IB Higher Level Extended Essay in Chemistry:

Investigating the effect of heat treatment on lycopene stability and extractability in tomatoes

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1. Introduction

Lycopene is one of the most abundant antioxidants and is the pigment that gives tomatoes their red colour. It reduces oxidative stress and limits cellular damage through free-radical quenching mechanisms (Rowles et al., 2021). Additionally, tomatoes are one of the most popular crops in the world that are high in phytochemicals, such as carotenoids and polyphenols, which have been linked to health benefits (Marti, Sanchez, & Garcia-Martinez, 2016). Within these compounds, the carotenoid lycopene is remarkable for its strong antioxidant properties, linked to its long chain of conjugated double bonds, which allow efficient singlet oxygen quenching (Perkins-Veazie & Collins, 2017). High dietary lycopene consumption has been proven to lower risks of cardiovascular disorders and several cancers, highlighting its nutritional and biomedical significance (Rowles et al., 2021).

Moderate heating favours isomerisation of all-trans lycopene to cis-isomers, which are more extractable (Cooperstone et al., 2015) and simplifies release from the tomato matrix through cell wall softening (Kessy et al., 2011). However, increasing temperatures can lead to oxidative degradation and a decrease in antioxidant activity (Abdullahi et al., 2020). Few studies have examined the effects of regulated short duration heating over specific temperature ranges (Kessy et al., 2011; Abdullahi et al., 2020), and even fewer have compared tomato cultivars with different pulp qualities (Ilahy et al., 2011; Gorecka et al., 2020).

Thus, this investigation examines how 30-minute heat treatments at 50.0 °C, 80.0 °C, 100.0 °C, and 120.0 °C (± 0.1 °C) affect the concentration of extractable lycopene (mg per 100 g of pulp) in round and plum tomatoes, using spectrophotometry at 503 nm (Poojary & Passamonti, 2015). This is relevant for both household cooking and food production, because such knowledge can inform about preparation methods that maximise lycopene intake from tomato-based foods.

It is hypothesised that round tomatoes, with their denser pulp, will thoroughly result in higher lycopene concentrations than plum tomatoes, and that moderate heating (e.g., around 80 °C) will increase extractable lycopene resulting from improved cell disruption and cis-isomer synthesis, whereas temperatures above that range will begin to breakdown lycopene. At temperatures between 100 °C and 120 °C lycopene levels are predicted to drop because the advantages of isomer production begin to be outweighed by oxidative and thermal degradation (Colle et al., 2010; Abdullahi et al., 2020).

1.1 Research Question

How does the concentration of extractible lycopene (mg per 100 g) in round tomatoes and plum-type tomatoes change following 30-minute heat treatments at 50.0 °C, 80.0 °C, 100.0 °C, and 120.0 °C (± 0.1 °C) as determined by spectrophotometric analysis at 503 nm?

2. Background Information

Lycopene, a cyclic carotenoid made up of a long polyene chain that contains eleven conjugated double bonds and two unconjugated double bonds. The extended π-electron cloud accounts for its intense red colour and high chemical reactivity. In addition, the concentration of lycopene and its antioxidant capacity are decreased at high temperatures due to polyene chain breakdown and the formation of oxidation products. Because lycopene is greatly lipophilic and basically insoluble in water, it partitions into lipid phases and non-polar extraction solvents, implying that both the tomato matrix and processing conditions have a high influence on how much lycopene can be extracted from tomato tissues (Gorecka et al., 2020).

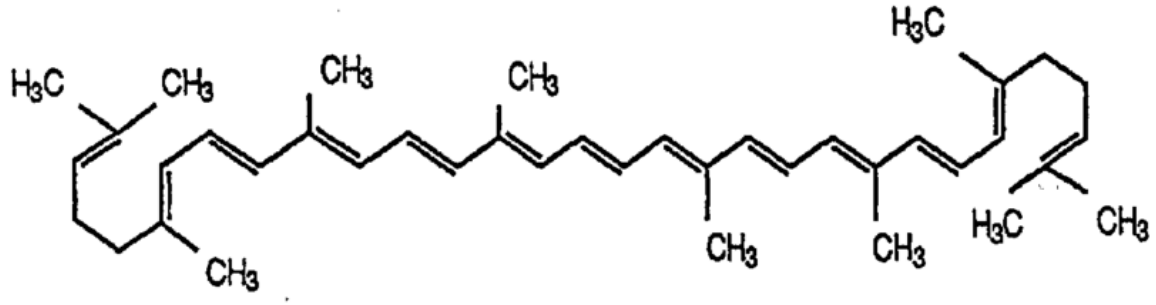


Figure 1. *Molecular structure of lycopene ($C_{40}H_{56}$), a linear polyene hydrocarbon responsible for the red pigmentation of tomatoes (adapted from Kumar et al., 2017).*

2.1 Isomerisation and extractability

The all-trans configuration is the most common type of lycopene in fresh tomatoes, which is thermodynamically stable but has low extractability because of its poor solubility in lipid membranes. Heat treatment causes cis-trans isomerisation, that results in cis-isomers which are easier for the human digestive tract to absorb (Cooperstone et al., 2015). Additionally, heating softens cell walls, also facilitates lycopene release from the tomato matrix, increasing its extractability. However, extended heating leads to oxidative degradation, decreasing both lycopene concentration and antioxidant activity (Colle et al., 2010).

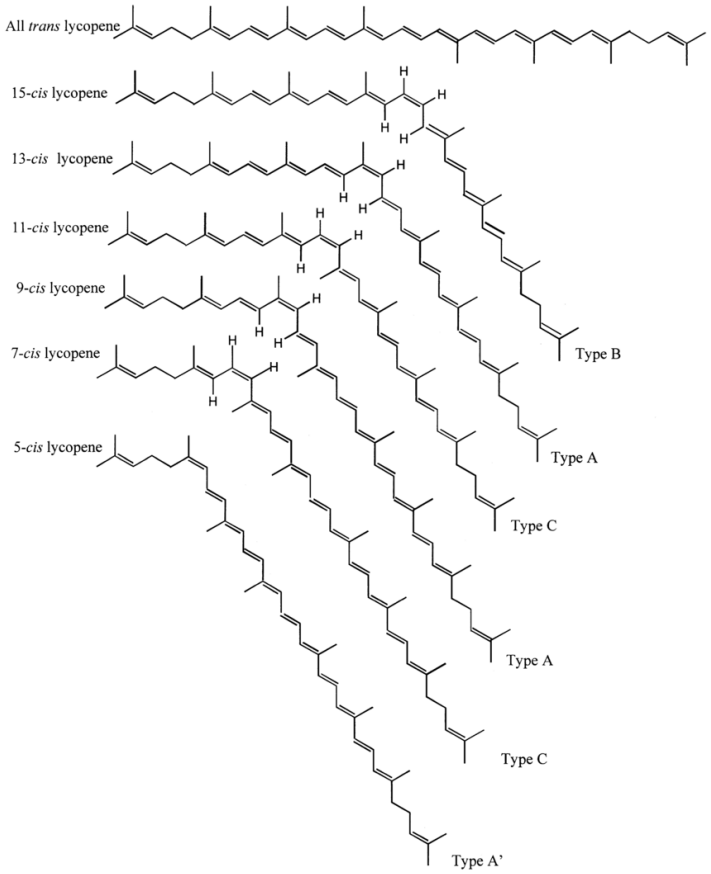


Figure 2. *Structural isomers of lycopene, including all-trans and cis-forms (5-cis, 7-cis, 9-cis, 11-cis, 13-cis, 15-cis). These isomers differ in the spatial arrangement around their conjugated carbon-carbon double bonds (adapted from Chasse et al., 2001).*

2.2 Varietal differences in tomato pulp

Physical characteristics like pulp viscosity, pigment density and water content affect solvent penetration and release of carotenoids (Ilahy et al. 2011; Knockaert et al., 2012). Round tomatoes, with their denser, more gelatinous pulp and orange-red pigmentation, retain lycopene differently compared to plum tomatoes, which have lighter red pigmentation, higher water content and lower structural density. These types of varietal differences in prior research have been shown to impact heat stability and antioxidant recovery (Gorecka et al., 2020).

