pale orange. The mixture darkened when the iodine solution was added. At the endpoint, the solution became greenish-brown. Upon full oxidation of ascorbic acid, the presence of excess iodine caused a color change. Once iodine and starch reacted, a visible iodine-starch complex was created. Using the Colour Picker AR app, the endpoint was recorded when the colour reading fell within a preset hex/RGB range (hex #755502 - #765709; RGB: R 117-118, G 85-87, B 2-9) reducing subjectivity.

2.2 Data analysis

Volumes of iodine solution used to reach the endpoint were recorded and averaged across five trials for each treatment to improve reliability. The average titres were then used to calculate vitamin C concentration (mg/100 cm³). Worked example of these calculations for 20,00 °C (control variable), including uncertainty propagation, is provided in the *Calculations* section.

The redox reaction used:

$$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2I^- \quad \textbf{Eq. 3}$$

This 1:1 molar ratio allowed the moles of iodine added to equal the moles of ascorbic acid in the sample:

$n = C \cdot V$

Mass of vitamin C:

Molar mass of vitamin C = 176.12 g mol^{-1}

$mass\ (mg) = n \cdot 176.12\ g\ mol^{-1} \cdot\ 1000$

Concentration:

$$mg/100\ cm\ = \left(\frac{mass(mg)}{25.00}\right) \cdot 100$$

2.3 Calculations

Sample average calculation (20 °C, stovetop heating)

To determine the average volume of iodine (I_2) solution required for titration at 20.00°C, five concordant trials were conducted. The values remained within ±0.060 cm^3 uncertainty. The average was conducted using the following formula:

$$Average\ volume\ (\bar{x}) = \frac{sum\ of\ all\ five\ trials}{number\ of\ values\ (n)}$$

$$\bar{x} = \frac{18.250 + 18.200 + 18.300 + 18.100 + 18.150}{5}$$

$$\bar{x} = \frac{91.000}{5} = 18.200\ cm^3$$

This method of averaging was applied for each temperature condition to ensure consistency and reliability in further stoichiometric and concentration calculation.

Calculating vitamin C (ascorbic acid) concentration

To determine the concentration of ascorbic acid in orange juice at 20.00 °C (stovetop sample), the average volume of iodine solution used was 18.200 cm^3 (±0.060 cm^3), equivalent to 0.018200 dm^3. The standardized concentration of the iodine solution was 0.00392 mol dm^{-3} (±0.00005 mol dm^{-3}). The number of moles of iodine added was calculated using the formula:

$$n(I_2):$$

$$V(I_2) = 18.200\ (\pm\ 0.060\ cm^3)$$

$$c(I_2) = 0.00392\ mol\ dm^{-3}\ (\pm 0.00005\ mol\ dm^{-3})$$

$$V(juice) = 25.00\ cm^3 (\pm\ 0.25\ mL)$$

$$n(I_2) = 0.00392 \cdot 0.018200 = 7.13 \cdot 10^{-5}\ mol$$

$$n(I_2) = n(C_6H_8O_6) = 7.13 \cdot 10^{-5}\ mol$$

$$m(C_6H_8O_6) = 7.13 \cdot 10^{-5} \cdot 176.12 = 1.256 \cdot 10^{-2}\ g = 12.56\ mg$$

To express the vitamin C content per 100 cm^3 of juice, the result was proportionally scaled:

$$\frac{12.56\ mg}{25.00\ cm^3} = \frac{x\ mg}{100.00\ cm^3}$$

$$x = \frac{12.56 \cdot 100.00}{25.00} = 50.24\ mg/100\ cm^3$$

Uncertainty calculations:

$$\%U(I_2\ volume) = \frac{0.060}{18.200} \cdot 100\% = 0.33\%$$

$$\%U(I_2\ conc.) = \frac{0.00002}{0.00392} \cdot 100\% = 0.5\%$$

$$\%U(Juice\ Volume) = \frac{0.25}{25.00} \cdot 100\% = 1\%$$

$$\%U(total\ of\ Vit.C) = 1\% + 0.5\% + 0.33\% = 1.63\% \approx 1.6\%$$

$$AU(Vit\ C\ conc.) = \frac{1.6\% \cdot 50.2\ mg}{100\%} = \pm 0.8\ mg$$

Vitamin C concentration with uncertainty is: $50.2 \pm 0.8\ mg/100\ cm^3$

2.3 Processed data

Temperature reached (°C)	Stovetop; vitamin C concentration (mg/100 cm^3)	Microwave; vitamin C concentration (mg/100 cm^3)
20.00	50.2 ±0.8	51.7 ±0.8
40.00	49.5 ±0.8	50.3 ±0.8
60.00	49.0 ±0.8	50.0 ±0.8
80.00	48.6 ±0.8	48.8 ±0.8

