Investigating the effect of subjecting Daucus carota to thermal processing at different temperatures, T, (25ºC, 40ºC, 55ºC, 70ºC, 85ºC) of distilled water on its calcium content

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Abstract: Calcium is a mineral that plays a crucial role in our organisms, not only in terms of healthy bones and teeth, but also in blood clotting, contraction of muscles and regulating normal heart rhythms or nerve functions. Taking into consideration the negative effects of calcium deficiency it is vital to deliver necessary amounts of this mineral to our organism by implementing a proper diet. One of the vegetables rich in calcium is carrot. The aim of our experiment was to study the effect of subjecting Daucus carota to thermal treatment of distilled water at different temperatures on the calcium content in it. Based on the experiment, it can be concluded that with an increase in the temperature of thermal processing of carrots, there was a visible decreasing tendency in the calcium content in carrots.

Keywords: Daucus carota; calcium content; thermal processing

1. Introduction

Calcium is a mineral that plays a crucial role in our organisms, not only in terms of healthy bones and teeth, but also in blood clotting, contraction of muscles and regulating normal heart rhythms or nerve functions. Nevertheless, barely 1% of calcium is stored in tissues other than bones (“Calcium”). Due to calcium deficiency osteoporosis can occur weakening them till the point they break. Chronic calcium deficiency can also cause depression, bipolar disorder as well as congestive heart failure, brain or renal calcification (National Institutes of Health Office of Dietary Supplements). Taking into consideration the negative effects of calcium deficiency it is vital to deliver necessary amounts of this mineral to our organism by implementing a proper diet. The diet can be supported by products rich in calcium, these include: different types of milks, cheese, yoghourts, almonds or vegetables (“Calcium”). A typical carrot is considered to have about 33 mg of calcium per 100 g of product in its mineral content (‘Carrots Raw Nutritional Values’), although this value may decrease with the increasing temperature (Kimura, M, and Y Itokawa). In almost

every vegetable, also carrots, oxalic acid can be found. Oxalic acid is an organic compound that tends to bind with minerals and create oxalates with them, for instance: calcium oxalate (Sherrell). Calcium oxalate is nearly insoluble in water, however, combined with oxalic acid creates a soluble compound (Berg et al.) which then can be easily leached from the carrot. Oxalic acid has been proven to be removed in greater amounts from vegetables that were thermally processed in higher temperatures (Ando et al.). Since, in my experiment, I check the calcium content of thermally processed carrots by first checking the calcium

content of water after draining the carrots, I have decided to use a method called complexometric titration. “Complexometric Titration is a type of volumetric analysis wherein the coloured complex is used to determine the endpoint of the titration” (‘Complexometric Titration’). A titrant normally used for the complexometric titration is ethylenediaminetetraacetic acid - EDTA. It is the compound that contains 2 amino groups and 4 carboxyl groups and acts as the Lewis base, which means that each nitrogen and oxygen atom have a lone pair of electrons. Those electrons allow for creating coordinate bonds that can eventually trigger forming complex ions with calcium metal ions. In the experiment, murexide - ammonium purpurate - will play a role of complexometric indicator, which combined with calcium ions creates a pink solution. After adding the EDTA-$Na_2 \cdot H_2O$ solution the end-point of titration will occur resulting in a purple colour of solution (Sangal). Research question: What is the effect of subjecting *Daucus carota* to thermal processing, for exact periods of time, t, (10mins) at different temperatures, T, (^oC) (25^oC, 40^oC, 55^oC, 70^oC, 85^oC), of distilled water on its calcium content, investigated by complexometric titration indicated with ethanolic murexide ($C_8H_8N_6O_6$) solution?

2. Materials and Methods

— Independent and dependent variables —

Independent variable: Different temperatures, T, (25^oC, 40^oC, 55^oC, 70^oC, 85^oC), of distilled water.

Dependent variable: Calcium content in water in which *Daucus carota* was thermally processed, found by the complexometric titration indicated with 5% ethanolic murexide solution.

— Controlled variables —

Table 1. Controlled variables, method of controlling variables and their importance for the investigation.