2.3 Quantification methods

The concentration of lycopene was measured using a spectrophotometric technique that follows the Beer-Lambert law, which describes the relationship between absorbance and solute concentration:

$$A = \varepsilon cl$$

Where A is the absorbance, ε the molar extinction coefficient ($L\ mol^{-1}cm^{-1}$), c the concentration ($mol\ L^{-1}$) and the path length of the cuvette (cm) (Atkins & de Paula, 2014).

For this investigation, a monochromator built into the spectrophotometer was used to isolate light at 503 nm, this represents lycopene's maximum absorption wavelength. Light was passed through a cuvette, which contained the lycopene extract in hexane-acetone-ethanol solvent. There the apparatus measured the incident light (I_0) intensity entering and the transmitted light (I) intensity exiting the sample. Absorbance is a logarithmic measure of light attenuation that indicates how much light beam is reduced in intensity as it travels through the sample and calculated as $A = \log_{10}(I_0/I)$ (Atkins & de Paula, 2014).

Since absorbance is directly proportional to concentration, this relationship made it possible to calculate the amount of lycopene in hexane using Beer-Lambert law and the literature molar extinction coefficient $\varepsilon = 172,000\ L\ mol^{-1}cm^{-1}$ for lycopene (Poojary & Passamonti, 2015). The gotten concentrations were converted to mg of lycopene per 100 g of tomato pulp using the molar mass of lycopene ($536.9\ g\ mol^{-1}$).

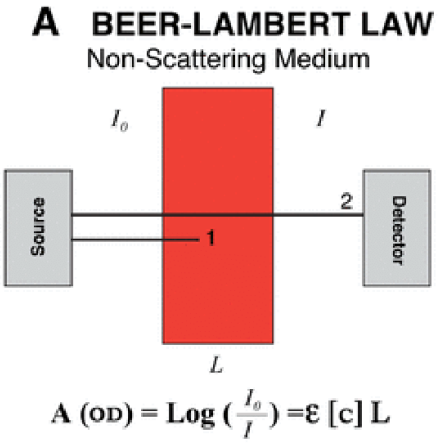


Figure 3. *Schematic presentation of the Beer-Lambert law showing the passage of monochromatic light through an absorbing solution (adapted from Barstow, 2019).*

The Beer-Lambert law uses published molar extinction coefficients (ε = 172,000 $L \cdot mol^{-1} \cdot cm^{-1}$ in hexane) to convert absorbance values into concentrations (Atkins & de Paula, 2014). Lycopene exhibits three peak levels of absorbance at 444 nm, 471 nm, and 503 nm, of which 503 nm is the best option for the measurement because of minimal overlap with β-carotene, that absorbs more strongly at shorter wavelengths (Poojary & Passamonti, 2015).

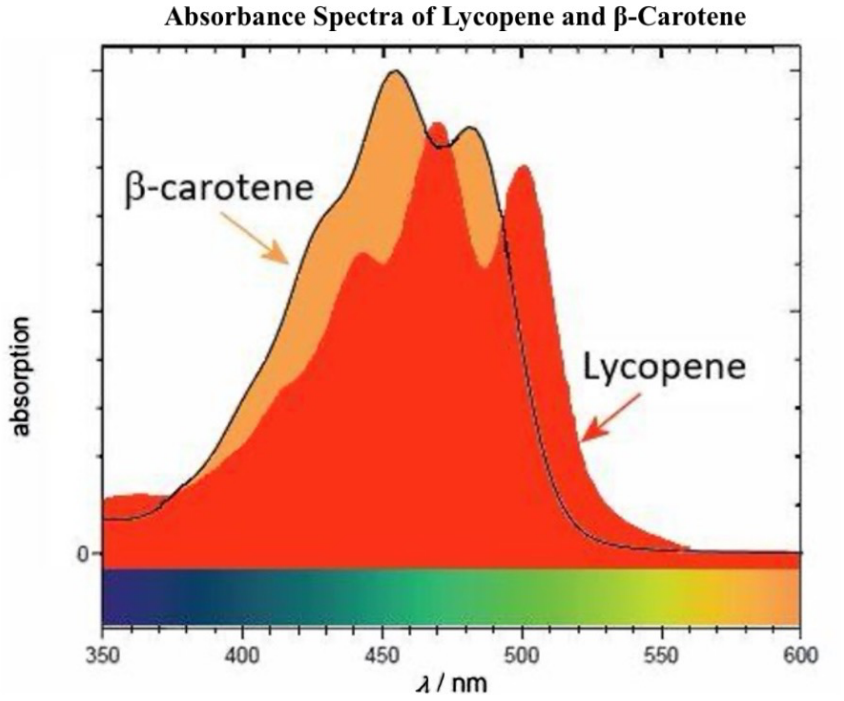


Figure 4. *Absorption spectra of lycopene and β-carotene, showing three characteristic maxima at 444, 471 and 503 nm, with lycopene's peak at 503 nm selected for spectrophotometric quantification (reprinted from "Lycopene: Molecule of the Month" School of Chemistry, University of Bristol).*

3. Variables

Independent variable	Temperature of heat treatment (°C)	Range: 50.0 °C, 80.0 °C, 100.0 °C, 120.0 °C (±0.1 °C). Each tomato pulp sample was heated in a temperature-stable oven for 30 minutes. Based on research (Knockaert et al., 2012) the temperatures were chosen, which suggested increasing lycopene extractability and gradual trans-to-cis isomerization with rising temperature, up to a point where oxidative degradation becomes significant.
Dependent variable	Extracted lycopene concentration (mg per 100 g)	Lycopene was extracted from each heated tomato pulp sample using a hexane:acetone:ethanol (2:1:1) solvent mixture that contained 0.05% BHT to limit oxidation. After phase separation, the upper hexane layer, containing dissolved lycopene, was analyzed using a UV-visible spectrophotometer at 503 nm (lycopene's absorption maximum in hexane). Absorbance values were converted to concentration using the Beer-Lambert Law (ε = 172,000 $L \cdot mol^{-1} \cdot cm^{-1}$; M = 536.9 g mol^{-1}). The results were expressed as milligrams of measured lycopene per 100 grams of tomato pulp, making them directly comparable.