90.00	47.7 ±0.8	47.8 ±0.8

Table 4. *Average concentration of ascorbic acid (mg/100cm^3) in orange juice samples heated by microwave and stovetop methods at varying temperatures. All uncertainties represent absolute uncertainty (AU) in vitamin C concentration.*

2.4 Statistical analysis

One-way ANOVA test

In this experiment, the One-Way ANOVA test was applied to investigate whether heating temperature (stovetop/microwave) has a measurable effect on the concentration of vitamin C in orange juice

For stovetop heating the ANOVA had a p-value of $1.11 \cdot 10^{-15}$ which is less than the significance level. Similarly, for microwave heating, the p-value is $4.99 \cdot 10^{-19}$. Therefore, the null hypothesis is rejected, indicating that heating temperature has a statistically significant detrimental effect on vitamin C concentration.

Kruskal-Wallis

This test was used to determine whether heating temperatures causes statistically significant differences in vitamin C concentration across multiple temperature groups for both stovetop and microwave heating. The p-value was obtained $1.2 \cdot 10^{-4}$, which is below $\alpha = 0.05$, stating statistically significant differences between groups. Thus, this confirms that heating temperature positively correlated to vitamin C degradation for both heating methods.

Two-Way Anova Test

This test was used to determine whether heating method, temperature and their interaction significantly affect vitamin C concentration. Both heating methods ($p < 1 \cdot 10^{-15}$) and temperature ($p < 1 \cdot 10^{-16}$) showed statistically significant effects and a

significant interaction was observed ($p = 2.9 \cdot 10^{-10}$). This indicates that vitamin C concentration depends on these two factors and that their effects are not independent. Although a statistically significant effect of heating method is identified, but the difference between microwave and stovetop heating is minimal and within the uncertainty range (± 0.8 mg) indicating that observed variation is not practically significant.

2.5 Graphical analysis

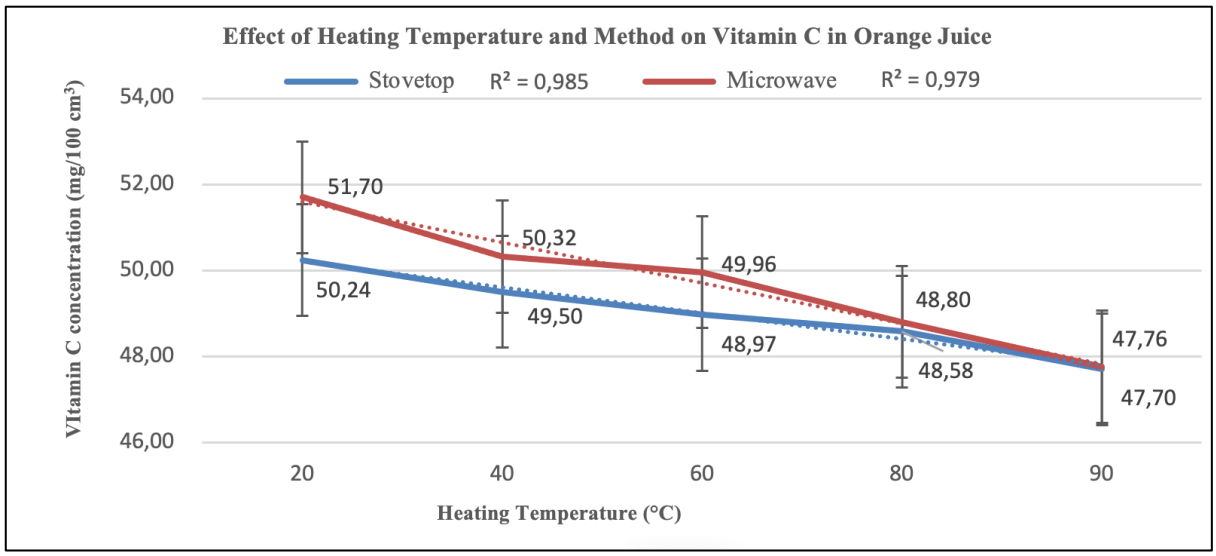


Figure 4. *Vitamin C concentration (mg/100 cm³) in orange juice after heating to different target temperatures (20,00 to 90,00 ℃) using microwave and stovetop methods. Data obtained by iodometric titration. Error bars represent absolute uncertainties in concentration.*

