The Extraction of β-Carotene from Orange Peels

What is the effect of temperature (22°C, 60°C, 90°C, 120°C, 150°C) on the concentration of β-carotene (ppm) extracted when orange peel powder is heated with ethanol under reflux for a retention time of 10 minutes?

Chemistry EE
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1. INTRODUCTION

The food industry generates millions of tons of orange peel waste (OPW) annually, 55 million tons of which is produced from orange juice production alone. A huge fraction of this OPW is unsustainably managed, creating a significant amount of problematic organic waste, which is landfilled or thrown into the ocean, resulting in dire consequences, including greenhouse gas emissions (Teigiserova et al., 2021). The classical methods of handling this waste, other than landfilling, are animal feeding, incineration, and ensiling. However, these approaches are not ideal, as they are costly and ineffective on a large scale. Instead, the most feasible method to address problems regarding waste management is using the organic waste, in this case, orange peels, to produce or extract valuable products, such as carotenoids. Because there is a remarkable upsurge in this approach (Mohsin et al., 2021), this essay focuses on the extraction of the carotenoid β-carotene from orange peels.

Although orange peels have various other beneficial constituents, such as flavonoids, pectin, and limonene (Mohsin et al., 2021), the carotenoid β-carotene was chosen due to its wide pharmaceutical applications as an antioxidant, which emphasise why its extraction is crucial. Since the purpose of this essay is to make the most out of the peels to reduce waste and the main factor affecting the concentration of β-carotene is heat, the research question driving this essay is: what is the effect of temperature (22°C, 60°C, 90°C 120°C, 150°C) on the concentration of β-carotene extracted by heating orange peel powder with ethanol under reflux for 10 minutes? Finding this relationship may aid in determining at what temperature the highest concentration of β-carotene can be extracted.

2. BACKGROUND INFORMATION

2.1. WHAT IS β-CAROTENE?

β-carotene is a red-orange pigment (Newman, 2017) found in yellow, orange and green leafy vegetables, and fruits (National Centre for Biotechnology Information, 2022). Because it is a precursor of vitamin A (converts into vitamin A in the human body), its appropriate consumption leads to healthy skin and mucus membranes, a strong immune system, as well as good eyesight. Moreover, β-carotene is an antioxidant, and hence, prevents other molecules from oxidizing; it defends the body against free radicals, which can damage cells, and eventually, cause chronic illnesses. As an antioxidant, β-carotene supplements may further prevent cognitive decline (Newman, 2017). In addition to being used as a nutrient supplement, it serves as a colour additive for food products and cosmetics (National Centre for Biotechnology Information, 2022).

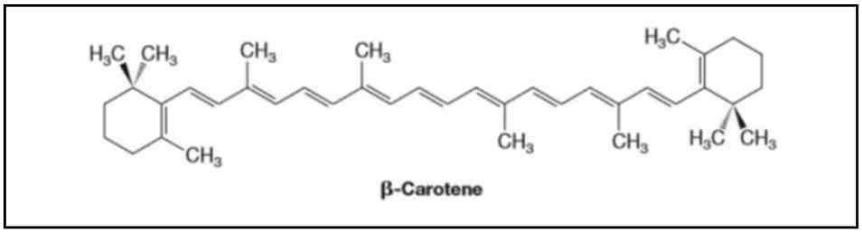


Figure 1: Chemical structure of β -carotene (CourseHero, n.d.)

The molecular formula of β-carotene is $C_{40}H_{56}$. As demonstrated by its structure depicted in Fig.1, its molecule consists of two retinyl groups (National Centre for Biotechnology Information, 2022) comprised of eight isoprene units and two β rings at the ends. It has a long polyene chain of conjugated double bonds, which causes its π electrons to delocalise, making the compound absorb light within the visible spectrum range (Pénicaud et al., 2011). β-carotene absorbs blue and purple light with peak absorbance at 455 nm, which is why it appears in red-orange colour (McMurry, 2008).

β-carotene is sensitive to heat and air. It degrades even at room temperature, producing various substances. Therefore, its concentration is affected as the temperature of extraction alters; with

higher temperatures, the extent and rate of degradation increase (Pénicaud et al., 2011) while the concentration decreases.

2.2. THE DEGRADATION OF β-CAROTENE

The general overview of β-carotene degradation is shown in Fig.2.

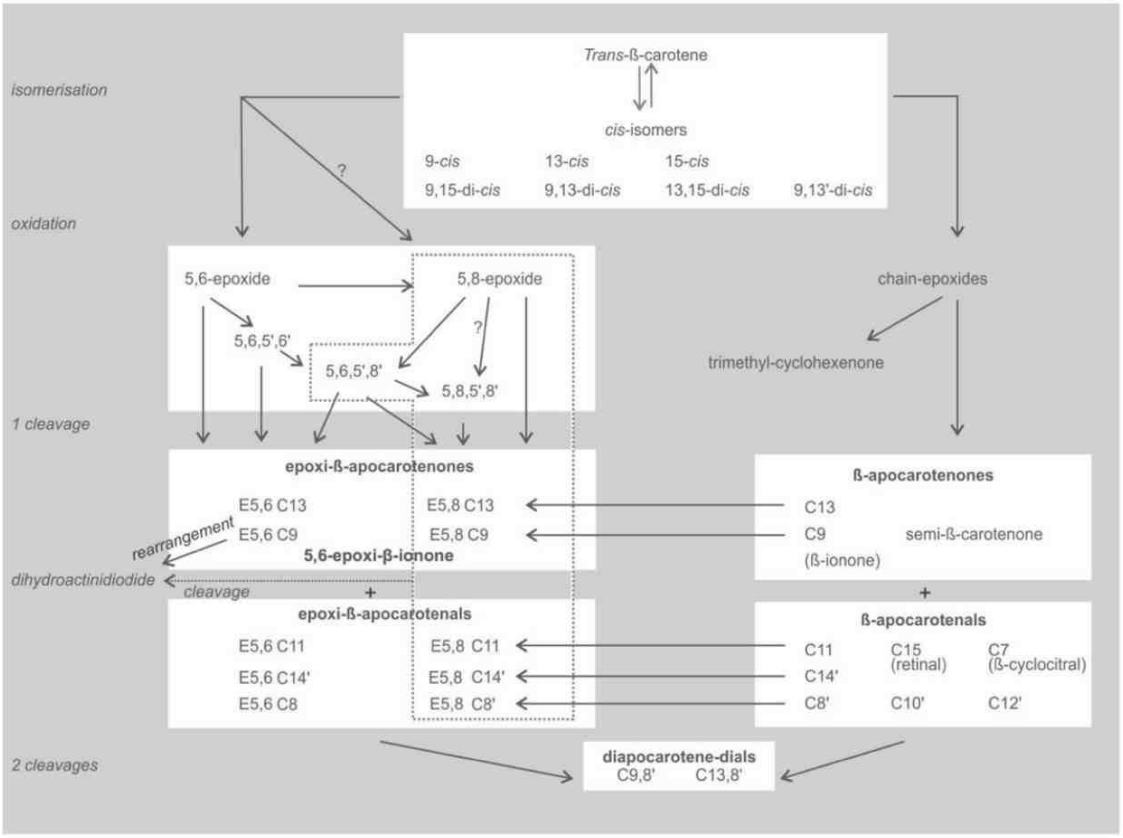


Figure 2: The general overview of the mechanism of β-carotene degradation (Pénicaud et al., 2011)

According to Fig.2, the first step of degradation is the isomerisation of all-*trans* to *cis* β-carotene. This step is a result of heat. As the *trans* isomer of this compound is predominant in nature and has greater antioxidant properties than the *cis* isomer (Pénicaud et al., 2011), this essay more specifically focuses on the extraction of all-*trans* β-carotene. These two isomers can be distinguished by their spectral properties; the *cis* isomer has a lower maximum absorbance wavelength (< 455 nm) than the all-*trans* one (Pénicaud et al., 2011).

Isomerisation is followed by oxidation (Pénicaud et al., 2011), which is shown in a more detailed and understandable manner in Fig.3.