Table 1. *The independent and dependent variables of the experiment.*

Controlled variable	Method of control	Reason for control
Sunlight exposure	Samples were prepared and heated in a dimly lit environment, avoiding direct sunlight.	Sunlight can photooxidise lycopene, reducing its concentration before analysis.
Tomato ripeness	Only tomatoes of similar ripeness and colour were selected for all trials.	Differences in ripeness change initial lycopene concentration, leading to inconsistent results.
Sample mass	10.000 g of tomato pulp was measured using an analytical balance (±0.001 g).	Changes in sample size would affect solvent-to-sample ratio and extraction efficiency.
Solvent volumes	Constant volumes of solvents were added for each extraction: 5.00 cm^3 BHT-acetone, 5.00 cm^3 ethanol, 10.00 cm^3 hexane, measured with graduated pipettes.	Inconsistent solvent volumes could affect lycopene extraction efficiency and concentration measurement.
Spectrophotometer settings	All absorbance readings were taken at 503 nm using a 1 cm cuvette, with hexane as the blank.	Different wavelengths or cuvette path lengths could lead to inaccurate absorbance and concentration values.

Table 2. *The controlled variables of the experiment, including the method and reason of control.*

4. Methodology

4.1 Materials

Glassware	Equipment	Chemicals
25.00 cm^3 (± 0.05 cm^3) measuring cylinder; 100.00 cm^3 (± 0.08 cm^3) volumetric flask; 250 cm^3 conical flasks; 10.00 cm^3 (± 0.05 cm^3) graduated pipette; 5.00 cm^3 (± 0.05 cm^3) graduated pipette; 100.0 cm^3 (± 0.5 cm^3) graduated cylinders; Cuvettes, 1 cm path length;	Analytical scale (± 0.001 g); Temperature stable oven (± 0.1 °C); Digital timer (± 1s); Thermometer (±0.30°C); Metal pot, 500 cm^3; Hand blender; Metal sieve; Aluminum foil roll; 1 dm^3 container for distilled water; Spectrophotometer "Vernier" (503 nm setting)	1 kg fresh tomatoes (round and plum varieties); Butylated Hydroxytoluene (BHT) powder; Acetone, 150 cm^3; 300 cm^3 of 99% hexane; 150 cm^3 of 95% ethanol; Distilled water

Table 3. *The glassware, equipment, and chemicals used in the experiment.*

4.2 Preliminary experiment

A preliminary experiment was done to test the viability of the process and find any possible problems. The goal was to determine whether heating in a conventional oven could maintain a steady temperature throughout the 30-minute treatment period and to confirm the effectiveness of lycopene extraction using organic solvents.

The sample was heated to 80.0 °C (± 0.1 °C) for 30 minutes, allowed to cool to the room temperature, and then mixed with ethanol, hexane, and a pre-made 0.05% BHT in acetone solution in the 2:1:1 ratio as stated in the literature. The composition of this solvent mixture

(hexane:acetone:ethanol in 2:1:1 ratio with 0.05% BHT) was adapted from Kumcuoglu, Yilmaz & Tavman (2014). Since no mechanical shaker was available at the school setting, all five trial flasks were placed on a chair and shaken manually in a consistent motion. After settling, a noticeable phase separation was seen. The upper hexane layer showed an apparent reddish color, confirming the presence of lycopene and thus this indicated that the chosen solvent system effectively extracted the pigment from the tomato matrix.

4.3 Experimental design

Tomato pulp preparation and heat treatment

Fresh plum (*Solanum lycopersicum*, Roma) and round tomatoes (*Solanum lycopersicum*; Adas) were purchased from a local supermarket, yet the exact cultivars were not labelled, so here they are treated as two generic types.

1. To create a homogenous pulp, fresh round and plum tomatoes were washed thoroughly with distilled water, peeled, deseeded and blended using a hand blender.
2. Using an electronic scale (±0.001 g), 10.000 g of pulp was weighed into a clean 250 cm^3 glass flask.
3. Each flask was labelled according to the trial temperature: control (unheated), 50.0 °C, 80.0°C, 100.0 °C, and 120.0 °C. For each temperature and tomato type, five trials were prepared (n = 5).
4. Samples were heated in a preheated oven set to the wanted temperature for 30 minutes. The oven had a built-in thermostat with (±0.1°C).
5. After heating, flasks were allowed to cool to room temperature (20.00 °C ±0.30 °C) before extraction.

Preparation of BHT solution and lycopene extraction

1. A 0.05% BHT solution was prepared by dissolving 0.050 g ± 0.001g of Butylated Hydroxytoluene (BHT) in 100.0 cm^3 ± 0.5 cm^3 of acetone (99%). The solution was refrigerated and protected from light using aluminium foil.

2. To the 10.00 g tomato pulp sample in each 250 cm^3 flask, the following reagents were added in this order:

   5.00 cm^3 ± 0.10 cm^3 of 0.05% BHT in acetone;

   5.00 cm^3 ± 0.10 cm^3 of 95% ethanol;

   10.00 cm^3 ± 0.10 cm^3 of 99% hexane;

3. Flasks were wrapped in aluminium foil immediately to avoid light-induced lycopene degradation.

4. Manual shaking was done for 30 minutes to ensure optimal lycopene extraction into the non-polar hexane layer and stabilization by BHT.

Phase separation and lycopene isolation

1. After shaking, 3.00 cm^3 ± 0.05 cm^3 of distilled water was added to each flask to facilitate phase separation.

2. The flasks were swirled and left undisturbed for 5 minutes.

3. Once a clear separation between the hexane layer (top) and aqueous pulp layer (bottom) was visible, the reddish hexane layer containing lycopene was carefully extracted using a glass pipette.

4. The hexane extract was transferred into a labelled spectrophotometry cuvette for absorbance measurements at 503 nm.

4.4 Risk assessment

Material name and chemical formula	Associated risks	Protective measures taken
Butylated hydroxytoluene (BHT), $C_{15}H_{24}O$	Very toxic to aquatic life with long-lasting effects; may cause mild skin/eye irritation. 	Wear gloves, lab coat, goggles. Avoid skin and eye contact; prevent release into the environment; dispose of according to hazardous waste protocols.
Acetone, C_3H_6O	Highly flammable liquid and vapor. Causes serious eye irritation, may cause drowsiness or dizziness. 	Wear gloves, lab coat, goggles. Ensure good ventilation, keep away from heat, sparks, open flames. Avoid inhalation and eye contact.
Hexane, C_6H_{14}	Highly flammable liquid and vapor. Causes skin irritation, may cause drowsiness or dizziness. Causes damage to organs through prolonged exposure. Toxic to aquatic life with long-lasting effects. 	Wear gloves, lab coat, goggles. Work in a fume hood or well-ventilated area. Avoid inhalation, ingestion, skin contact. Dispose of as hazardous waste

Ethanol, C_2H_5OH	Highly flammable liquid and vapor. Causes serious eye irritation. 	Wear gloves, lab coat, goggles, avoid ingestion and eye contact. Ensure ventilation. Keep away from heat, sparks, flames.

Table 4*. The risk assessment of all chemicals employed in the experiment, listing the associated risks and protective measures taken (adapted from PubChem, National Institutes of Health (NIH)).*

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.

5. Results and analysis

5.1 Calculations

Outlier identification

Potential outliers in the absorbance dataset were identified using the Grubbs test at a 95% confidence level. This statistical method was selected because it is well-suited for the small sample sizes (n = 5 trials per condition) conducted in this investigation, and tests whether the most extreme value in a dataset is significantly different from the others.

The null hypothesis (H_0) stated that no outlier was present, while the alternative hypothesis (H_1) stated that at least one outlier existed. The Grubbs test statistic is calculated as:

$$G = \frac{|suspected\ outlier - \bar{x}|}{s}$$

Where $\bar{x}$ is the sample mean and s is the standard deviation and was compared to the critical value G_{table} from published Grubbs tables for n = 5 at a 95% confidence level. If

$G_{calculated} > G_{table}$, the null hypothesis was rejected and the value was excluded from further analysis.