Controlled variables	Importance of the variables	Method of controlling
Source of carrots.	Different sources of carrots can provide carrots of different species, not necessarily *Daucus carota*, which may mean different calcium content. This in turn can lead to inconsistencies in amounts of EDTA-Na_2·H_2O used, thus, falsification of the results.	Buying only one species of carrot, *Daucus carota*, and only from one grocer - the same supplier - ABC grocer.
Mass of carrots.	Different masses of carrots in samples can lead to different calcium contents in those samples, hence, inconsistencies in amounts of EDTA-Na_2·H_2O used and inaccuracies of the results.	Using an electronic scale to weigh exactly 150g of grated carrots for each set of trials.
Volume of filtrate used to prepare the analyte.	Different volumes of filtrate used to prepare analytes would cause the inconsistencies of calcium concentration in those analytes, which would cause the falsification of the results.	Using the same amount of filtrate, 25cm^3, for each trial, measured by 25cm^3 pipette.
Volume of distilled water used for thermal processing of carrots.	Norm has to be maintained between trials. Using too much distilled water can dilute the solution, hence, making calcium concentration in water lower than in other samples. At the same time smaller amounts of water can make calcium concentration in samples too high. Both can lead to inaccurate outcomes.	Using the same amount of water, 250cm^3, measured by measuring cylinder for each thermal processing of carrot.
Concentration of	Norm has to be maintained between trials. Different concentrations of EDTA-Na_2·H_2O solution can lead to facilitation of results. If a diluted solution would be used on one sample, it would cause ions not to bond. It means that EDTA-Na_2·H_2O would not be in excess, resulting in a different colour of solution during titration.	There was only one solution of 0.01 moles dm^{-3} EDTA-Na_2·H_2O used for all the trials.
Volume of distilled water used to prepare the analyte.	Volume of water presenced in the solution does not affect the titration itself, however, it dilutes the whole solution, making its colour more watery. This in turn can lead to inaccuracies in terms of EDTA-Na_2·H_2O used in order to see colour change.	Using the same amount of water, 100cm^3, measured by measuring cylinder for each solution.
Concentration of NaOH solution used to prepare the analytes.	Concentration of NaOH solution changes the pH of the analyte. Since pH affects the way EDTA-Na_2· H_2O creates bonds with calcium, different pH of analytes would falsify the results.	There was only one solution of 0.1 moles dm^{-3} NaOH used for all the trials.
Volume of NaOH used to prepare the analytes.	Volume of NaOH added to the analyte affects the final pH of the analyte, which in turn disrupts the bonding of EDTA-Na_2·H_2O with calcium and makes the results inaccurate.	Adding exactly the same volume of NaOH solution to each analyte, 10cm^3, measured by the 10cm^3 pipette.
Volume of murexide solution used in the titration	Amount of murexide affects the colour of the initial solution. Different volumes of murexide solution would cause differences in colours of solutions, leading to misinterpretation in determining end-point.	Only 2 drops of ethanolic murexide solution was added by drop pipette to each analyte.

— Apparatus used in the investigation —-

Table 2. Apparatus and its uncertainty used during the investigation. Number of apparatus needed given in brackets.

Apparatus	Uncertainty	Apparatus	Uncertainty
(5) 250cm^3 Erlenmeyer flask	-	(1) Dropping pipette	-
(1) 500cm^3 Erlenmeyer flask	-	(1) Spatula	-
(1) 25cm^3 burette	± 0.050cm^3	(1) Sheet of filter paper	-
(1) 250cm^3 measuring cylinder	± 2.000cm^3	(1) Clamp stand	-
(1) 100cm^3 measuring cylinder	± 1.000cm^3	(1) Grater	-
(1) 500cm^3 volumetric flask	± 0.500cm^3	(1) Cutting board	-
(1) 250cm^3 volumetric flask	± 0.300cm^3	(1) Electronic scale	± 0.001g
(1) 10cm^3 graduated pipette	± 0.040cm^3	(1) Water bath	± 0.100°C
(1) 25cm^3 graduated pipette	± 0.060cm^3	(1) Timer	± 0.010s
(1) 200cm^3 beaker	-	(1) Protective gloves, glasses and coat	-
(1) Pipette pump	-	(1) Funnel	-

— Materials used in the investigation —-

Table 3. Materials used in the investigation.

Materials	Amounts per one set of trials*	Amounts for entire experiment**
Daucus carota	150.00g	750.00g
Distilled water	800.00cm^3	7750.00cm^3
Sodium hydroxide (NaOH)	-	2.00g
EDTA-Na_2·2H_2O ($C_{10}H_{14}N_2O_8Na_2$·2H_2O)	-	0.93g
Murexide ($C_8H_8N_8O_6$)	-	1.00g
Ethanol (C_2H_6O)	-	19.00cm^3

***** Amounts needed to conduct 5 trials (all in one temperature group). Does **not** include distilled water needed to prepare solutions of NaOH (500cm^3) and EDTA (250cm^3) and to fill a 3dm^3 water bath.

****** Amounts needed for the entire experiment (25 trials together). Does include distilled water needed to prepare solutions of NaOH (500cm^3) and EDTA (250cm^3) and to fill a 3dm^3 water bath.