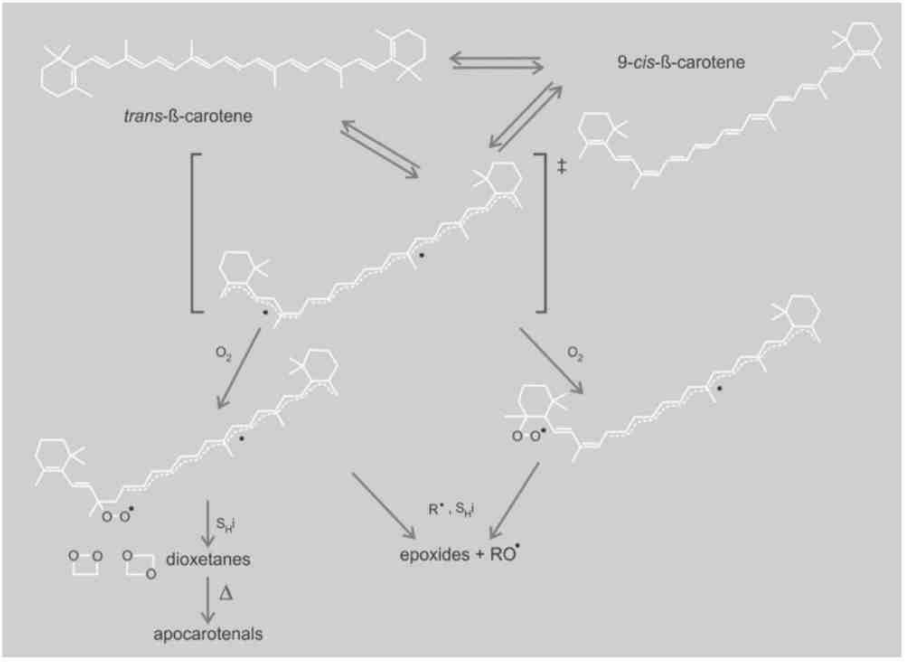


Figure 3: Isomerisation followed by oxidation of β -carotene (Pénicaud et al., 2011)

From Fig.3, it is noted that after isomerisation, a diradical of β-carotene is formed, which can easily be attacked by oxygen. Oxygen can either attack the β-ring or the chain of the diradical. If done on the β-ring, after a homolytic internal substitution, epoxides are produced as the initial products of oxidation. They are established from the *trans* isomer of β-carotene. The detailed formations of these epoxides are shown in Fig.4. On the other hand, if the radical attack is done on the chain of β-carotene, in-chain epoxides are formed, which are derived from the *cis* isomer instead of the *trans* one. The stable final products or the cleavage products of the degradation reaction include apocarotenones and apocarotenals, both of which are deduced from the generated epoxides, as remarked in Fig.4. If they are formed from the in-chain epoxides, they are called β-apocarotenals and β-apocarotenones. If produced by a second addition of oxygen on a 5,6-epoxide, they are called epoxy-β-apocarotenals and epoxy-β-apocarotenones respectively (Pénicaud et al., 2011). This distinction is clearly depicted in Fig.2.

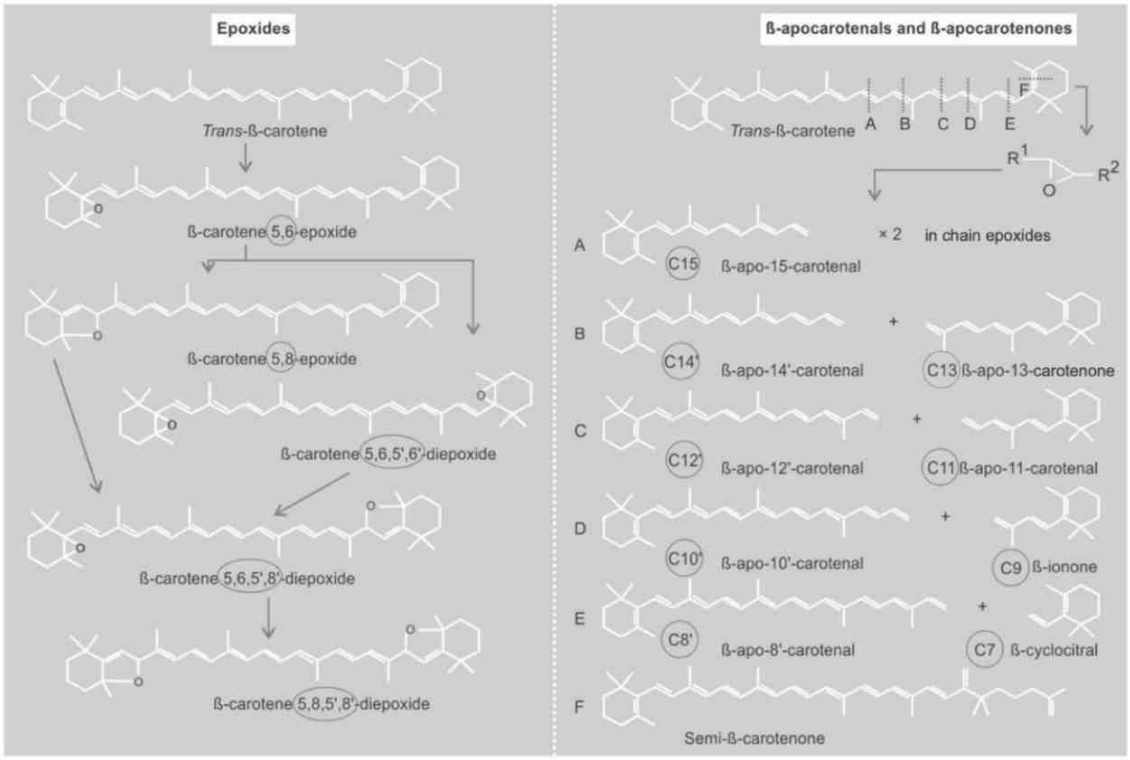


Figure 4: The detailed formations of epoxides, β-apocarotenals and β-apocarotenones (Pénicaud et al., 2011)

Although the overview of the degradation of β-carotene in Fig.2 is generalised, it shows the formation of numerous products as a response against heat and air. Because most of these product groups have different peak absorbance wavelengths, for example β-apo-8'-carotenal has a peak absorbance at around 475 nm (Durojaye et al., 2019) while for β-carotene-5,6-epoxide it is at approximately 446.2 nm (Bradbury et al., 2012), the absorption spectra of the solutions obtained from the experiment of this investigation are expected to consist of various peaks. These peaks will be indicating the presence of the reaction products. The wavelengths at which they will occur may differ for every temperature, since the extent of degradation will be different, and consequently, different substances will be produced.

3. HYPOTHESIS

It is hypothesised that the concentration of β-carotene will increase with temperature because more of it will dissolve in ethanol upon exceeding 22°C; heat improves solubility of solute if the dissolution reaction is endothermic (Lu et al., 2021), which in this case it is, as the heat is used to break the intermolecular forces between the β-carotene molecules. Though this compound is sensitive to heat, its degradation will not be extensive until a certain temperature at which the concentration will begin to decline. This is due to the short retention time of 10 minutes. There have been multiple other research investigations regarding the extraction and degradation of β-carotene in one of which the β-carotene existed in solutions at 150°C for up to an hour (Lemmens et al., 2010). In another research paper, good concentrations of carotenoids were extracted from citrus peels kept at temperatures ranging from 10-40°C for up to 72 hours. As a result, concentration was found to increase with temperature (Shahba & Rafid, 2018). Based on these investigations, it can be deduced that a 10-minute heat exposure should be insignificant in terms of degradation until some high temperature above 40°C.

4. EXPERIMENT

4.1. INDEPENDENT AND DEPENDENT VARIABLES

The independent variable:

As an essential factor in β-carotene extraction, temperature in degrees Celsius was selected as the independent variable of this experiment. The temperatures considered were 22°C (room temperature), 60°C, 90°C, 120°C, and 150°C.

When conducting research, majority of explorations were found focused on temperatures between 10°C and °C, while a couple had them up to 130°C and 150°C. Based on reasonable grounds of their results, temperatures below room temperature are inefficient for extraction. Therefore, 22°C was fixed as the lowest endpoint. To procure significant results with clear differences between data

points from different treatments, the wide interval of 30°C was selected to lead to a maximum temperature of 150°C.

The dependent variable:

The dependent variable of this experiment was the concentration of β-carotene extracted in parts-per-million (ppm). Although ppm is not an SI-unit, it was appropriate for this investigation, as otherwise, the expression in molarity would have resulted in lengthy decimal numbers smaller than one.

The concentration was determined through spectrophotometry. Another method could have been high performance liquid chromatography (HPLC) and to detect as well as diagnose the different organic compounds extracted, particularly β-carotene, the FT-IR spectroscopy detector could have been utilised (Shahba & Rafid, 2018). However, they were unavailable in the school lab due to safety hazards. Therefore, spectrophotometry was the safest and most practical method.

4.2. CONTROLLED VARIABLES

Table 1: Controlled variables, reason for control and method of control

Controlled variable:	Reason for control:	Method of control:
Solvent	The solubility of β-carotene changes from solvent to solvent.	Ethanol was used throughout, as it dissolves β-carotene well (V. Popova, 2016), and it is an organic solvent with multiple industrial applications (Solventis World Class Chemical Solutions, n.d.). The other options were petroleum ether, ethyl acetate and acetone (Hecker, 2014), all of which were accessible in the lab and equally require safety precautions. However, due to ethanol being considered as one of the universal solvents for extraction (Seidu, 2021), it was finalised for this research.
Volume of ethanol	The volume of ethanol affects how diluted the solutions would be.	20 cm^3 of ethanol was used to prepare the mixtures for every trial.
Mass of orange peel powder	The more the mass of orange peel powder, the higher the concentration of β-carotene extracted, as more of it would dissolve in ethanol.	Two grams of orange peel powder was added to 20 cm^3 of ethanol for every trial. These values were decided upon after the pre-tests, which first experimented with five grams of orange peel powder and 35 cm^3 of ethanol. Nevertheless, this led to highly concentrated solutions. Hence, the mass of powder was reduced from five to two grams, and the volume of ethanol was lowered from 35 cm^3 to 20 cm^3. This was also to reduce the volume of waste.
Retention time	If one sample would be given a longer retention time (time spent under reflux), more β-carotene would dissolve in the ethanol or for higher temperatures, β-carotene may degrade more.	The retention time was 10 minutes. It initially decreased from two hours due to retaining saturated solutions.
Equipment	Though used for the same purpose, different equipment have different uncertainties.	The same equipment was used throughout the experiment.
Speed of the magnetic stirrer	The faster the magnet spins, the more the β-carotene may dissolve in ethanol.	The magnet span at 700 revolutions per minute for every trial.
Type of orange	The type of orange may influence the amount of β-carotene its peels contain.	Oranges grown in South Africa, bought from the same store at the same time, were used.