Example calculation for the control variables, plum tomato condition:

Absorbance readings (A_{503}) = {0.605, 0.596, 0.594, 0.571, 0.586}

Mean:

$$\bar{A} = \frac{0.605 + 0.596 + 0.594 + 0.571 + 0.586}{5} = \frac{2.952}{5} = 0.5904$$

Standard deviation:

$$s = \sqrt{\frac{\sum(A_i - \bar{A})^2}{n-1}} = \sqrt{\frac{(0.0146)^2 + (0.0056)^2 + (-0.0194)^2 + (0.0036)^2 + (-0.0044)^2}{4}}$$

$$= \sqrt{\frac{0.00021316 + 0.00003136 + 0.00037636 + 0.00001296 + 0.00001936}{4}}$$

$$= \sqrt{\frac{0.0006532}{4}} = \sqrt{0.0001633} = 0.01278$$

Test statistic, suspected outlier = 0.571, the lowest value:

$$G = \frac{|0.571 - 0.5904|}{0.01278} = \frac{0.0194}{0.01278} = 1.52$$

For n = 5 at 95% confidence, $G_{table} \approx 1.715$

Since $G_{calc} = 1.52 < 1.715$, no outlier is removed, all five values are retained.

Converting absorbance to molar concentration (Beer-Lambert):

The Beer Lambert Law relates the absorbance of a solution to the concentration of the absorbing species. For lycopene in hexane, the maximum absorbance occurs at 503 nm due to its extensive conjugated π-electron system, which allows for a direct and proportional

relationship between absorbance and concentration when path length and molar absorptivity are constant.

$$A = \varepsilon\, b\, c \;\rightarrow c = \frac{A}{\varepsilon\, b}$$

A = measured absorbance;

ε = molar extinction coefficient, $1.72 \cdot 10^5\ L\ mol^{-1}cm^{-1}$ (lycopene in hexane);

b = path length of the cuvette, 1.00 cm;

c = molar concentration ($mol\ L^{-1}$) of lycopene.

Using the mean absorbance $\bar{A} = 0.5904$:

$$c = \frac{0.5904}{(1.72 \cdot 10^5)(1.00)} = 3.43 \cdot 10^{-6}\ mol\ L^{-1}$$

Convert to mg L^{-1} and then mg per 100 g pulp:

Mass concentration in mg L^{-1} is obtained by multiplying c by the molar mass (M) of lycopene and by 1000 to convert gram to milligrams:

M = molar mass of lycopene = 536.9 g mol^{-1}

$$C_{mg/L} = c \cdot M \cdot 1000$$

$$C_{mg/L} = (3.43 \cdot 10^{-6}) \cdot 536.9 \cdot 1000$$

$$C_{mg/L} = 1.84\ mg\ L^{-1}$$

Express per 100 g fresh pulp using total hexane extract volume $V = 0.010\ L$ and sample mass $m = 10.00\ g$:

$$Lycopene\ (mg/100\ g) = C_{mg/L} \cdot \frac{V}{m} \cdot 100$$

$$Lycopene = 1.84 \cdot \frac{0.010}{10.00} \cdot 100$$

$$Lycopene = 0.184\ mg\ per\ 100\ g$$

This calculation procedure was done to all tomato-types and temperature combinations to obtain lycopene concentrations in mg per 100 g of pulp.

Temperature °C (± 0.30)	Lycopene concentration in plum tomatoes (mg/100 g)					Lycopene concentration in round tomatoes (mg/100 g)				
	1st trial	2nd trial	3rd trial	4th trial	5th trial	1st trial	2nd trial	3rd trial	4th trial	5th trial
20.00 (control)	0.205	0.201	0.193	0.200	0.198	0.043	0.038	0.041	0.036	0.040
50.00	0.259	0.264	0.267	0.248	0.260	0.076	0.087	0.086	0.085	0.088
80.00	0.465	0.469	0.452	0.441	0.463	0.143	0.139	0.089	0.145	0.137
100.00	0.470	0.471	0.473	0.473	0.476	0.178	0.203	0.187	0.186	0.176
120.00	0.499	0.488	0.510	0.495	0.508	0.214	0.209	0.217	0.214	0.215

Table 5. *Lycopene concentration in plum and round tomatoes at different heating temperatures. Values are expressed in mg lycopene per 100 g of tomato pulp, calculated from absorbance readings at 503 nm using the Beer-Lambert Law. Each value represents an individual trial (n = 5 per condition).*

Table 5 shows potential outliers which were identified for each condition using the Grubbs test at a 95% confidence level and highlighted in the dataset (shaded orange cells). Outliers were excluded prior to calculation of mean lycopene concentrations and uncertainties.

Example calculation of mean lycopene concentration (after outlier removal):

Round tomatoes, 50.0 °C (n = after outlier removal). Remaining data (mg/100 g) includes 0.087, 0.086, 0.085, 0.088:

$$\bar{x} = \frac{sum\ of\ values}{number\ of\ values}$$

$$\bar{x} = \frac{0.087 + 0.086 + 0.085 + 0.088}{4}$$

$$\bar{x} = \frac{0.346}{4} = 0.0865\ mg/100\ g$$

The same method was applied to all other conditions after outlier removal to obtain mean lycopene concentrations, which are reported in *Table 7 (Processed data)*.

One-Way ANOVA test

A one-way ANOVA (Analysis of Variance) test was used to determine if different heating temperatures have a statistically significant effect on the concentration of extractable lycopene in tomato pulp. Separate tests were done for plum and round tomatoes.

For plum tomatoes, the ANOVA had a p-value of $7.4 \cdot 10^{-25}$ which is less than the significance level. Similarly, for round tomatoes, the p-value is $2.1 \cdot 10^{-15}$. Therefore, the null hypothesis is rejected, indicating that increasing temperature correlates with increasing extractible lycopene concentration.

Kruskal-Wallis

This test was used to determine whether heating causes statistically significant differences in lycopene concentration across both groups - plum and round tomatoes as opposed to not heating (control). The p-value was obtained $1.2 \cdot 10^{-4}$, stating statistically significant differences between groups. Thus, this confirms that heating affects lycopene extractability in both tomato types.

Two-Way Anova Test

This test was used to determine whether tomato type, heating temperature and their interaction significantly affect lycopene concentration. Both tomato types ($p = 2.5 \cdot 10^{-47}$)

and temperature ($p = 4.2 \cdot 10^{-40}$) showed statistically significant effects and a significant interaction was observed ($p = 5.6 \cdot 10^{-23}$). This indicates that lycopene extractability depends on these two factors and that their effects are not independent. Heating had a stronger effect on lycopene concentration in plum tomatoes.