— Safety, environmental and ethical issues —-

Safety issues: While holding the Erlenmeyer flasks taken from water bath at temperatures higher or equal 70°C gloves with additional heat protection were worn, since these temperatures can burn the skin; Safety glasses, protective gloves and lab coat were worn through the entire experiment, since sodium hydroxide is a substance with corrosive properties, hence, can cause skin damage and eyes damage; Increased caution was maintained while dealing with fragile glassware, which could cut the skin if broken, as well as while grating the carrot on sharp grater, which could also cut the skin if used not properly.

Environmental issues: Remaining EDTA-Na_2·H_2O solution was even more diluted with water and then poured down to the sink, at the end the sink was again poured down with water. EDTA-Na_2·H_2O solution cannot be poured down to soil due to its harmful effect on the natural environment.

Ethical issues: There were not any ethical issues during the conduction of this experiment, since there were not any animal species involved in the investigation.

------- Method used in the experiment ----------

— Preparation of Standard Solution of 0.01 mol dm^{-3} EDTA-$Na_2 \cdot H_2O$ —

- To prepare a Standard Solution of 0.01 mol dm^{-3} EDTA-$Na_2 \cdot H_2O$, the relative molecular mass of EDTA-$Na_2 \cdot H_2O$ was calculated to find the amount of solid substance needed to prepare a solution. It was done by adding molecular masses of its elements (taken from Section 6 of Chemistry Data Booklet):

$\mathbf{M_{EDTA} = 10(12.01) + 18(1.01) + 2(14.01) + 10(16.00) + 2(22.99) = 372.28 g\ mol^{-1}}$

- Then relative molecular mass was multiplied by 0.01, which is desired concentration:

$\mathbf{M_{EDTA\ needed} = 372.28 g\ mol^{-1} \cdot 0.01 mol\ dm^{-3} \approx 3.72 g\ dm^{-3}}$

- 250cm^3 of EDTA solution was prepared, hence 3.72 g has to be multiplied by 0.25:

$\mathbf{M_{EDTA\ needed} = 3.72 g\ dm^{-3} \cdot 0.25 dm^3 = 0.930 g}$

- After obtaining results, 0.930 g of solid EDTA-$Na_2 \cdot H_2O$ was measured with the use of an electronic scale.

- Weighed EDTA-$Na_2 \cdot H_2O$ was put into 250cm^3 volumetric flask and poured with distilled water until the 250cm^3 graduation mark was reached; Then the flask plugged and circularly moved until the powder was completely dissolved in water; Turning upside down is desired if the powder does not easily dissolve.

— Preparation of 0.1 mol dm^{-3} NaOH solution —

- Molecular masses of sodium, oxygen and hydrogen were added to calculate molecular mass of NaOH:

$\mathbf{M_{NaOH} = 22.99 + 16.00 + 1.01 = 40.00 g\ mol^{-1}}$

- Then needed mass of solid NaOH was calculated (above mentioned steps shortened into one question):

$\mathbf{M_{NaOH} = 40.00 g\ mol^{-1} \cdot 0.10 mol\ dm^{-3} \cdot 0.50 dm^3 = 2.000 g}$

- 2.000 g of solid NaOH were weighed and put into 500cm^3 volumetric flask; Distilled water was poured into the flask until 500cm^3 graduation mark was reached; Then the flask was plugged and circularly moved until NaOH grains were completely dissolved in water; Turning upside down is desired if the powder does not easily dissolve.

— Preparation of 20cm^3 of 5% ethanolic murexide solution —

- 5% ethanolic murexide solution was obtained accordingly to the following formula:

$\mathbf{\%C = \frac{mass\ of\ solid\ murexide\ (g)}{mass\ of\ the\ solution\ (g)} \cdot 100\%}$ so $\mathbf{\%5 = \frac{mass\ of\ solid\ murexide\ (g)}{20g} \cdot 100\%}$, hence, $\mathbf{0.05 \cdot 20g = 1.000g}$

- 1.000g of murexide ($C_8H_8N_8O_6$) was poured with 19.000g of ethanol and resulted in the 5% solution.

— Preparation of carrots' content solution —

NOTE: Before preparing the carrots' content solution, 3dm^3 of distilled water was poured into the water bath; Then the bath was set on a desired temperature in each sample.

(This method was applied for the 25°C sample and was repeated with the remaining samples.)

- Water bath was set on 25°C and left for heating up.

- Carrots were grated on the cutting board and systematically weighed until obtaining 150g.

- 150g of grated carrots was put into the 500cm^3 Erlenmeyer flask and when the water bath reached the temperature of 25°C it was poured down with 250cm^3 of distilled water.