4.3. RISK ASSESSMENT

Table 4: Safety data table for ethanol and β-carotene

Chemical:	Hazard:	Precaution:	Emergency action:	Disposal:
Ethanol  (ThermoFisher Scientific, 2009)	Ethanol is highly flammable and may irritate the eyes. It may damage organs and cause drowsiness.	Use gloves, goggles, and a lab coat to avoid contact.	If exposed on skin or eyes, wash or rinse with water immediately. If eyes continue to irritate, receive medical advice.	Dispose of to a waste disposal plant to avoid exposing it to the environment.
β-carotene (ThermoFisher Scientific, 2021)	β-carotene is not deemed hazardous.	Use gloves, goggles, and a lab coat as early safety precaution.	If exposed on skin or eyes, wash or rinse for at least 15 minutes. If symptoms occur, receive medical advice.	Can be drained down the sink.

4.4. METHODOLOGY

This methodology was inspired and adapted by the experiment done by Shahba Rafie Abdullah & Rafid Khalil Abdul Razaq.

Preparing the orange peel powder:

Eight oranges were washed, peeled, and left to dry at room temperature for two days. They were ground using a grinder to form powder, which was sifted through a sieve and stored in a sealed glass container until use.

Extraction:

A water bath (or paraffin oil bath for temperatures 120°C and 150°C) was placed on a hot plate magnetic stirrer. A thermometer was set inside the water bath (or oil bath) to monitor the temperature with the help of a clamp on a stand. The heat was turned on and temperature was adjusted.

In an Erlenmeyer flask, two grams of orange peel powder was measured on an analogue scale and 20 cm^3 of ethanol was added into it. This Erlenmeyer flask with the mixture was placed in the water bath with a magnet inside and a condenser attached to it using a clamp on a stand for stability. The experimental setup can be seen from Fig.5.

Figure 5: The experimental setup

The magnetic stirrer was turned on at 700 revolutions per minute. After 10 minutes, heat was turned down and vacuum filtration was conducted by running the solution through a Büchner funnel on a Büchner flask. For a few of the trials, the solution had to be filtered twice due to some of the orange peel powder particles leaking in through the edges of the filter paper.

Determining the concentration of the extracted β-carotene:

Because most spectrophotometers are most stable within the absorbance range of 0.1-1.0 (Vernier, 2020) but the filtered solutions obtained from this experiment led to absorbance values greater than 2, they were diluted. The dilution was performed in the ratio of 1:9; for every 1 cm^3 of solution, 9 cm^3 of ethanol was added. The solutions were prepared in 10 cm^3 volumetric flasks. Please note

that before the dilution, the volume of the filtered solution was measured to ensure whether the ethanol had evaporated, resulting in inaccurate concentration values.

The spectrophotometer was connected to a laptop, which had the application Vernier Spectral Analysis. After calibrating it with ethanol, the diluted solution was poured into a cuvette and inserted into the device. From the produced absorption spectrum, the peak absorbance value was read. The concentration of the prepared solution was determined by examining the standard curve of β-carotene. This concentration was later used to calculate the concentration of the original solution.

The standard curve of β-carotene:

β-carotene solutions of concentrations of 6.4 ppm, 40 ppm, 50 ppm and 60 ppm were prepared. These values were selected based on the standard curve obtained from another research conducted at the Omar El-Mokhtar University, which included concentrations from 10-80 ppm. Since the stable absorbance range of the spectrophotometer was from 0.1-1.0, the highest concentration chosen was 60 ppm, as it had an absorbance of around 1.05 (Hasan et al., 2019).

An unmeasurable amount of β-carotene powder was required to prepare solutions of concentrations as small as 6.4 and 60 ppm, for 100 cm^3 of 100 ppm solution was initially prepared. Serial dilution was performed to produce the final standard solutions.

The formula used to deduce the measurements for preparing the solutions were:

$$c(solution)\ in\ ppm = \frac{m(\beta-carotene)}{V(solution)} \times 1\ 000\ 000 \qquad [1]$$

&

$$c_1V_1 = c_2V_2 \qquad [2]$$

EXAMPLE CALCULATIONS:

Preparing 100 cm³ of 100 ppm solution:

$$c_1 = 100\ ppm$$
$$V_1 = 100.0\ cm^3\ (\pm 0.1\ cm^3)$$
$$\therefore 100\ ppm = \frac{m(\beta - carotene)}{100.0\ cm^3} \times 1\ 000\ 000$$
$$m(\beta - carotene) = 0.0100\ g$$

∵ 0.0100 g of β-carotene was measured using an analytical scale with an uncertainty of 0.0001 g

∴ (0.0100 ± 0.0001) g of β-carotene was dissolved in 100.0 cm³ of ethanol

Preparing 20 cm³ of 60 ppm solution from the 100 ppm solution:

$$c_1V_1 = c_2V_2$$
$$100\ ppm \times V_1 = 60\ ppm \times 20\ cm^3$$
$$V_1 = 12\ cm^3$$

∴ 12 cm³ of 100 ppm solution was mixed with 8 cm³ of ethanol to produce 20 cm³ of 60 ppm solution.

Uncertainty propagation for the concentrations:

$$\because Propagated\ error = \sqrt{(\frac{\delta a}{a})^2 + (\frac{\delta b}{b})^2 + (\frac{\delta c}{c})^2}$$, (Hogan, 2014)

where $\delta = uncertainty, a = concentration\ of\ previously\ diluted\ solution,$

$b = volume\ of\ previously\ diluted\ solution, c = volume\ of\ ethanol$

$$\& \because Propagated\ error\ (100.0\ ppm) = \sqrt{(\frac{0.0001g}{0.0100g})^2 + (\frac{0.1cm^3}{100.0cm^3})^2} \approx 0.010 = 1.0\%$$

$$\therefore Propagated\ error\ (60.0\ ppm) = \sqrt{(\frac{0.010\ldots \times 100.000\ ppm}{100.000\ ppm})^2 + (\frac{0.15\ cm^3}{12.00\ cm^3})^2 + (\frac{0.075\ cm^3}{8.000\ cm^3})^2} *$$
$$\approx 0.019 = 1.9\%$$

$$Propagated\ error\ (50.0\ ppm) = \sqrt{(0.019\ldots)^2 + (\frac{0.075\ cm^3}{10.000\ cm^3})^2 + (\frac{0.075\ cm^3}{10.000\ cm^3})^2}$$
$$\approx 0.021 = 2.1\%$$

$$Propagated\ error\ (40.0\ ppm) = \sqrt{(0.021\ldots)^2 + (\frac{0.15\ cm^3}{16.00\ cm^3})^2 + (\frac{0.045\ cm^3}{4.000\ cm^3})^2}$$
$$\approx 0.026 = 2.6\%$$

$$Propagated\ error\ (6.4\ ppm) = \sqrt{(0.026\ldots)^2 + (\frac{0.045\ cm^3}{1.600\ cm^3})^2 + (\frac{0.075\ cm^3}{8.400\ cm^3})^2}$$
$$\approx 0.039 = 3.9\%$$

Because the uncertainty estimation for the values can be increased, the overall uncertainty of the standard solution concentrations will be considered 3.9%. A shared uncertainty was required to estimate the uncertainty of the concentrations of the β-carotene solutions obtained from the experiment.

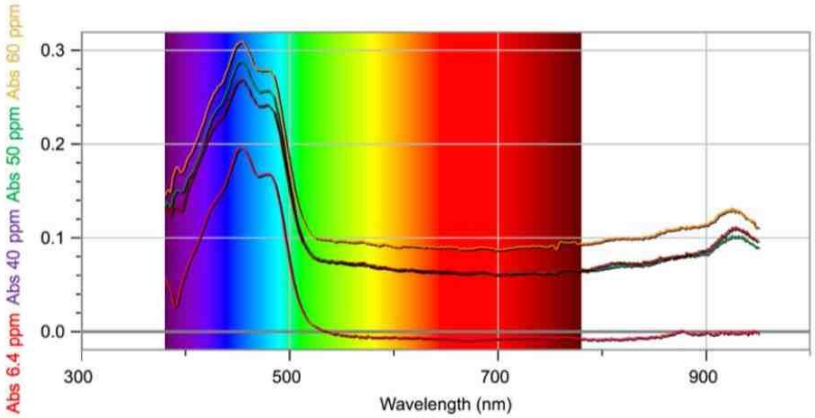
*The volumes of solution and ethanol composing all the standard solutions can be found from Appendix III with the calculation procedures. The uncertainties for the measurements are based on those of the equipment used, which can be found from Appendix I.

The prepared standard solutions of desired concentrations were inserted into the spectrophotometer to produce absorption spectra. Peak absorbance values were inserted into LoggerPro with their corresponding concentration values. The absorbances made up the y-coordinates while the concentrations were on the x-axis. Best fit line was plotted to complete the standard curve of β-carotene.