5.2 Calculation of measurement uncertainty

Point of instrument use	Measuring instrument	Absolute uncertainty (±AU)	Measured value	% Uncertainty (±PU)
Measuring tomato pulp	Analytical balance	±0.001 g	10.000 g	$\left(\frac{0.001}{10.000}\right) \cdot 100 = 0.01\%$
Preparation of the BHT solution	Analytical balance	±0.001 g	0.050 g	$\left(\frac{0.001}{0.050}\right) \cdot 100 = 2.0\%$
	Volumetric flask	±0.08 cm^3	100.00 cm^3	$\left(\frac{0.08}{100.00}\right) \cdot 100 = 0.08\%$
Samples extraction (BHT solution, hexane, ethanol, distilled water)	25.00 cm^3 graduated cylinder	±0.17 cm^3	5.00 cm^3	$\left(\frac{0.17}{5.00}\right) \cdot 100 = 3.4\%$
	25.00 cm^3 graduated cylinder	±0.17 cm^3	5.00 cm^3	$\left(\frac{0.17}{5.00}\right) \cdot 100 = 3.4\%$
	25.00 cm^3 graduated cylinder	±0.17 cm^3	10.00 cm^3	$\left(\frac{0.17}{10.00}\right) \cdot 100 = 1.7\%$
	10.00 pipette	±0.05 cm^3	3.00 cm^3	$\left(\frac{0.05}{3.00}\right) \cdot 100 = 1.7\%$

Time control during heating	Digital timer	±0.01 sec	30 min or 1800 seconds	$\left(\frac{0.01}{1800}\right) \cdot 100 = 0.0006\%$
Temperature measurement during heating	Built-in thermostat	±0.1 °C	100.0 °C	$\left(\frac{0.1}{100.0}\right) \cdot 100 = 0.1\%$
Transferring sample extract to cuvette	10.00 cm^3 pipette	±0.05 cm^3	5.00 cm^3	$\left(\frac{0.05}{5.00}\right) \cdot 100 = 1.0\%$
		Overall percentage uncertainty of measurement:		$\sum PU = 13.39\%$ $\approx 13.4\%$

Table 6. *Percentage uncertainty of measurement calculation, showing the largest percentage uncertainties of each measurement taken.*

The combined measurement uncertainty (13.4%) was treated as an approximate relative uncertainty for all trials. For example, for plum tomatoes at 20 °C, with a mean lycopene concentration of 0.199 mg/100 g, the propagated measurement uncertainty is:

$$\Delta c = 0.199 \cdot 0.134 = 0.0267 \approx 0.03\ mg/100\ g$$

The result can be expressed as 0.199 ± 0.03 mg/100 g.

5.3 Processed data

Temperature °C (± 0.30)	Plum tomatoes (mg/100 g, mean ±SD)	Round tomatoes (mg/100 g, mean ±SD)
20.00 (control)	0.199 ±0.004	0.040 ±0.003
50.00	0.260 ±0.007	0.087 ±0.002
80.00	0.458 ±0.011	0.141 ±0.004
100.00	0.473 ±0.002	0.186 ±0.011
120.00	0.500 ±0.009	0.214 ±0.003

Table 7. *Mean lycopene concentration (mg/100 g) in plum and round tomatoes at different heating temperatures. Uncertainties represent standard deviation.*

6. Discussion of results

6.1 Trends in lycopene concentration

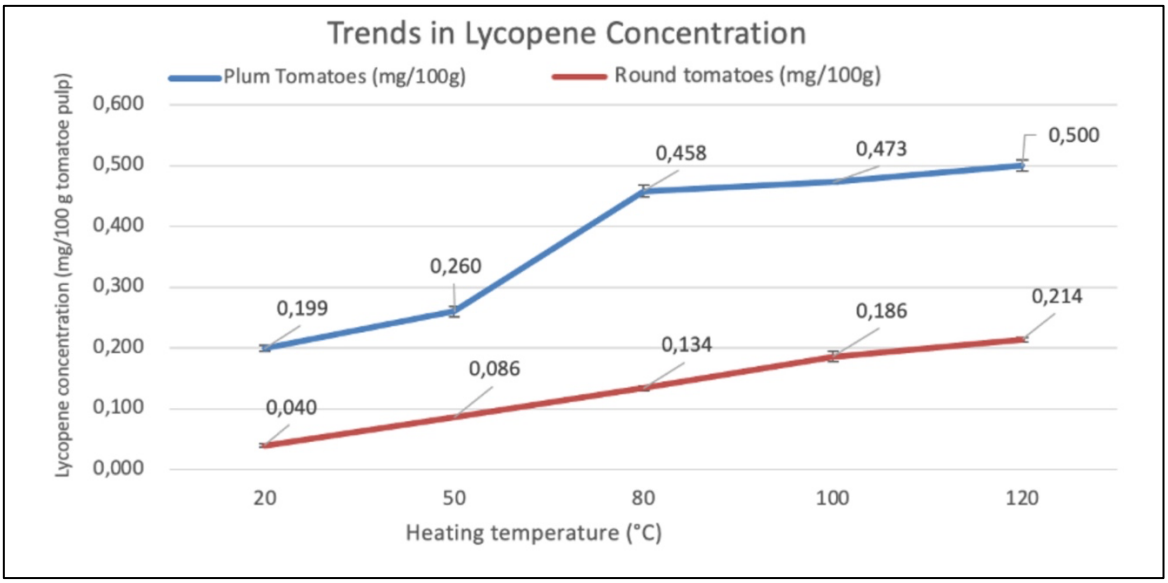


Figure 5. *Trends in lycopene concentration (mg/100 g) in round and plum tomatoes at increasing heating temperatures (20 ℃ -120 °C).*

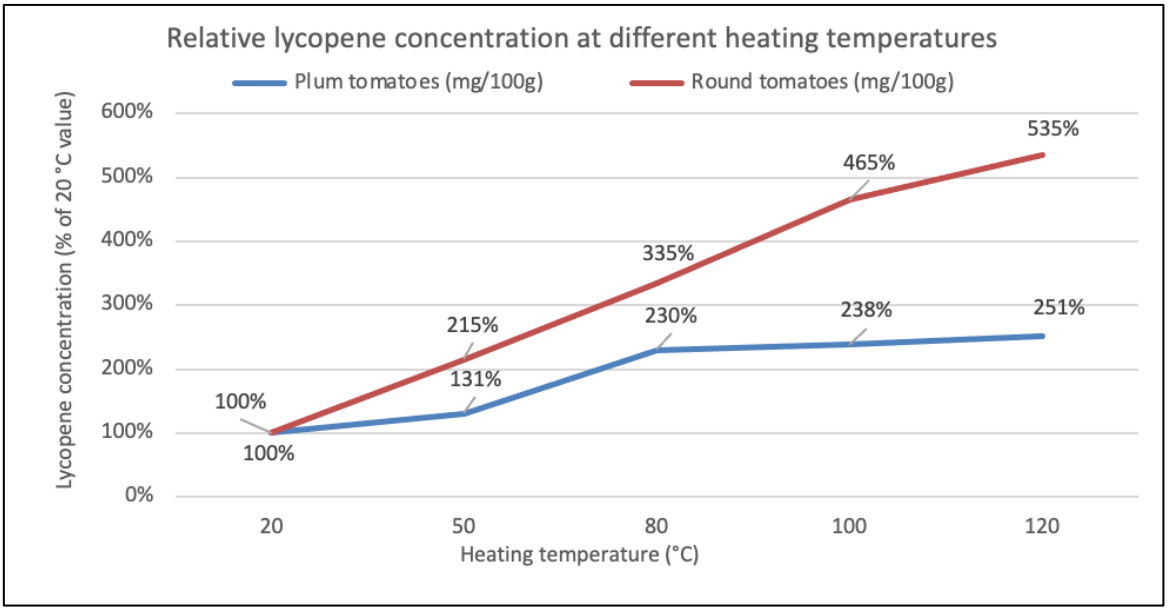


Figure 6. *Relative lycopene concentration (% of 20 ℃ value) in plum and round tomatoes at different temperatures (20 ℃ – 120 ℃). Values are expressed as a percentage of the mean concentration at 20 ℃ for each cultivar.*