- Prepared Erlenmeyer flask was put in the water bath and covered to prevent any evaporation of solution.

- The timer was set for 10 minutes; After this time the Erlenmeyer flask was removed from the water bath.

— Preparation of analytes —

(This method was applied for the 25ºC sample and was repeated with the remaining samples.)

- Content of coolled sample was gradually poured onto a folded circle of filter paper put in the funnel, and filtered into the beaker; It was necessary to not clog the pipette with the remains of carrots.
- When the beaker was filled with around 150cm^3 of filtered solution, 25cm^3 of the solution was measured 5 times with the use of 25cm^3 pipette and poured to 5 different 250cm^3 Erlenmeyer flasks.
- To each Erlenmeyer flask 100cm^3 of distilled water measured with the 100cm^3 measuring cylinder was added; Then 10cm^3 of sodium hydroxide solution was added by 10cm^3 pipette in order to create a basic environment; Entire solution was gently moved in circular pattern during the process.
- At the end 2 drops of ethanolic murexide solution was added; The solution was circulatory moved until all the compounds were mixed.

— Conduction of the complexometric titration —

(This method was applied for the 25ºC sample and should be repeated with remaining samples).

- EDTA-$Na_2 \cdot H_2O$ solution was poured into the burette placed in the clamp stand until 0.00cm^3 was reached.
- A white piece of paper was placed on the table, and then the previously prepared analyte was put on it; The paper created a bigger contrast between table and analyte, which allowed for faster recognition of the colour change, hence, obtaining more accurate results.
- The initial volume of the titrant was written down.
- The titration was carefully started by allowing drops of titrant to drip slowly into the analyte; Analyte was gently mixed through the process of titration.
- The tap has been immediately closed after the colour change of analyte from pink to purple had occurred.
- The final volume of titrant presenced in the burette was written down.
- The process was conducted for the remaining trials in this temperature group with taking the final volume of the prior titration as the initial volume for the next titration.

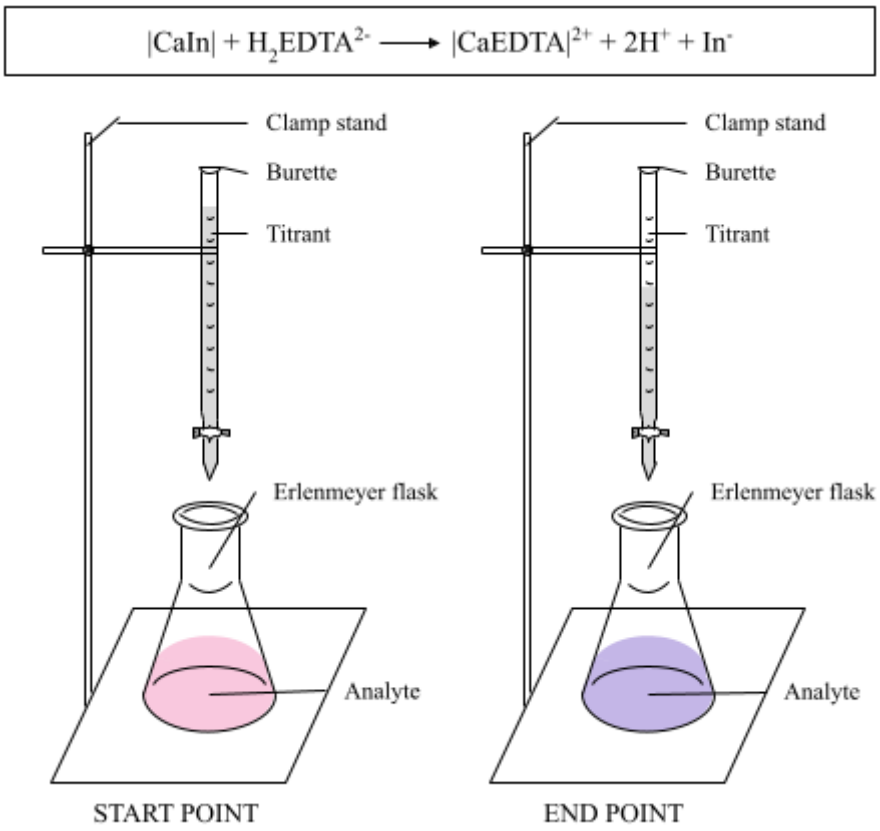

Figure 1. Process of complexometric titration

3. Results and Discussion

— Observations —

- Carrots that underwent thermal procedure at higher temperatures were more soft and mushy than those that were thermally processed at lower temperatures.
- Carrots' solutions were more orangish after processing at higher temperatures than at lower temperatures.
- More EDTA-$Na_2 \cdot H_2O$ was used during titration of analytes prepared from carrots's content solutions processed at higher temperatures.
- Filtration of 150cm^3 carrots' calcium solutions processed at higher temperatures took much more time than filtration of those processed at lower temperatures.