5. RESULTS AND DISCUSSION

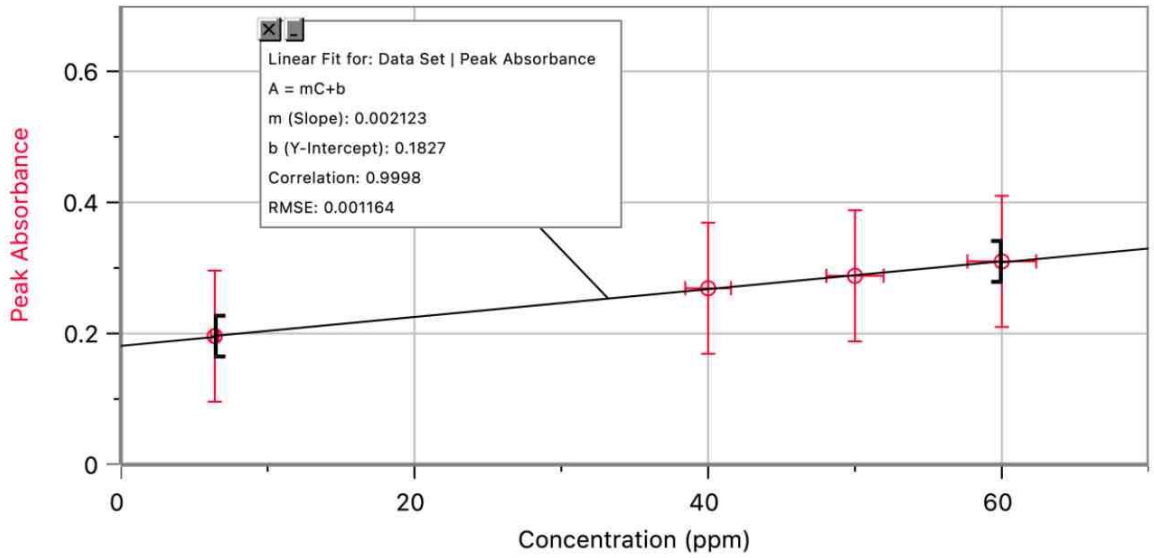
5.1. THE STANDARD CURVE OF β-CAROTENE

Graph 1: Absorption spectra of standard β-carotene solutions of concentrations 6.4 ppm, 40 ppm, 50 ppm and 60 ppm



As can be seen from Graph 1, the four absorption spectra of the standard solutions have one clear peak each, all of which occur at wavelength 453.7 nm. This value is very close to the literature maximum wavelength of 455 nm, indicating that the absorbance values obtained from Graph 1 well represent the concentrations of particularly β-carotene, and not of other compounds. However, the absorbances are much lower than the ones acquired from the standard curve of Hasan et al., 2019; 60 ppm solution had an absorption of 1.05, while in Graph 1, it is 0.31. This could be because in this experiment, the β-carotene did not completely dissolve in ethanol, despite vigorous hand-mixing with a glass rod and shaking of the flasks. On the other hand, the study mentioned earlier used a different solvent, chloroform, and proper equipment may have been used for the dissolution.

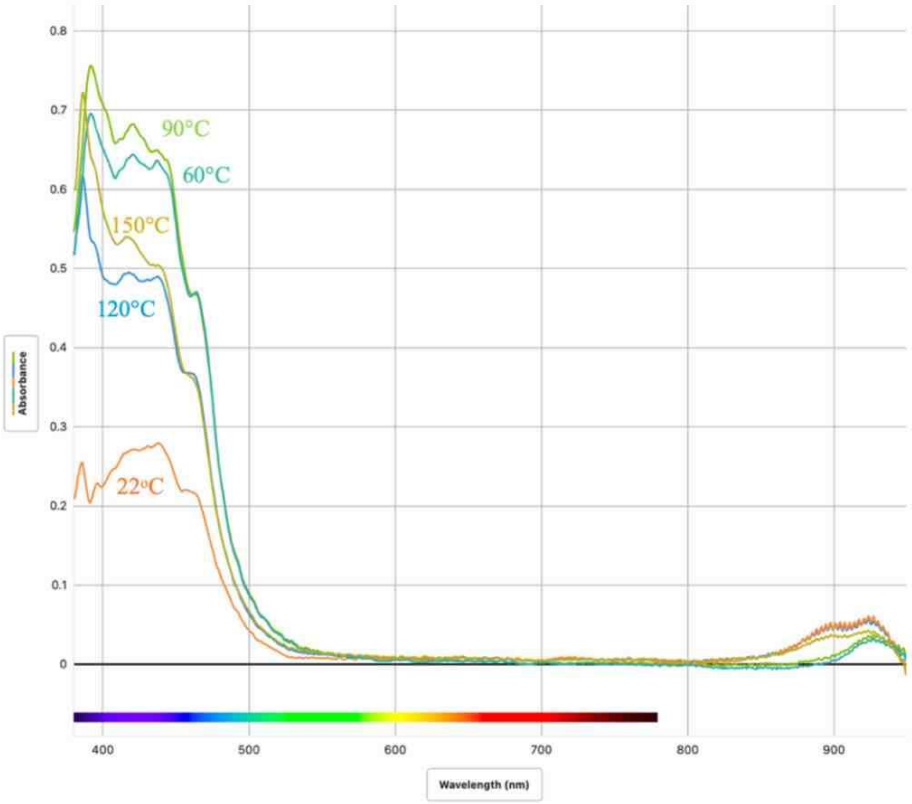
Graph 2: The standard curve of β-carotene acquired from this experiment



Theoretically, no absorbance should occur at a concentration of zero ppm. In spite of this, it is discernible from Graph 2 that the best-fit line of the standard curve does not pass through the origin. This alludes the occurrence of systematic errors related to the incomplete dissolution of β-carotene in ethanol and the time-consuming preparation of the solutions. The β-carotene was long exposed to air, consequently, lowering peak absorbance values and giving increased concentration values on Graph 2. However, these errors seem to have affected values in a fixed and predictable manner. Therefore, a linear best-fit for the standard curve was acquired with an almost perfect correlation coefficient of 0.9998.

5.2. QUANTITATIVE RAW DATA AND ANALYSIS

Graph 3: Absorption spectra of trial 1 of every treatment (the absorption spectra of trial 2 and 3 can be seen from appendix II)



As expected, there are various peaks on the spectra on Graph 3, some of which appear at different wavelengths for every temperature, indicating the presence of degradation products. Because the rate of degradation and the extent to which β-carotene degrades vary with temperature, the shape of the absorption spectra differ, not only from each other, but also from those on Graph 1. In addition to this, other constituents of orange peels may have dissolved in ethanol, resulting in the emergence of extra peaks.

For the trial of the treatment 22°C, the difference between the absorbance values of the highest peak at 438 nm and of the one occurring at 455.5 nm, which may represent β-carotene, is not very great, as is visibly perceived from Graph 3. This means that the concentration of β-carotene and of another principal substance are mostly balanced in the solution. However, this difference increases with

temperature, ultimately, resulting in a shift of peak to the left, and implying that the concentration of β-carotene in the solution has substantially decreased, while the concentration of another product has become dominant. For example, for the solution at 60°C, the highest absorbance peak has shifted to be at 391.6 nm. Based on these remarks, it can be deduced that degradation of β-carotene was least significant in the solution kept at 22°C, while for the higher temperatures, it was notable. These results seem to conform the hypothesis.

As the peak absorbances of the standard β-carotene solutions appeared at 453.7 nm, the peak absorbance values occurring at this wavelength on Graph 3 were employed to compose the raw data on Table 2.

Table 2: Raw data comprised of temperature (°C) and absorbance values

	TRIAL 1	TRIAL 2	TRIAL 3
Temperature ± 2 (°C):	**Absorbance ± 0.100:**	**Absorbance ± 0.100:**	**Absorbance ± 0.100:**
22	0.219	0.405	0.398
60	0.505	0.499	0.486
90	0.521	0.518	0.523
120	0.372	0.462	0.488
150	0.380	0.305	0.377

On Table 2, there are three anomalies marked in red: trial 1 (22°C), trial 1 (120°C) and trial 2 (150°C). These anomalies are a result of random errors, which are discussed in the Evaluation section. Please note that the uncertainty of the absorbances was ensured from Vernier, n.d..

5.3. QUALITATIVE RAW DATA

While conducting pre-tests, the filtered solutions were kept in containers to dispose of them separately when colour change was noticed between two trials of the same treatment. The solution of trial 1 was stored in a container at room temperature for three hours, while trial 2 was stored for 30 minutes. The orange colour of the solution of trial 1 was lighter than that of trial 2, perhaps due to the loss of β-carotene. This can be perceived from Fig.6.

Figure 6: Trial 1 and trial 2 of 22°C from pre-tests

While conducting the experiment, the orange colour of all the solutions was found to be vibrant (Fig.8), suggesting the presence of β-carotene and other orange peel constituents. Moreover, the solutions filtered were not always transparent (Fig.7). Very tiny particles began forming, probably because the solutions were saturated. After decreasing the mass of the orange peel powder for the final experiment, some of them were still blurry, while some from the same treatment were transparent. The reason behind this is yet undetermined.

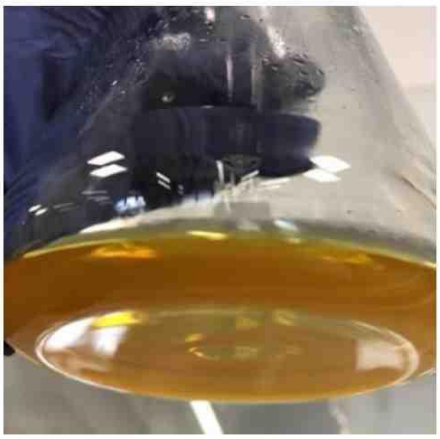


Figure 7: Unclear, saturated solution of treatment 60°C from the pre-test

Figure 8: Transparent solution of treatment 60°C (trial 1) from the final experiment

5.4. PROCESSED DATA

The absorbances from Table 2 were used to determine the concentrations of the diluted solutions from the standard curve of β-carotene. As these solutions were diluted by mixing one cm^3 of the originally filtered solutions with nine cm^3 of ethanol, the original solutions should be ten times as concentrated as the diluted ones.