Relative values were calculated using:

$$Relative\ concentration\ (\%) = \frac{C_T}{C_{20\,℃}} \cdot 100$$

where:

C_T = mean concentration at temperature (T);

$C_{20\,℃}$ = mean concentration at 20 °C for that cultivar

For exaple (plum, 50 ℃):

$$\frac{0.260}{0.199} \cdot 100 = 131\%$$

Figure 5 shows how lycopene concentration in round and plum tomatoes changes as heating temperatures increase (20 °C - 120 °C). In both cultivars, lycopene concentration rose with temperature, supporting the idea that moderate heating enhances extractability through cell wall softening and cis-isomer formation. Plum tomatoes consistently contained higher concentrations than round tomatoes across all treatments, beginning at 0.199 mg/100 g at 20 °C and rising to 0.500 mg/100 g at 120 °C, whereas round tomatoes started at 0.040 mg/100 g

and reached to 0.214 mg/100 g under the same circumstances. Yet, varietal differences in pulp structure and water content likely explain this pattern: the more watery texture of plum tomatoes allowed for solvent penetration and lycopene diffusion during extraction, whereas the denser, more gelatinous pulp of round tomatoes may have retained lycopene, limiting its transfer into the hexane phase. Notably, lycopene levels in both tomato types continued to increase across the full temperature range (20 °C -120 °C), with gains of +0.301 mg/100 g (251%) in plum tomatoes and +0.174 mg/100 g (535%) in round tomatoes, although the increase was smaller between 100 °C and 120 °C, suggesting that higher temperatures slowed the rate of increase. Despite lower absolute values, the much higher percentage gain in round tomatoes sindicates that once their denser matrix was effectively disrupted by heating, lycopene became available more rapidly rather than in plum tomatoes. Overall, the data indicate that temperature strongly influences lycopene availability; nevertheless, varietal differences remain a key factor determining the magnitude of this effect.

7. Conclusion

This investigation examined how thermal treatment at 50.0 °C, 80.0 °C, 100.0 °C, and 120.0 °C (± 0.1 °C) influences the concentration of extractable lycopene (mg per 100 g of pulp) in round and plum tomatoes, as determined spectrophotometrically at 503 nm.

The results showed a clear temperature-dependent trend: lycopene concentrations increased steadily from 20 °C to 120 °. This supports the prediction that moderate heating enhances lycopene extractability due to cis-isomer formation and cell wall disruption. Unlike some studies reporting a decline at very high temperatures, lycopene levels in this experiment did not decrease within the 30 min heating duration. But, the increase between 100 °C and 120 °C was a bit smaller, consistent with the onset of oxidative degradation without a net loss of lycopene (Colle et al., 2010; Abdullahi et al., 2020). Although round tomatoes had lower

absolute concentrations, they showed a larger percentage increase with temperature. Whereas, across all conditions, plum tomatoes displayed consistently higher lycopene levels than round tomatoes. Thus, the prediction that moderate heating would increase extractable lycopene was supported, but the hypothesis that round tomatoes would contain more lycopene than plum tomatoes and that lycopene would decline between 100 °C and 120 °C were not.

The results are consistent with existing literature, which reported increased lycopene extractability with moderate heat treatment followed by degradation at excessive temperatures (Abdullahi et al., 2020; Colle et al., 2010). Importantly, the observed varietal differences highlight how that not only temperature but also cultivar-specific characteristics such as water content, pulp viscosity and pigment density strongly determine lycopene outcomes. Nevertheless, the results ensure that short-term heating up to 120 °C of tomatoes can be used to enhance lycopene availability in both home cooking and manufacturing. Plum tomatoes remain superior, which implies that choosing cultivars with more hydrated pulp and higher baseline lycopene could enhance the nutritional value of tomatoe-based products without requiring major changes to recipes.

8. Evaluation

8.1 Limitations of the experimental procedure

8.1.1 Sources of random error

Several uncontrolled variables likely caused data scatter and decreased the accuracy of the final lycopene concentrations. Even though tomatoes were chosen to have similar ripeness and colour, slight variations in maturity and redness are expected to influence each sample's initial lycopene concentration, adding variability which is unrelated to heating conditions.

Since hexane, ethanol, acetone, and the BHT solutions were measured using graduated cylinders rather than volumetric equipment, minor variations in solvent volumes may have also impacted the consistency of extraction among trials.

Furthermore, some deterioration might have happened during transfer, despite wrapping flasks in foil. According to literature (Poojary & Passamonti, 2015) the inclusion of BHT (0.05% in acetone) helped to lessen this by slowing oxidative chain reactions and serving as a radical scavenger. However, a tiny amount of oxidative loss most likely contributed to the data's random distribution.

8.1.2 Major sources of systematic error

The absolute lycopene concentration measurements stated in this experiment might have been affected by systematic errors. At first, the oven's internal air temperature display was used to determine the heating process rather than measurements of the pulp itself. Given that thermal conductivity differs between watery plum tomatoes and the denser round tomatoes, the actual internal temperature of the samples may have been slightly overheated or underheated compared to the intended values. This introduces a bias that remains consistent throughout all trials.

Moreover, samples were heated in unsealed containers without stirring, which meant that heat distribution was not uniform. This could have resulted in temperature differences in denser round tomatoes pulp, causing partial degradation in hotter parts or partial isomerisation in cooler areas of the same sample.

Another systematic source of error was the possibility that lycopene was not fully incorporated into the hexane layer during the extraction process with only 30 minutes of shaking. Some lycopene may have been trapped in the pulp matrix, particularly in the harder round tomatoes samples, which led to a systematic underestimation of the true lycopene amount.

The extinction coefficient used for Beer-Lambert calculations is another cause of inaccuracy. This experiment used a mixed solvent system of hexane, acetone, and ethanol, although the

value applied ($\varepsilon = 172{,}000\ L \cdot mol^{-1} \cdot cm^{-1}$) is published for lycopene dissolved in pure hexane. According to Atkins & de Paula (2014) even slight variations in solvent polarity can change extinction coefficients or shift absorbance maxima, that the computed concentrations may have a systematic offset from their actual values.

Lastly, while BHT was added to stop oxidation and does not absorb at 503 nm, turbidity might have been brought on by partial phase separation or pulp remaining traces in the hexane layer. These particles' ability to scatter light may cause absorbance measurements to rise slightly overestimating the amount of lycopene present under all circumstances.

8.2 Impact of errors on conclusions

Since the rise was significantly larger than the estimated uncertainty (for instance, plum tomatoes increased by 0.301 mg/100 g, more than 20 times the ±0.010 margin), the overall pattern of increasing lycopene content from 20 °C to 120 °C should be considered reliable. It also suggests that lycopene release is improved by mild heating. In round tomatoes, the smaller increase between 80 °C and 100 °C indicates that lycopene release didn’t start declining, instead had a slower rate.

8.3 Improvements

To improve the reliability of this investigation, solvent volumes could be distributed using calibrated micropipettes. Additionally, instead of shaking by hand, a motorised shaker would provide a more complete transfer of lycopene into the non-polar solvent phase. To increase heating accuracy, a temperature sensor probe could be inserted straight into the pulp to measure the interior temperature, avoiding the need to make assumptions based on oven air temperature. At last, turbidity from suspended pulp remains would be removed by centrifuging extraction mixtures prior to solvent separation, lowering the possibility of light scattering and artificially high absorbance values.