— Raw data —

Table 4. Volume, (V), ± 0.05cm^3 of EDTA-Na_2·H_2O solution used in each trial during the complexometric titration.

	25ºC		40ºC		55ºC		70ºC		85ºC	
	Initial (V)	Final (V)	Initial (V)	Final (V)	Initial (V)	Final (V)	Initial (V)	Final (V)	Initial (V)	Final (V)
Trial 1	0.00	1.10	0.00	1.20	0.00	1.80	0.00	2.00	0.00	2.50
Trial 2	1.10	2.00	1.20	2.40	1.80	3.70	2.00	4.10	2.50	4.90
Trial 3	2.00	3.00	2.40	3.70	3.70	5.60	4.10	6.20	4.90	7.30
Trial 4	3.00	4.10	3.70	4.80	5.60	7.40	6.20	8.30	7.30	9.70
Trial 5	4.10	5.10	4.80	5.90	7.40	9.20	8.30	10.30	9.70	12.10

------- Processed data ----------

— Establishment of calcium ions content in carrots' content solutions —

- At first, the amount of EDTA-Na_2·H_2O used for each trial was calculated by subtraction of Initial*(V)* value from the Final*(V)* value; Values are presented in *Table 4*; All example calculations based on 25ºC set of trials:

Example: 1.10cm^3 - 0.00cm^3 = 1.10cm^3

- After calculating the amount of EDTA-Na_2·H_2O solution used in each trial, all the volumes were converted from cm^3 to dm^3 as dm^3 is a standard volume unit; All the values were divided by 1000:

Example: 1.10cm^3 : 1000 = 0.0011dm^3

- As information from each single trial does not allow for conducting the analysis as properly as averaged values permit, the mean value of EDTA solution used in titration was calculated for each set of trials:

Mean = $\frac{\Sigma\ (volume\ of\ EDTA\ used\ in\ a\ trial)\ [dm3]}{number\ of\ trials}$, hence, **Mean** = $\frac{0.0011+0.0009+0.0010+0.0011+0.0010}{5}$ **= 0.00102dm^3.**

- At the end, uncertainty of mean of volume of EDTA solution added was determined:

ΔV = $\frac{maximum\ value\ of\ EDTA\ added - minimum\ value\ of\ EDTA\ added}{2}$, hence, **ΔV** = $\frac{0.0011 - 0.0009}{2}$ **= 0.0001dm^3.**

Table 5. Average volume, V, (dm^3) of EDTA solution added into tirand and uncertainties, ΔV, (dm^3) for its calculations.

Temperature, T, (ºC), (± 0.1ºC)	Average volume, V, (dm^3) of EDTA solution added	Uncertainty of average volume, ΔAv_V, (dm^3) of EDTA solution added
25.0	0.00102	± 0.00010
40.0	0.00118	± 0.00010
55.0	0.00184	± 0.00005
70.0	0.00206	± 0.00005
85.0	0.00242	± 0.00005

- In research from *Rajkovic* it was mentioned that EDTA has a 1:1 stoichiometric ratio with all cations. As the complexometric titration goes along with the equation shown on *Figure 1* in my investigation, it was concluded that the number of EDTA-Na_2·H_2O moles is equal to the number of calcium moles.

- If the number of EDTA-Na_2·H_2O moles is known, the number of moles of calcium can be easily determined. Thus, the number of moles of EDTA-Na_2·H_2O was calculated with the following formula:

number of moles, n = concentration, C · volume, V

- Since $\mathbf{n_{EDTA} = n_{Ca2+}}$ then $\mathbf{C_{EDTA} \cdot V_{EDTA} = C_{Ca2+} \cdot V_{Ca2+}}$

- So $\mathbf{C_{Ca2+}} = \frac{C\ of\ EDTA \cdot V\ of\ EDTA}{V\ of\ Ca2+}$

- Therefore, $\mathbf{C_{Ca2+}} = \frac{0.0100 mol/dm3 \cdot 0.00102 dm3}{0.0250 dm3} = \mathbf{0.000408 mol\ dm^{-3}}$