After computing the final concentrations, the average concentrations of every treatment were also calculated. Example calculations regarding this are shown below.

EXAMPLE CALCULATIONS:

This example calculation refers to trial 1 ($22°C$).

$$A(absorbance) = 0.219$$
$$c(diluted\ solution) = 17.0984456\ ppm \text{ (determined from the standard curve)}$$
$$\because c(original\ solution) = 10 \times c(diluted\ solution)$$
$$\therefore c(original\ solution) = 10 \times 17.0984456\ ppm \approx 171.0\ ppm$$

The following formula was used to calculate the average concentrations (Ac).

$$Ac = \frac{c(original\ solution)_1 + c(original\ solution)_2 + c(original\ solution)_3}{3}$$

$$\because c(original\ solution)_1 = 170.984456\ ppm$$
$$c(original\ solution)_1 = 1047.103156\ ppm$$
$$c(original\ solution)_1 = 1014.130947\ ppm$$

$$\therefore\ Ac\ (22°C) = \frac{170.984\ldots\ ppm + 1047.103\ldots ppm + 1014.130\ldots\ ppm}{3} \approx 744.0\ ppm$$

The uncertainty of the average concentrations was calculated using the following formula.

$$Uncertainty = \frac{maximum\ value - minimum\ value}{2}$$

$$\therefore Uncertainty = \frac{1047.103\ldots ppm - 170.984\ldots ppm}{2} \approx 438.1\ ppm$$

The following processed data was generated after conducting the calculations.

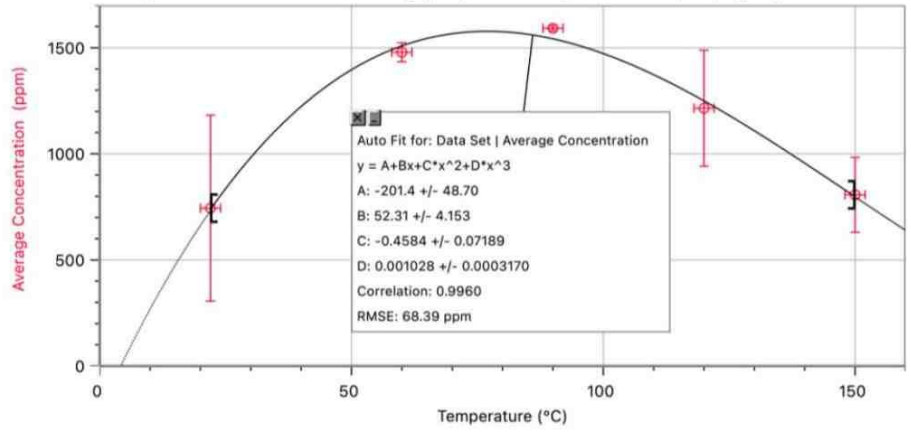
Table 3: Processed data consisting of temperature (°C), the corresponding concentrations of the original solutions and average concentrations in ppm

	TRIAL 1		TRIAL 2		TRIAL 3		
Temperature ± 2 (°C):	**Concentration of prepared solution (ppm) ± 3.9%:**	**Concentration of original solution (ppm) ± 3.9%:**	**Concentration of prepared solution (ppm) ± 3.9%:**	**Concentration of original solution (ppm) ± 3.9%:**	**Concentration of prepared solution (ppm) ± 3.9%:**	**Concentration of original solution (ppm) ± 3.9%:**	**Average concentration (ppm):**
22	17.1	171.0	104.7	1047.1	101.4	1014.1	744.1 ± 438.1
60	151.8	1518.1	149.0	1489.9	142.9	1428.6	1478.9 ± 44.7
90	159.3	1593.5	157.9	1579.4	160.3	1602.9	1591.9 ± 11.8
120	89.2	891.7	131.6	1315.6	143.8	1438.1	1215.1 ± 273.2
150	92.9	929.3	57.6	576.1	91.5	915.2	806.9 ± 176.6

Although anomalies were identified from Table 2, they were not disregarded in the data processing, since otherwise, the averages would have been calculated from only two concentration values, which is an unreliable number. Moreover, the anomalies would not have significantly influenced the overall trend between the two variables.

On Graph 6, to illustrate the effect of temperature on the concentration of β-carotene, the average concentrations were placed on the y-axis, while their corresponding temperatures were on the x-axis. The cubic function was plotted, as it best fit the data points.

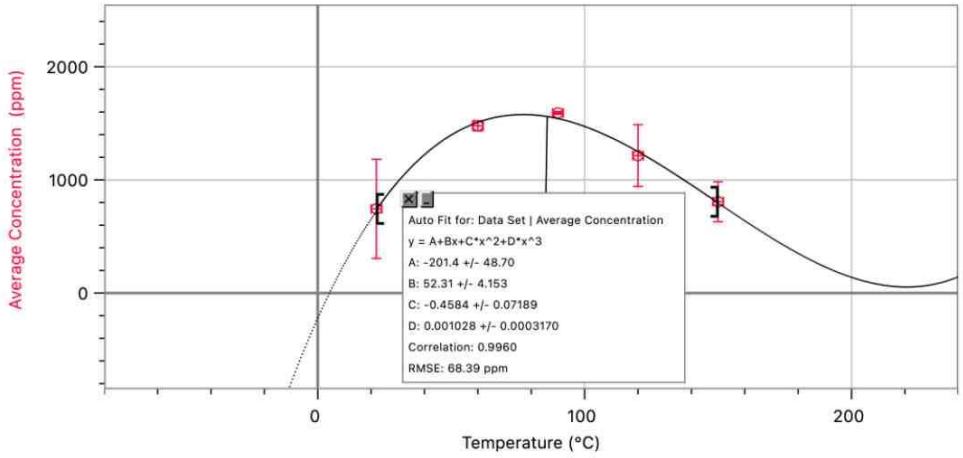
Graph 6: Concentration (ppm) vs. temperature (°C) graph



5.5. DISCUSSION

As seen from Graph 6, the cubic function connects the data points with an almost perfect correlation coefficient of 0.9960. Hence, it well describes the relationship between the temperature and concentration of β-carotene from the experiment, but only on the limited domain of $4°C \leq x \leq 150°C$. Beyond this domain, the average concentration either reaches negative values or begins to increase.

Graph 7: Zoomed out version of Graph 6



From Graph 7, it is examined that after temperature 150°C, the average concentration first declines, after which it re-elevates. However, this is unlikely to happen, since as established before, the β-carotene will continue to degrade with temperature. On the other hand, it is possible for the concentration to be zero ppm at °C, as the β-carotene can be sufficiently frozen, thus unable to dissolve in ethanol. In fact, while preparing the standard solutions, it was challenging to dissolve the β-carotene, as it was stored in a cool place prior to use.

Moreover, from Graph 7, it is observed that from 4.0°C to 76.5°C, the degradation is not impactful, therefore, the concentration increases. The maximum concentration is achieved between 76.5°C and 77.5°C with an average of 1577.3 ppm. Referring to the aim of this essay, which was to make the most out of the peels and reduce OPW, this information is essential, since this is the optimal

temperature range for β-carotene extraction. When temperature exceeds 77.5°C, degradation becomes remarkable, and the concentration decreases.

6. CONCLUSION

The aim of this research, which was to investigate the effect of temperature on the concentration of β-carotene extracted from orange peels, has been fulfilled. According to the results, the concentration increases with temperature, but after the temperature of 77.5°C, the concentration declines. More specifically, the effect was described to be cubic on the specific domain of $4°C \leq x \leq 150°C$. Although the relationship was not hypothesised to be cubic, the overall description of the effect matches the conjecture made previously. Therefore, the results support the hypothesis.

The outcome of this experiment is supplemented by results acquired from other similar explorations. According to one research paper, the highest yield of β-carotene extracted from ripe bitter melon pericarp was achieved at a temperature of approximately 70°C, but as the temperature fell or exceeded this value, the yield dropped (Patel et al., 2019). This correlation is like the one demonstrated on Graph 7, but instead of 70°C, the optimal concentration is achieved between 76.5°C and 77.5°C.

Another research was conducted on the effect of thermal processing on the isomerisation of β-carotene. Solutions of β-carotene were kept at 130°C and 140°C for 10 minutes. 15% and 30% of β-carotene *cis* isomers were found from the solutions, respectively, suggesting that isomerisation, the first step of degradation, occurred. On the other hand, the solutions kept at 100°C and 120°C did not experience extensive *trans* to *cis* isomerisation (Marx et al., 2003). This accommodates the results of this research; Graph 7 presents this trend, as it displays that the concentration of the pigment decreases with temperature.

Because no research paper was found to include values for concentrations of extracted β-carotene between the temperature range of this experiment, the results could not be directly quantitively compared with secondary data. However, in one of the investigations, it was discovered that every 100 grams of orange peel constitutes of 14.51 milligrams of β-carotene (Abd El-Rahman et al., 2019). This is 0.01451% of the orange peel mass:

$$\%\ content\ of\ \beta - carotene = \frac{14.51 \times 10^{-3}\ g}{100\ g} \times 100\% = 0.01451\% \approx 0.014\%$$

To refer to this percentage, the percentage yield of β-carotene extracted from every trial was calculated using the following calculation procedure.