8.4 Further research

Future research could examine how heating time at a constant temperature affects the balance between cis-trans isomerisation and oxidative degradation. Furthermore, using more sophisticated analytical techniques, like HPLC (Khoo et al., 2020), and comparing a larger variety of tomato cultivars as well as heating procedures could help ascertain whether structural differences or processing techniques play the dominant role in lycopene extractability.

9. Appendices

Appendix A

Raw absorbance readings

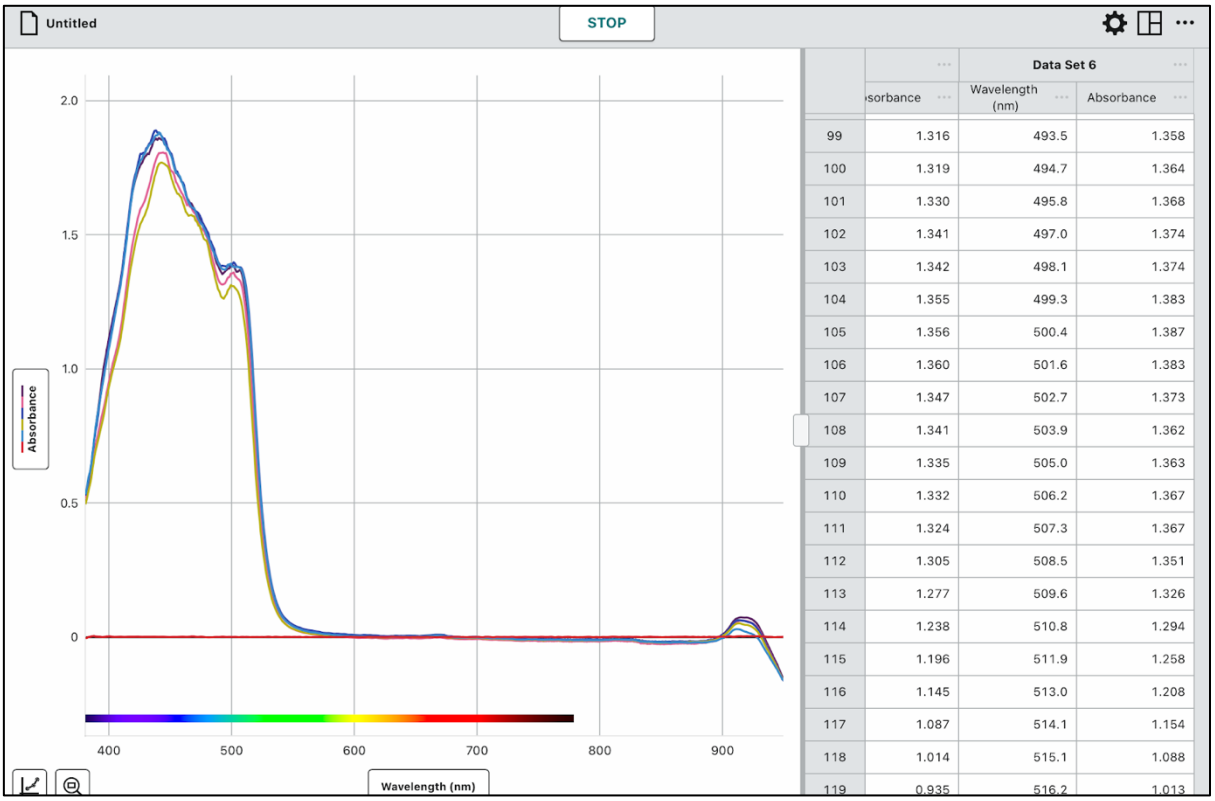
Temperature °C (± 0.30)	Absorbance of plum tomatoes					Absorbance of round tomatoes				
	1st trial	2nd trial	3rd trial	4th trial	5th trial	1st trial	2nd trial	3rd trial	4th trial	5th trial
20.00 (control)	0.656	0.643	0.618	0.640	0.634	0.138	0.122	0.131	0.115	0.128
50.00	0.829	0.845	0.854	0.794	0.832	0.243	0.278	0.275	0.272	0.282
80.00	1.488	1.502	1.446	1.411	1.481	0.458	0.445	0.285	0.464	0.438
100.00	1.504	1.507	1.510	1.510	1.521	0.570	0.650	0.598	0.595	0.563
120.00	1.597	1.561	1.632	1.584	1.625	0.685	0.669	0.694	0.685	0.688

Table A1. *Raw absorbance readings for plum and round tomatoes at different heating temperatures. Values are expressed in absorbance at 503 nm for each trial (n = 5).*

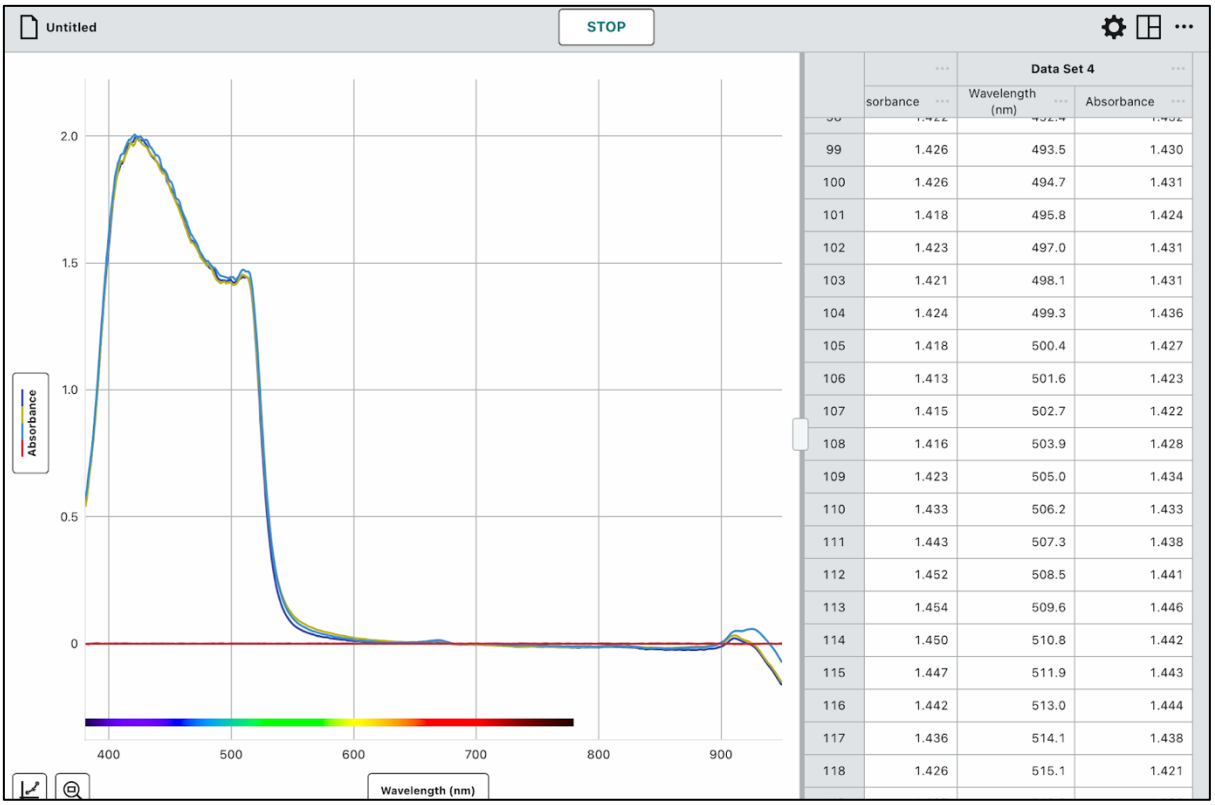
Appendix B

Spectrophotometer output

The following screenshots show representative spectrophotometric scans used to determine absorbance values at 503 nm for lycopene extracts. Each coloured line corresponds to one trial within the same treatment condition.