Temperature of analyte (± 0.30 °C)	**Concentration of vitamin C (mg/100 cm³)**		**Percentage uncertainty of concentration (± 2 s. f.)**		**Absolute uncertainty of concentration (mg/cm³ ± 2 s. f.)**	
	Stovetop heating	Microwave heating	Stovetop heating	Microwave heating	Stovetop heating	Microwave heating
20.00	50.24	51.70	±3 %	±3 %	±1.5	±1.6
40.00	49.50	50.32	±3 %	±3 %	±1.5	±1.5

60.00	48.97	49.96	±3 %	±3 %	±1.5	±1.5
80.00	48.58	48.80	±3 %	±3 %	±1.5	±1.5
90.00	47.70	47.76	±3 %	±3 %	±1.4	±1.4

Table 5. *Processed data showing the concentration if vitamin C in orange juice at different temperatures with associated uncertainties for stovetop and microwave heating.*

3. Conclusion

The concentration of vitamin C reduced roughly linearly with temperature for both heating methods and the trendlines exhibited high coefficients of determination ($R^2 = 0.985$ for stovetop and $R^2 = 0.979$ for microwave). The high R^2 values support the relevance of a linear model, since statistical significance was established using One-Way ANOVA. At 90 °C, the concentration dropped to 47.7 mg/100 cm³ (stovetop) and 47.8 mg/100 cm³ (microwave), compared with 50.2 mg/100 cm³ and 51.7 mg/100 cm³ at 20 °C, showing a small decrease in vitamin C concentration. This matches to an approximate decrease of 5% (from 50.2 to 47.7 mg/100 cm^3), showing a small practical effect under short heating conditions and this is consistent with increased reaction rates at higher temperatures, leading to faster oxidation of ascorbic acid. Given the overlapping uncertainties (±0.8 mg/100 cm³) between microwave and stovetop results, there is no meaningful difference between the two heating methods. The results align with more recent work, such as Dhenge et al. (2022) showing low ascorbic acid breakdown during short heating, indicating that heating temperature and time have a bigger impact than methodology itself. Trial-to-trial variation was small, however, potential systematic error, such as uncertainty in endpoint detection, may have influenced the values measured. The findings indicate that under typical household conditions, reheating juice for a short time is unlikely to significantly affect its nutritional value.

4. Evaluation

Low percentage uncertainties and strong control of variables (juice volume and reagent concentration) increased validity by reducing preperation-related variation. Nonetheless, several limitations still reduced precision and accuracy.

A major limitation was the lack of direct temperature monitoring during microwave heating. Target temperatures were reached using microwave's built-in infrared sensor rather than with a probe, so internal temperature may have varied between trials because sensor measures surface temperature and microwave heating can be non-uniform. If certain samples reached slightly higher temperatures than expected, this then represents a major source of systematic error, most likely resulting in an underestimation of vitamin C concentration, since higher temperatures accelerate oxidative degradation. Thus, that weakens control of the independent variable and limits the validity of temperature-concentration relationship. Validity could be increased by constantly recording internal temperature during heating using a fiber-optic probe that is compatible with microwaves, ensuring consistent thermal conditions across all trials.

Endpoint detection, although improved with the Colour Picker AR app, still carried an aspect of subjectivity. Variations in lighting conditions, camera position, and natural juice colour could influence when the endpoint was considered to have been reached. To increase consistency between trials, all colour measurements were taken with the phone placed at 90° above the marked same spot and completed within a 15-minute span, minimising daylight interference. In some trials, the starch-iodine complex began fading soon after appearing, creating uncertainty over whether the reaction was complete. A way to increase reproducibility could be by using an objective endpoint approach such as colorimeter/spectrophotometer or regulated lighting (e.g., constant LED or white background).

Lastly, environmental factors including pH and oxygen exposure were not directly controlled. Degradation during heating and titration may have contributed to somewhat lower observed values, as vitamin C oxidises more easily in alkaline or oxygen-rich settings. However, pre-treatment oxidation was minimised by squeezing all oranges on the same day, pooling and mixing the juice, then keeping the container sealed while using it immediately for trials. For further research, this effect could be reduced by recording/standardising pH and limiting oxygen exposure throughout heating and titration. Additionally, the investigation was limited to 20-90 °C temperature range and short heat durations, which limits the extent to which the conclusions can be applied to prolonged heating conditions.