EXAMPLE CALCULATION:

This example calculation refers to trial 1 (22°C).

$$c = 170.984456\ \ ppm$$
$$V(solution) = 15.30\ cm^3\ (\pm 0.15\ cm^3)*$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\ 000\ 000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\ 000\ 000} = \frac{170.984456\ \ ppm \times 15.30\ cm^3}{1\ 000\ 000}$$
$$\therefore m(\beta - carotene) \approx 2.616 \times 10^{-3} g = 2.616\ mg$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{2.616 \times 10^{-3}\ g}{2.0\ g} \times 100\% \approx 0.13\%$$

Uncertainty propagation:

$$Propagation\ error\ (22°C)_1 = \sqrt{(\frac{0.039\ldots \times 170.984\ldots ppm}{170.984\ldots ppm})^2 + (\frac{0.15\ cm^3}{15.30\ cm^3\ *})^2} \approx 0.0402 = 4.02\%$$

$$Propagation\ error\ (22°C)_2 = \sqrt{(0.039\ldots)^2 + (\frac{0.15\ cm^3}{14.40\ cm^3})^2} \approx 0.0404 = 4.04\%$$

$$Propagation\ error\ (22°C)_3 = \sqrt{(0.039\ldots)^2 + (\frac{0.15\ cm^3}{15.60\ cm^3})^2} \approx 0.0402 = 4.02\%$$

$$Propagation\ error\ (60°C)_1 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{15.60\ cm^3})^2} \approx 0.0402 = 4.02\%$$

$$Propagation\ error\ (60°C)_2 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{15.90\ cm^3})^2} \approx 0.0401 = 4.01\%$$

$$Propagation\ error\ (60°C)_3 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{16.60\ cm^3})^2} \approx 0.0400 = 4.00\%$$

$$Propagation\ error\ (90°C)_1 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{15.00\ cm^3})^2} \approx 0.0403 = 4.03\%$$

$$Propagation\ error\ (90°C)_2 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{16.10\ cm^3})^2} \approx 0.0401 = 4.01\%$$

$$Propagation\ error\ (90°C)_3 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{15.20\ cm^3})^2} \approx 0.0402 = 4.02\%$$

$$Propagation\ error\ (120°C)_1 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{14.90\ cm^3})^2} \approx 0.0403 = 4.03\%$$

$$Propagation\ error\ (120°C)_2 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{13.80\ cm^3})^2} \approx 0.0405 = 4.05\%$$

$$Propagation\ error\ (120°C)_3 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{13.20\ cm^3})^2} \approx 0.0406 = 4.06\%$$

$$Propagation\ error\ (150°C)_1 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{13.50\ cm^3})^2} \approx 0.0406 = 4.06\%$$

$$Propagation\ error\ (150°C)_2 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{11.40\ cm^3})^2} \approx 0.0412 = 4.12\%$$

$$Propagation\ error\ (150°C)_3 = \sqrt{(0.039)^2 + (\frac{0.15\ cm^3}{11.00\ cm^3})^2} \approx 0.0413 = 4.13\%$$

$\therefore$ The uncertainty of the mass percentages is 4.13%

*Although the volume of the solutions was originally 20 cm^3, after keeping them under reflux and after the filtration, some of the ethanol evaporated, decreasing the volume. 15.30 cm^3 is the volume of the solution, whose concentration was determined, thus, it is considered in the calculations. Note: the volume was different for every solution.

Table 4: Volumes of solution (cm^3) after filtration and the percentage mass of β-carotene extracted at temperatures 22°C, 60°C, 90°C, 120°C and 150°C

	TRIAL 1		TRIAL 2		TRIAL 1	
Temperature ± 2 (°C):	**Volume of solution ± 0.15 (cm^3):**	**Percentage mass of β-carotene ± 4.13 (%):**	**Volume of solution ± 0.15 (cm^3):**	**Percentage mass of β-carotene ± 4.13 (%):**	**Volume of solution ± 0.15 (cm^3):**	**Percentage mass of β-carotene ± 4.13 (%):**
22	15.30	0.13	14.40	0.75	15.60	0.79
60	15.60	1.18	15.90	1.18	16.60	1.19
90	15.00	1.20	16.10	1.27	15.20	1.22
120	14.90	0.66	13.80	0.91	13.20	0.95
150	13.50	0.63	11.40	0.33	11.00	0.50

The percentage masses of β-carotene presented in Table 4 are all greater than 0.01415%. This may be because in the experiment by Abd El-Rahman et al., 2019, the solutions were first purified, ensuring it is the β-carotene in particular whose concentration is determined. However, in the experiment of this investigation, the solutions were not purified, and exploring the effect of temperature led to the formation of degradation products, which interfered with the absorbance readings. Nevertheless, this does not imply that the effect depicted on Graph 6 is invalid. This "error" affected all the concentrations to be higher, thereby, causing the graphed cubic effect itself to still be somewhat valid, although the values are inaccurate.

7. EVALUATION

This experiment had strengths in terms of its methodology, but it also involved several errors, which lowered the accuracy and reliability of the acquired results.

7.1. SYSTEMATIC ERRORS

The spectrophotometer was calibrated with ethanol instead of an empty cuvette. This reduced systematic errors by ensuring the concentrations of the compounds present in the solution were determined without the influence of the solvent used, improving the accuracy of results.

Moreover, cuvettes were cleaned and rinsed with ethanol to ensure readings are not disturbed by the residue from previous experiments, nor by dust particles, which also improved the accuracy.

7.2. RANDOM ERRORS

The thermometer used gave more accurate and stable readings for lower temperatures than for the higher ones. The readings especially fluctuated for 150°C, despite the heat from the hot plate remaining untouched. This may be because the highest temperature the thermometer could measure was 150°C, and consequently, upon reaching this value, the readings became unstable. Alternatively, the hot plate could have been malfunctioning due to not being properly heated before the experiment. It may have been due to this that the anomalies of trial 1 (120°C) and trial 2 (150°C) in Table 2 occurred. To overcome this, a thermometer with a higher range could have been used and all the equipment could have been warmed up prior to experimentation.

Trial 1 of 22°C (room temperature) is also an anomaly. However, this was not caused by equipment since hot plate was not required. Instead, the time spent between the filtration of the solution to using the spectrophotometer varied. The difference in time was caused by filtering the solution of trial 1 twice, as some particles leaked into the solution. It is also because of this time factor that the results for all the trials of the same treatment are not exact.

To measure 2 grams of orange peel powder, an analogue scale was used. Since it is an electronic scale with an accuracy of 0.01 grams, the readings for the same mass could have varied. This suggests that some of the trials may have had a greater amount of orange peel powder than the others, increasing the concentration of extracted β-carotene. This may have caused slight imprecision of results of trials of the same treatment.

Moreover, 0.0100 grams of β-carotene was measured to prepare standard solutions. An analytical scale was used, but the readings are very often interfered by air currents, affecting the balance. Despite ensuring the reading was 0.0100 grams, the actual mass may have been greater or less than the required value, introducing uncertainty into the concentration values.

7.3. IMPROVEMENTS

Many more trials could have been conducted to improve the accuracy of the results. Moreover, the equipment, such as the hot plate and thermometer, could have been warmed up before starting the extraction process to reduce random errors.

Moreover, the solutions could have been purified to get results of only β-carotene, without the interference of other substances. The solutions could have been purified through thin layer chromatography (L. Jeyanthi Rebecca et al., 2014). Alternatively, the orange peel powder could have been macerated in a blender with acetone and ascorbic acid. After filtering this solution and using the rotary vacuum evaporator, the extract could have been saponified, followed by the washing of it with methanolic potassium hydroxide solution and water in a separatory funnel. Adding hexane and washing the extract with dihydrogen potassium phosphate solution would have resulted in some yield of pure β-carotene (Abd El-Rahman et al., 2019). However, it would have have to be ensured that the solution is heated, and is kept at that specific temperature throughout the process of purification, which should have taken approximately the same amount of time for every

trial. Moreover, safety precautions would have have to be taken very seriously due to the hazardous chemicals used.

7.4. EXTENSIONS

A 10-minute retention time is a small duration of time to extract β-carotene from orange peels, especially at an industrial level. Therefore, to extend this research further, the effect of retention time on concentration could be investigated. Moreover, the extraction could be conducted using different solvents to investigate, which one gives the highest concentration or yield of β-carotene.