	Data Set 6		
	sorbance	Wavelength (nm)	Absorbance
99	1.316	493.5	1.358
100	1.319	494.7	1.364
101	1.330	495.8	1.368
102	1.341	497.0	1.374
103	1.342	498.1	1.374
104	1.355	499.3	1.383
105	1.356	500.4	1.387
106	1.360	501.6	1.383
107	1.347	502.7	1.373
108	1.341	503.9	1.362
109	1.335	505.0	1.363
110	1.332	506.2	1.367
111	1.324	507.3	1.367
112	1.305	508.5	1.351
113	1.277	509.6	1.326
114	1.238	510.8	1.294
115	1.196	511.9	1.258
116	1.145	513.0	1.208
117	1.087	514.1	1.154
118	1.014	515.1	1.088
119	0.935	516.2	1.013



	Data Set 4		
	sorbance	Wavelength (nm)	Absorbance
99	1.426	493.5	1.430
100	1.426	494.7	1.431
101	1.418	495.8	1.424
102	1.423	497.0	1.431
103	1.421	498.1	1.431
104	1.424	499.3	1.436
105	1.418	500.4	1.427
106	1.413	501.6	1.423
107	1.415	502.7	1.422
108	1.416	503.9	1.428
109	1.423	505.0	1.434
110	1.433	506.2	1.433
111	1.443	507.3	1.438
112	1.452	508.5	1.441
113	1.454	509.6	1.446
114	1.450	510.8	1.442
115	1.447	511.9	1.443
116	1.442	513.0	1.444
117	1.436	514.1	1.438
118	1.426	515.1	1.421

Appendix C

Experimental set-up



***Figure C1**. Conical flasks containing 10.000 g tomato pulp samples, wrapped in aluminium foil, labelled to minimize light exposure and put on the chair for mechanical shaking.*

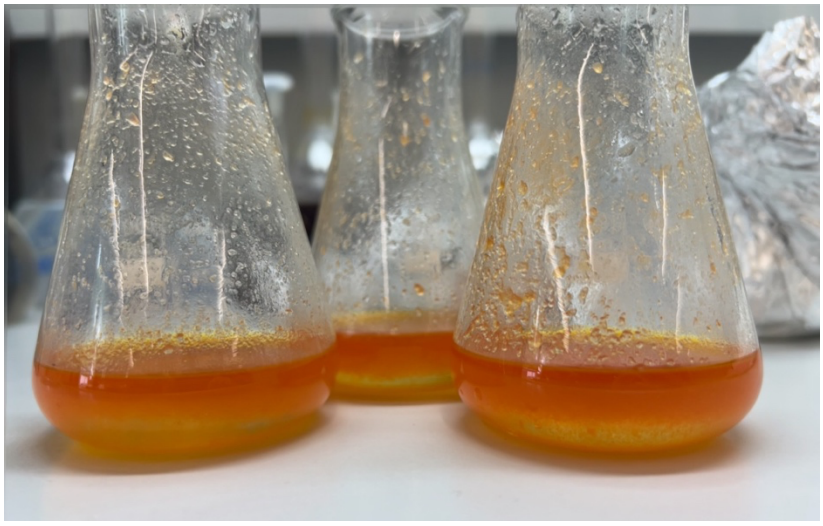


***Figure C2**. Tomato solvent mixtures after heating and extraction, showing a clear phase separation.*

Appendix D

Statistical Test Outputs

One-Way ANOVA Calculator

Temperature (°C)	Count	Mean lycopene (mg/100 g)	Variance
20	5	0.199	$1.93 \cdot 10^{-5}$
50	5	0.260	$5.23 \cdot 10^{-5}$
80	5	0.458	$1.30 \cdot 10^{-4}$
100	5	0.473	$5.30 \cdot 10^{-6}$
120	5	0.500	$8.35 \cdot 10^{-5}$

Table D1. *Summary statistics for lycopene concentration in plum tomatoes at different heating temperatures.*

Temperature (°C)	Count	Mean lycopene (mg/100 g)	Variance
20	5	0.040	$7.30 \cdot 10^{-6}$
50	5	0.084	$2.33 \cdot 10^{-5}$
80	5	0.131	$5.51 \cdot 10^{-}$
100	5	0.186	$1.14 \cdot 10^{-4}$
120	5	0.214	$8.70 \cdot 10^{-6}$

Table D2. *Summary statistics for lycopene concentration in round tomatoes at different heating temperatures.*

Source of Variation	SS	DF	MS	F statistic	P-value
Betweem Groups	0.3807	4	0.09519	1638.89	$7.4 \cdot 10^{-25}$
Within Groups	0.001162	20	0.00005808	-	-
Total	0.3819	24	-	-	-

Table D3. *One-Way ANOVA Table conducted by Microsoft Excel Data Analysis "Anova: Single Factor" Tool; plum tomatoes.*

Source of Variation	SS	DF	MS	F statistic	P-value
Betweem Groups	0.102	4	0.02551	181.2687	$2.1 \cdot 10^{-15}$
Within Groups	0.002814	20	0.00001407	-	-
Total	0.1048	24	-	-	-

Table D4. *One-Way ANOVA Table conducted by Microsoft Excel Data Analysis "Anova: Single Factor" Tool; round tomatoes.*

Kruskal-Wallis test

Parameter	Value for plum tomatoes	Value for round tomatoes
Groups (temperatures, °C)	20 (control); 50; 80; 100; 120	20 (control); 50; 80; 100; 120
n per group	5	5
Test statistic H	23.0858	23.0858
Degrees of freedom (df)	4	4
p-value	$1.2 \cdot 10^{-4}$	$1.2 \cdot 10^{-4}$
Effect size η^2	0.95	0.95

Table D5. *Kruskal-Wallis test for the effect of heating temperature on lycopene concentration in plum and round tomatoes.*

Two-Way Anova Test

Source	**df**	**SS**	**MS**	**F Statistic (df_1, df_2)**	**p-value**
Factor A – tomato type (plum vs round)	1	0.7629	0.7629	7674.64 (1, 40)	$2.5 \cdot 10^{-47}$
Factor B – temperature (20, 50, 80, 100, 120 °C)	4	0.4270	0.1067	1073.84 (4, 40)	$4.2 \cdot 10^{-40}$
Interaction $A \cdot B$ – type · temperature	4	0.05582	0.01396	140.39 (4, 40)	$5.6 \cdot 10^{-23}$

Error	40	0.00398	$9.94 \cdot 10^{-5}$	-	-
Total	49	1.2496	0.0255	-	-

Table D6. *Two-way ANOVA results for the effect of tomato type and heating temperature on lycopene concentration.*

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