Overall, validity could be improved with direct temperature monitoring, objective endpoint detection and stricter environmental factor control. With these improvements, findings would more closely reflect the actual impact of heating on orange juice's vitamin C content by lowering both systematic and random errors.

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6. Appendices

Appendix A

Colour Picker AR application screenshots

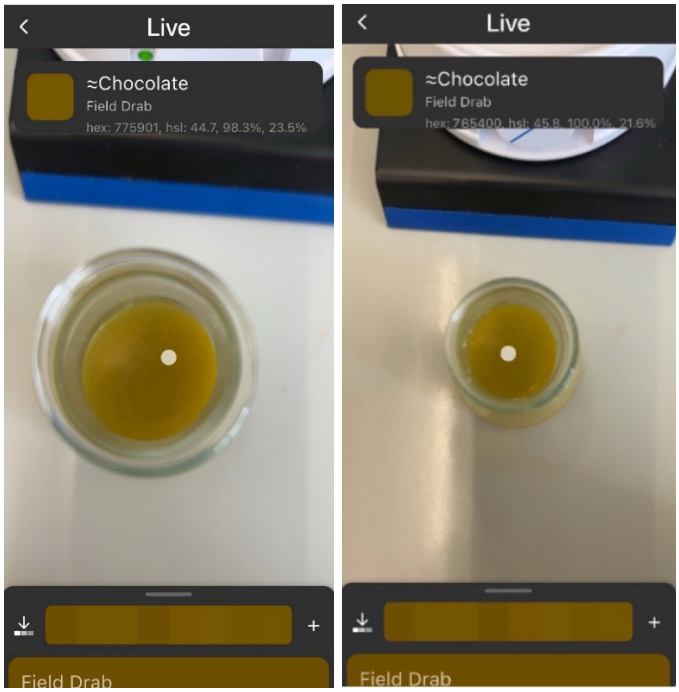


Figure A1 and A2. *Screenshots oft he Colour Picker AR app used to standardise endpoint detection by matching the solution colour to a predefined reference colour code (hex #755901 and #765400),*

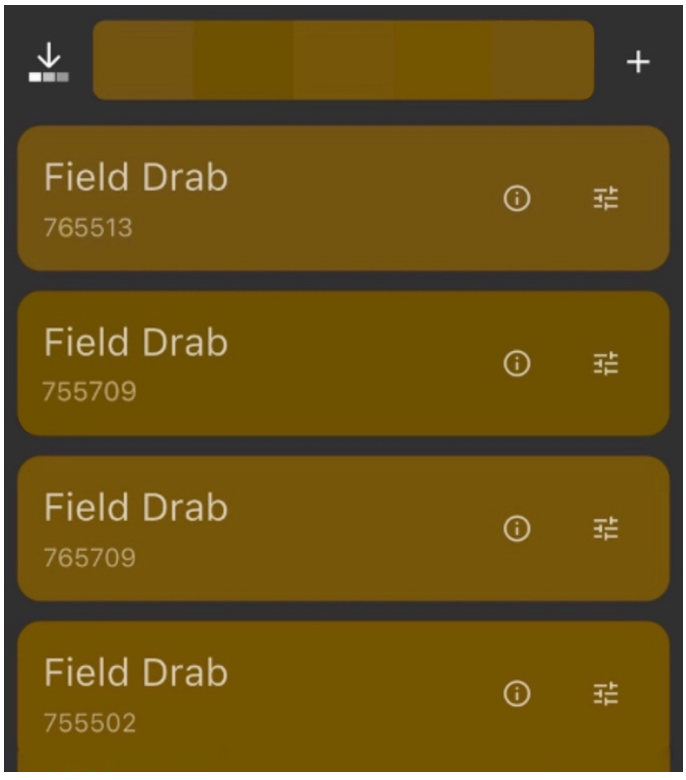


Figure A3*. Colour Picker AR endpoint readings across trials, where endpoint was accepted when the measured colour fell within a predifined hex/RGB range (*hex #755502 - #765709; RGB: R 117-118, G 85-87, B 2-9*).*