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9. APPENDICES

9.1. APPENDIX I - APPARATUS AND SUBSTANCES

Apparatus:

- 5 cm^3 graduated pipette ± 0.045 cm^3
- 10 cm^3 graduated pipette ± 0.075 cm^3
- 20 cm^3 graduated pipette ± 0.15 cm^3
- 10 cm^3 volumetric flask ± 0.038 cm^3
- 100 cm^3 volumetric flask ± 0.10 cm^3
- Analytical scale ± 0.0001 g
- Analogue scale ± 0.01g
- Thermometer $\pm 0.5^oC$
- Glass rod for mixing the standard solutions
- Erlenmeyer flask
- Hot plate magnetic stirrer
- Water bath/paraffin oil bath
- Clamps and stands
- Condenser
- Magnet
- Rubber tubes
- Spectrophotometer with cuvettes
- Laptop with LoggerPro and Vernier Spectral Analysis software
- Büchner flask
- Büchner funnel
- Filter paper
- Sealed glass container to store the orange peel powder
- Plastic spoon to add orange peel powder into the Erlenmeyer flask
- Grinder
- Sieve

Substances:

- 30 g orange peel powder
- β-carotene
- Ethanol
- Distilled water (to wet the filter paper before filtration)

9.2. APPENDIX II – ABSORPTION SPECTRA

Table 5: Absorption spectra of trial 2 of every treatment

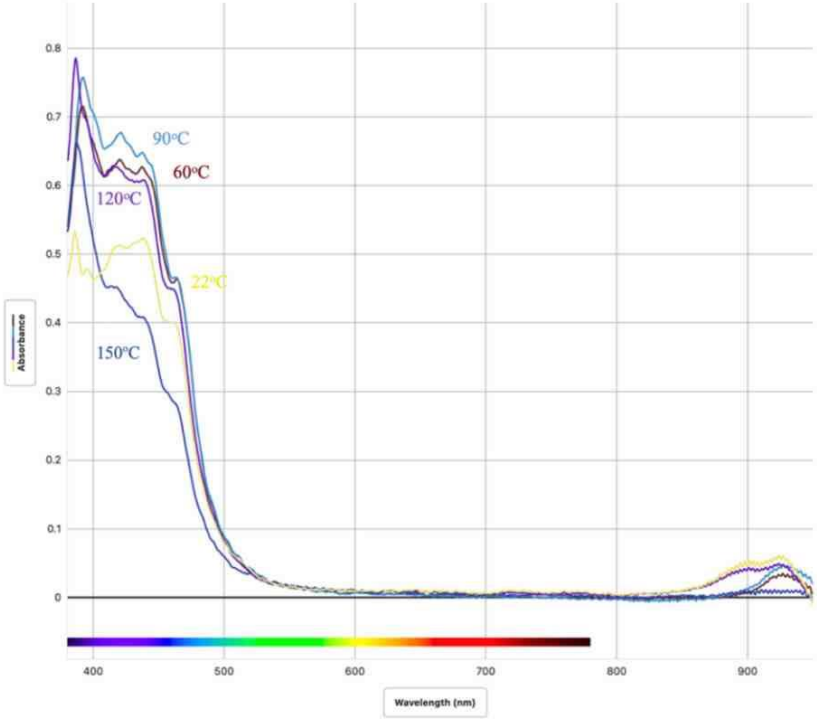
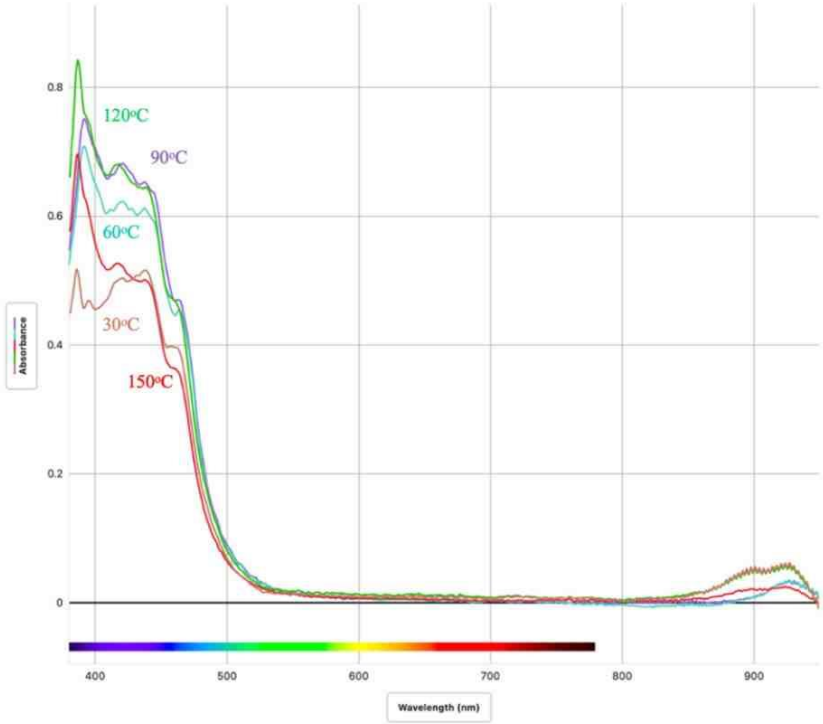


Table 6: Absorption spectra of trial 3 of every treatment



9.3. APPENDIX III – STANDARD SOLUTION CALCULATIONS

Preparing 100 cm^3 of 100 ppm solution:

$$c_1 = 100\ ppm$$
$$V_1 = 100.0\ cm^3\ (\pm\ 0.1\ cm^3)$$
$$\therefore 100\ ppm = \frac{m(\beta - carotene)}{100.0\ cm^3} \times 1\ 000\ 000$$
$$m(\beta - carotene) = 0.0100\ g$$

∵ 0.0100 g of β-carotene was measured using an analytical scale with an uncertainty of 0.0001 g

∴ (0.0100 ± 0.0001) g of β-carotene was dissolved in 100.0 cm^3 of ethanol

Preparing 20 cm^3 of 60 ppm solution from the 100 ppm solution:

$$c_1V_1 = c_2V_2$$
$$100\ ppm \times V_1 = 60\ ppm \times 20\ cm^3$$
$$V_1 = 12\ cm^3$$

∴ 12 cm^3 of 100 ppm solution was mixed with 8 cm^3 of ethanol to produce 20 cm^3 of 60 ppm solution.

Preparing 20 cm^3 of 50 ppm solution from the 60 ppm solution:

$$c_1V_1 = c_2V_2$$
$$60\ ppm \times V_1 = 50\ ppm \times 20\ cm^3$$
$$V_1 = 10\ cm^3$$

∴ 10 cm^3 of 60 ppm solution was mixed with 10 cm^3 of ethanol to produce 20 cm^3 of 50 ppm solution.

Preparing 20 cm^3 of 40 ppm solution from the 50 ppm solution:

$$c_1V_1 = c_2V_2$$
$$50\ ppm \times V_1 = 40\ ppm \times 20\ cm^3$$
$$V_1 = 10\ cm^3$$

∴ 10 cm^3 of 50 ppm solution was mixed with 10 cm^3 of ethanol to produce 20 cm^3 of 40 ppm solution.

Preparing 10 cm^3 of 6.4 ppm solution from the 40 ppm solution:

$$c_1V_1 = c_2V_2$$
$$40\ ppm \times V_1 = 6.4\ ppm \times 10\ cm^3$$
$$V_1 = 1.6\ cm^3$$

∴ 1.6 cm^3 of 40 ppm solution was mixed with 8.4 cm^3 of ethanol to produce 10 cm^3 of 6.4 ppm solution.

9.4. APPENDIX IV – CALCULATING CONCENTRATIONS OF THE ORIGINAL SOLUTIONS

Trial 1 (22°C):

$$c(diluted\ solution) = 17.0984456\ ppm$$
$$\therefore c(original\ solution) = 10 \times 17.0984456\ \ ppm \approx 171.0\ ppm$$

Trial 2 (22°C):

$$c(diluted\ solution) = 104.7103156\ ppm$$
$$\therefore c(original\ solution) = 10 \times 104.7103156\ \ ppm \approx 1047.1\ ppm$$

Trial 3 (22°C):

$$c(diluted\ solution) = 101.4130947\ ppm$$
$$\therefore c(original\ solution) = 10 \times 101.4130947\ \ ppm \approx 1014.1\ ppm$$

The following formula was used to calculate the average concentration (Ac) for 22°*C*.

$$Ac_{22°C} = \frac{c(original\ solution)_1 + c(original\ solution)_2 + c(original\ solution)_3}{3}$$

$$\therefore\ Ac_{22°C} = \frac{170.984\ \ldots\ ppm + 1047.103\ \ldots ppm + 1014.130\ \ldots\ ppm}{3} \approx 744.1\ ppm$$

The uncertainty of $Ac_{22°C}$ was calculated using the following formula:

$$Uncertainty = \frac{maximum\ value - minimum\ value}{2}$$

$$Uncertainty = \frac{1047.103\ \ldots ppm - 170.984\ \ldots ppm}{2} \approx 438.1\ ppm$$

$$\therefore\ Ac_{22°C} = (744.0 \pm 438.1)\ ppm$$

Trial 1 (60°C):

$$c(diluted\ solution) = 151.8134715\ ppm$$
$$\therefore c(original\ solution) = 10 \times 151.8134715\ \ ppm \approx 1518.1\ ppm$$

Trial 2 (60°C):

$$c(diluted\ solution) = 148.9872821\ ppm$$
$$\therefore c(original\ solution) = 10 \times 148.9872821\ \ ppm \approx 1489.9\ ppm$$

Trial 3 (60°C):

$$c(diluted\ solution) = 142.8638719\ ppm$$
$$\therefore c(original\ solution) = 10 \times 142.8638719\ \ ppm \approx 1428.6\ ppm$$

The following formula was used to calculate the average concentration (Ac) for 60°C.

$$Ac_{60°C} = \frac{c(original\ solution)_1 + c(original\ solution)_2 + c(original\ solution)_3}{3}$$

$$\therefore\ Ac_{60°C} = \frac{1518.134\ldots\ ppm + 1489.872\ldots ppm + 1428.638\ldots\ ppm}{3} \approx 1478.9\ ppm$$