- To provide fully proper estimations of calcium ions concentrations, absolute uncertainty was calculated:

$$\frac{\Delta C\ of\ Ca2+}{C\ of\ Ca2+} = \frac{\Delta C\ of\ EDTA}{C\ of\ EDTA} + \frac{\Delta V\ of\ EDTA}{V\ of\ EDTA} + \frac{\Delta V\ of\ Ca2+}{V\ of\ Ca2+}$$

$$\frac{\Delta C\ of\ Ca2+}{0.000408 mol/dm3} = \frac{\pm 0.0005000 mol/dm3}{0.010000 mol/dm3} + \frac{\pm 0.000050 dm3}{0.001020 dm3} + \frac{\pm 0.000060 dm3}{0.025000 dm3}$$

$$\frac{\Delta C\ of\ Ca2+}{0.000408 mol/dm3} = \mathbf{0.10141961}$$

- Thus, $\Delta C_{Ca2+} = \mathbf{0.10141961 \cdot 0.000408 mol\ dm^{-3} = 0.00004138 mol\ dm^{-3}}$

- Results from the above mentioned calculations were rounded to the same number of significant figures to make results more clear to analyse (*Table 6*):

Table 6. Average concentrations, C, (mol dm^{-3}) of Ca^{2+} and uncertainties of calculations obtained during the investigation.

Temperature, T, (°C), (± 0.1°C)	Concentration, C, (mol dm^{-3}) of Ca^{2+} obtained	Absolute uncertainty of calculations, ΔAu_{Ca2+}, (mol dm^{-3})
25.0	0.00041	± 0.00004
40.0	0.00047	± 0.00004
55.0	0.00074	± 0.00006
70.0	0.00082	± 0.00006
85.0	0.00097	± 0.00007

- From the calculated Ca^{2+} concentrations the mass of Ca^{2+} which leached during thermal processing is calculated; Molecular mass of calcium is taken from Section 6 of Chemistry Data Booklet:

$\mathbf{m_{Ca2+} = C_{Ca2+} \cdot V_{Ca2+} \cdot M_{Ca2+}}$

$\mathbf{m_{Ca2+} = 0.00041 mol\ dm^{-3} \cdot 0.025 dm^3 \cdot 40.08 g\ mol^{-1} = 0.00041082 g}$

- With known mass of Ca^{2+} absolute uncertainty can be calculated:

$$\frac{\Delta m\ of\ Ca2+}{m\ of\ Ca2+} = \frac{\Delta C\ of\ Ca2+}{C\ of\ Ca2+} + \frac{\Delta V\ of\ Ca2+}{V\ of\ Ca2+} + \frac{\Delta M\ of\ Ca2+}{M\ of\ Ca2+}$$

$$\frac{\Delta m\ of\ Ca2+}{0.00041082 g} = \frac{\pm 0.00004 mol/dm3}{0.00041 mol/dm3} + \frac{\pm 0.000060 dm3}{0.025000 dm3} + \frac{\pm 0.010000 g/mol}{40.08000 g/mol}$$

$$\frac{\Delta m\ of\ Ca2+}{0.00041082 g} = \mathbf{0.10021048}$$

$\mathbf{\Delta m_{Ca2+} = 0.1021048 \cdot 0.00041082 g = 0.00004195 g}$

- Results from the above mentioned calculations were rounded to the same number of significant figures to make results more clear to analyse (*Table 7*):

Table 7. Average masses, m, (g) of Ca^{2+} left in the water, and uncertainties of calculations obtained during the investigation.

Temperature, T, (°C), (± 0.1°C)	Mass, m, (g) of Ca^{2+} obtained	Absolute uncertainty of calculations, ΔAu_{Ca2+}, (g)
25.0	0.00041	± 0.00004
40.0	0.00047	± 0.00004
55.0	0.00074	± 0.00006
70.0	0.00082	± 0.00006
85.0	0.00097	± 0.00007

— Establishment of remaining mass of calcium ions in carrots —

- To establish the mass of calcium that remained in the carrot, the information about carrots' calcium content was taken from *Background knowledge*: carrots tend to have about 33mg of calcium per 100g. Examples based on the 25ºC group. Calcium content in 1g was obtained by dividing 33mg by 100g:

33mg : 100g = 0.33mg per gram

- 150g of carrots were prepared for 5 trials, so each trial contained the amount of calcium corresponding to the amount of calcium prescended in 30g of carrots. Calcium content in 30g of carrots were calculated:

0.33mg · 30 = 9.9mg

- In 25ºC, on average, 0.41mg leached from carrots (*Table 7.*) This value refers to calcium that leached from 25cm^3 carrots' content solution. Since for each set of trials, carrots were boiled together in 250cm^3 of distilled water, the value of 0.41mg was multiplied by 10:

0.41mg · 10 = 4.1mg

- Then calcium content that leached from carrot was subtracted from the overall carrots' calcium content:

9.9mg - 4.1mg = 5.8mg (average amount of calcium that is left in the carrot after processing at 25ºC).