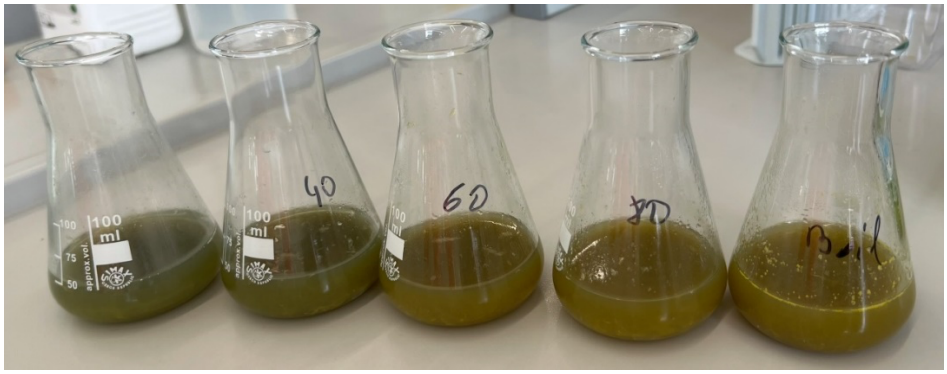


Figure A4. *Orange juice samples (20℃ – 90℃) after the titration and endpoint identification using the Colour Picker AR app.*

Apendix B

Statistical Test Outputs

One-Way Anova Test

Temperature (°C)	Count	Mean vitamin C (mg/100 cm^3)	Variance
20.00	5	50.24	$5.63 \cdot 10^{-3}$
40.00	5	49.50	$3.46 \cdot 10^{-3}$
60.00	5	48.97	$2.50 \cdot 10^{-3}$
80.00	5	48.58	$2.55 \cdot 10^{-3}$
90.00	5	47.70	$2.88 \cdot 10^{-3}$

Table B1. *Summary statistics for vitamin C concentration (mg/100 cm^3) in orange juice at different heating temperatures using stovetop heating.*

Temperature (°C)	Count	Mean vitamin C (mg/100 cm^3)	Variance
20.00	5	51.70	$3.25 \cdot 10^{-3}$
40.00	5	50.32	$3.88 \cdot 10^{-3}$
60.00	5	49.96	$2.50 \cdot 10^{-3}$
80.00	5	48.80	$3.25 \cdot 10^{-3}$

90.00	5	47.76	$6.25 \cdot 10^{-3}$

Table B2. *Summary statistics for vitamin C concentration (mg/100 cm³) in orange juice at different heating temperatures using microwave heating.*

Source of variation	SS	DF	MS	F statistic	p-value
Between groups	2.9022	4	0.7255	194.204	$1.11 \cdot 10^{-15}$
Within groups	0.07472	20	0.003736	-	-
Total	2.9769	24	-	-	-

Table B3. *One-Way ANOVA test results for the effect of heating temperature on vitamin C concentration in orange juice; stovetop heating.*

Source of variation	SS	DF	MS	F statistic	p-value
Between groups	6.0266	4	1.5066	424.4084	0
Within groups	0.071	20	0.00355	-	-
Total	6.0976	24	-	-	-

Table B4. *One-Way ANOVA test results for the effect of heating temperature on vitamin C concentration in orange juice; microwave heating.*

Kruskal-Wallis test

Parameter	Value for stove-top heating	Value for microwave heating
Groups (temperatures, °C)	20 (control); 40; 60; 80; 90	20 (control); 40; 60; 80; 90
n per group	5	5
Test statistic H	22.9401	23.1214
Degrees of freedom (df)	4	4
p-value	$1.3 \cdot 10^{-4}$	$1.2 \cdot 10^{-4}$
Effect size η^2	0.95	0.96

Table B5. *Kruskal-Wallis test for the effect of heating temperature on vitamin C concentration in orange juice using stovetop and microwave heating.*

Two-Way Anova Test

Source	**df**	**SS**	**MS**	**F Statistic (df_1, df_2)**	**p-value**
Factor A – heating method (stove-top vs microwave)	1	0.8505	0.8505	235.677 (1, 40)	$p < 1 \cdot 10^{-15}$
Factor B – temperature (20, 40, 60, 80, 90 °C)	4	8.5682	2.142	593.5917 (4, 40)	$p < 1 \cdot 10^{-16}$
Interaction $A \cdot B$ – method · temperature	4	0.3512	0.0878	24.3306 (4, 40)	$p = 2.9 \cdot 10^{-10}$
Error	40	0.1443	$3.6 \cdot 10^{-3}$	-	-
Total	49	9.9142	0.2023	-	-

Table B6. *Two-way ANOVA results for the effect of heating method and temperature on vitamin C concentration in orange juice.*