The uncertainty of $Ac_{60°C}$ was calculated using the following formula:

$$Uncertainty = \frac{maximum\ value - minimum\ value}{2}$$

$$Uncertainty = \frac{1518.134\ldots ppm - 1428.638\ldots ppm}{2} \approx 44.7\ ppm$$

$$\therefore\ Ac_{22°C} = (1478.9 \pm 44.7)\ ppm$$

Trial 1 (90°C):

$$c(diluted\ solution) = 159.3499764\ ppm$$
$$\therefore c(original\ solution) = 10 \times 159.3499764\ ppm \approx 1593.5\ ppm$$

Trial 2 (90°C):

$$c(diluted\ solution) = 157.9368818\ ppm$$
$$\therefore c(original\ solution) = 10 \times 157.9368818\ \ ppm \approx 1579.4\ ppm$$

Trial 3 (90°C):

$$c(diluted\ solution) = 160.2920396\ ppm$$
$$\therefore c(original\ solution) = 10 \times 160.2920396\ \ ppm \approx 1602.9\ ppm$$

The following formula was used to calculate the average concentration (Ac) for 90°C.

$$Ac_{90°C} = \frac{c(original\ solution)_1 + c(original\ solution)_2 + c(original\ solution)_3}{3}$$

$$\therefore\ Ac_{90°C} = \frac{1593.499\ \ldots\ ppm + 1579.368\ \ldots ppm + 1602.920\ \ldots\ ppm}{3} \approx 1591.9\ ppm$$

The uncertainty of $Ac_{90°C}$ was calculated using the following formula:

$$Uncertainty = \frac{maximum\ value - minimum\ value}{2}$$

$$Uncertainty = \frac{1602.920\ \ldots ppm - 1579.368\ \ldots ppm}{2} \approx 11.8\ ppm$$

$$\therefore\ Ac_{90°C} = (1591.9 \pm 11.8)\ ppm$$

Trial 1 (120°C):

$$c(diluted\ solution) = 89.16627414\ ppm$$
$$\therefore c(original\ solution) = 10 \times 89.16627414\ ppm \approx 891.7\ ppm$$

Trial 2 (120°C):

$$c(diluted\ solution) = 131.5591145\ ppm$$
$$\therefore c(original\ solution) = 10 \times 131.5591145\ \ ppm \approx 1315.6\ ppm$$

Trial 3 (120°C):

$$c(diluted\ solution) = 143.805935\ ppm$$
$$\therefore c(original\ solution) = 10 \times 143.805935\ \ ppm \approx 1438.1\ ppm$$

The following formula was used to calculate the average concentration (Ac) for 120°C.

$$Ac_{120°C} = \frac{c(original\ solution)_1 + c(original\ solution)_2 + c(original\ solution)_3}{3}$$

$$\therefore\ Ac_{120°C} = \frac{891.662\ldots\ ppm + 1315.591\ldots ppm + 1438.059\ldots\ ppm}{3} \approx 1215.1\ ppm$$

The uncertainty of $Ac_{120°C}$ was calculated using the following formula:

$$Uncertainty = \frac{maximum\ value - minimum\ value}{2}$$

$$Uncertainty = \frac{1438.059\ldots ppm - 891.662\ldots ppm}{2} \approx 273.2\ ppm$$

$$\therefore\ Ac_{120°C} = (1215.1 \pm 273.2)\ ppm$$

Trial 1 (150°C):

$$c(diluted\ solution) = 92.93452661\ ppm$$
$$\therefore c(original\ solution) = 10 \times 92.93452661\ ppm \approx 929.3\ ppm$$

Trial 2 (150°C):

$$c(diluted\ solution) = 57.60715968\ ppm$$
$$\therefore c(original\ solution) = 10 \times 57.60715968\ ppm \approx 576.1\ ppm$$

Trial 3 (150°C):

$$c(diluted\ solution) = 91.52143194\ ppm$$
$$\therefore c(original\ solution) = 10 \times 91.52143194\ ppm \approx 915.2\ ppm$$

The following formula was used to calculate the average concentration (Ac) for 150°C.

$$Ac_{150°C} = \frac{c(original\ solution)_1 + c(original\ solution)_2 + c(original\ solution)_3}{3}$$

$$\therefore\ Ac_{150°C} = \frac{929.345\ \ldots\ ppm + 576.071\ \ldots ppm + 915.214\ \ldots\ ppm}{3} \approx 806.9\ ppm$$

The uncertainty of $Ac_{150°C}$ was calculated using the following formula:

$$Uncertainty = \frac{maximum\ value - minimum\ value}{2}$$

$$Uncertainty = \frac{929.345\ \ldots ppm - 576.071\ \ldots ppm}{2} \approx 176.6\ ppm$$

$$\therefore\ Ac_{150°C} = (806.9 \pm 176.6)\ ppm$$

9.5. APPENDIX V – β-CAROTENE MASS PERCENTAGE CALCULATIONS

Trial 1 (22°C):

$$c = 170.984456\ ppm$$
$$V(solution) = 15.30\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{170.984456\ ppm \times 15.30\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 2.616 \times 10^{-3} g = 2.616\ mg$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{2.616 \ldots \times 10^{-3}\ g}{2.0\ g} \times 100\% \approx 0.13\%$$

Trial 2 (22°C):

$$c = 1047.103156\ ppm$$
$$V(solution) = 14.40\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{1047.103156\ ppm \times 14.40\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.01508\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.01508 \ldots\ g}{2.0\ g} \times 100\% \approx 0.75\%$$

Trial 3 (22°C):

$$c = 1014.130947\ ppm$$
$$V(solution) = 15.60\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{1014.130947\ ppm \times 15.60\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.01582\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.01582 \ldots\ g}{2.0\ g} \times 100\% \approx 0.79\%$$

Trial 1 (60°C):

$$c = 1518.134715\ ppm$$
$$V(solution) = 15.60\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{1518.134715\ ppm \times 15.60\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.02368\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.02368 \ldots\ g}{2.0\ g} \times 100\% \approx 1.18\%$$

Trial 2 (60°C):

$$c = 1489.872821\ ppm$$
$$V(solution) = 15.90\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{1489.872821\ ppm \times 15.90\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.02369\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.02369 \ldots g}{2.0\ g} \times 100\% \approx 1.18\%$$

Trial 3 (60°C):

$$c = 1428.638719\ ppm$$
$$V(solution) = 16.60\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{1428.638719\ ppm \times 16.60\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.02372\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.02372 \ldots\ g}{2.0\ g} \times 100\% \approx 1.19\%$$

Trial 1 (90°C):

$$c = 1593.499764\ ppm$$
$$V(solution) = 15.00\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{1593.499764\ ppm \times 15.00\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.02390\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.02390\ g}{2.0\ g} \times 100\% \approx 1.20\%$$

Trial 2 (90°C):

$$c = 1579.368818\ ppm$$
$$V(solution) = 16.10\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{1579.368818\ ppm \times 16.10\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.02543\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.02543 \ldots g}{2.0\ g} \times 100\% \approx 1.27\%$$

Trial 3 (90°C):

$$c = 1602.920396\ ppm$$
$$V(solution) = 15.20\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{1602.920396\ ppm \times 15.20\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.02436\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.02436 \ldots\ g}{2.0\ g} \times 100\% \approx 1.22\%$$

Trial 1 (120°C):

$$c = 891.6627414\ ppm$$
$$V(solution) = 14.90\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{891.6627414\ ppm \times 14.90\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.01329\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.01329\ldots\ g}{2.0\ g} \times 100\% \approx 0.66\%$$

Trial 2 (120°C):

$$c = 1315.591145\ ppm$$
$$V(solution) = 13.80\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{1315.591145\ ppm \times 13.80\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.01816\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.01816\ldots g}{2.0\ g} \times 100\% \approx 0.91\%$$

Trial 3 (120°C):

$$c = 1438.05935\ ppm$$
$$V(solution) = 13.20\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{1438.05935\ ppm \times 13.20\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.01898\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.01898\ldots\ g}{2.0\ g} \times 100\% \approx 0.95\%$$

Trial 1 (150°C):

$$c = 929.3452661\ ppm$$
$$V(solution) = 13.50\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{929.3452661\ ppm \times 13.50\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.01255\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.01255\ \ldots\ g}{2.0\ g} \times 100\% \approx 0.63\%$$

Trial 2 (150°C):

$$c = 576.0715968\ ppm$$
$$V(solution) = 11.40\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{576.0715968\ ppm \times 11.40\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 6.567 \times 10^{-3}\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{6.567\ \ldots \times 10^{-3}\ g}{2.0\ g} \times 100\% \approx 0.33\%$$

Trial 3 (150°C):

$$c = 915.2143194\ ppm$$
$$V(solution) = 11.00\ cm^3$$
$$\because c = \frac{m(\beta - carotene)}{V(solution)} \times 1\,000\,000$$
$$\therefore m(\beta - carotene) = \frac{c \times V(solution)}{1\,000\,000} = \frac{915.2143194\ ppm \times 11.00\ cm^3}{1\,000\,000}$$
$$\therefore m(\beta - carotene) \approx 0.01007\ g$$

In percentage, this is:

$$\%\ content\ of\ \beta - carotene = \frac{0.01007\ \ldots\ g}{2.0\ g} \times 100\% \approx 0.50\%$$