— Establishing all the errors —

- Exemplar calculations were prepared for the 25ºC set of trials. Preparation of EDTA-Na_2·H_2O, NaOH and murexide solutions were conducted only once for all the trials.

- Random error for the set of trials was obtained by applying the following formula:

$$\mathbf{E}_{\mathbf{R}} = \frac{Apparatus\ uncertainty\ (+)}{Mass\ of\ measured\ material} \cdot \mathbf{100\%}$$

Table 8. Calculations of random errors that could affect the experiment.

Apparatus	Materials used	Random error
Electronic scale	EDTA-Na_2·H_2O powder	± 0.001g : 0.930g · 100% = 0.108%
Volumetric flask	EDTA-Na_2·H_2O solution	± 0.300cm^3 : 250cm^3 · 100% = 0.120%
Electronic scale	NaOH grains	± 0.001g : 2.00g · 100% = 0.050%
Volumetric flask	NaOH solution	± 0.500cm^3 : 500cm^3 · 100% = 0.100%
Electronic scale	Solid murexide	± 0.001g : 1.00g · 100% = 0.100%
Electronic scale	Ethanol	± 0.001g : 19.0g · 100% = 0.005%
Measuring cylinder 250cm^3	Distilled water	± 2.00cm^3 : 250cm^3 · 100% = 0.800%
Measuring cylinder 100cm^3	Distilled water	± 1.00cm^3 : 100cm^3 · 100% = 1.00%
Pipette	NaOH solution	± 0.040cm^3 : 10.0cm^3 · 100% = 0.400%
Pipette	Carrots' solution	± 0.060cm^3 : 25.0cm^3 · 100% = 0.240%
Burette	Titrant	± 2 · 0.050cm^3 : 1.02cm^3 · 100% = 9.80%

- Percentage uncertainty, %, was calculated by summing up all the random errors; Values were rounded, since there is only one decimal place prescended in the value of calcium ions that left in carrots:

% uncertainty = 0.108% + 0.120% + 0.050% + 0.100% + 0.100% + 0.005% + 0.800% + 1.00% + 0.400% + 0.240% + 9.80% = 12.683% ≈ 12.7%

- Amount of calcium content left multiplied by percentage uncertainty provide the absolute uncertainty:

5.8mg · 12.7%, hence, **5.8mg · 0.127 = ± 0.7366 ≈ ± 0.7mg**

Table 9. Mass, m, (mg) of Ca^{2+} left in the carrots; Absolute and percentage uncertainties included.

Temperature, T, (°C), (± 0.1°C)	Mass, m, (mg) of Ca^{2+} left in carrots	Percentage uncertainty (%)	Absolute uncertainty (mg)
25.0	5.8	12.7	± 0.7
40.0	5.2	12.7	± 0.7
55.0	2.5	12.7	± 0.3
70.0	1.7	12.7	± 0.2
85.0	0.2	12.7	± 0.1

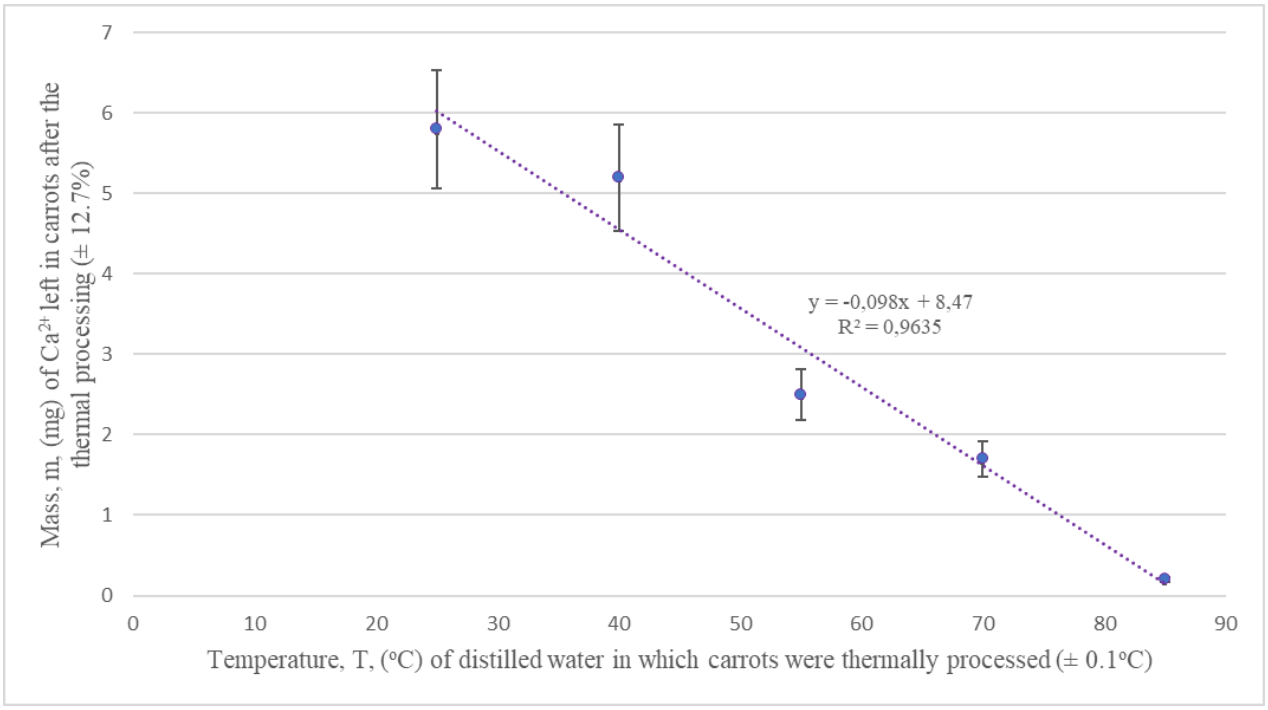

Figure 2. Relationship between the different temperatures, T, (°C) (25°C, 40°C, 55°C, 70°C, 85°C) of distilled water and the content, m, (mg) of calcium which has not been released from the carrots during the thermal procedure.

------- Analysis of the results ----------

At first, the validity of the results was examined. For this purpose, the ANOVA test was conducted. The main role of ANOVA test is to analyse and state statistical differences between non-identical samples.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
25	5	5,1	1,02	0,007
40	5	5,9	1,18	0,007
55	5	9,2	1,84	0,003
70	5	10,3	2,06	0,003
80	5	12,1	2,42	0,002

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7,0016	4	1,7504	397,8181818	9,44221E-19	2,866081402
Within Groups	0,088	20	0,0044			
Total	7,0896	24				

Figure 3. ANOVA test result

The test has been conducted in Microsoft Excel with the use of Analysis ToolPak. Data used in the test was based on results presented in *Table 4*. In order to analyse correlations introduced by ANOVA test properly, two hypotheses have been formed:

H_0: There is no visible relationship between calcium content and different temperatures of distilled water.

H_1: There is a significant relationship between calcium content and different temperatures of distilled water.

— Interpretation of the results —

- Results obtained from ANOVA test, (*Figure 3*), indicated value of F = 397.82 and value of p-level = $9.44E^{-19}$. Thus, as the probability level was tremendously low ($p < 10E^{-8}$), the **H_0 (null hypothesis)** was rejected and **H_1** was confirmed, which led to the conclusion that there exists a significant relationship between calcium content and different temperatures of distilled water in my experiment. This in turn greatly enhanced the reliability of my results.

- In correlation with ANOVA test results, *Figure 3* has also shown the relationship between different temperatures of distilled water, and calcium content of thermally processed carrots by forming a trendline of negative gradient. In addition the R^2 was observed to be close to 1. Such phenomena suggest that changes in dependent variables on the graph occur due to changes taking place in independent variables.

- Relatively small % uncertainty is a result of properly chosen laboratory glassware, and high precision maintained during the course of the experiment. Small uncertainties make outcomes more reliable.

- The quantitative data presented in *Table 9* shows that in 25°C 5.8mg (± 0.7mg) of calcium remained in the carrot, so around 58.6% of its original content. When compared to higher temperatures, it turned out that in 85°C only 0.2mg (± 0.1mg) was left in the carrot, so barely 2.0% of the initial content suggested by literature.

- *Table 7* shows that the increase of temperature results in the increase of Ca^{2+} that leaches into the water in which the carrots were thermally processed.

- There are no outstanding differences in EDTA-$Na_2 \cdot H_2O$ solution used in the titration, and Ca^{2+} that leached into the water in trials conducted in temperatures lower or equal 55°C and higher or equal 70°C (*Table 5; Table 7)*, so the second part of the hypothesis has to be rejected.

— Justification of the results —

Provided information has its reflection in scientific sources. As mentioned in *Background knowledge*, the increase of temperature causes higher amounts of calcium to react with oxalic acid:

$$\mathbf{Ca^{2+} + C_2O_4H_2 \rightarrow CaC_2O_4 \downarrow + 2H^+}$$

Calcium oxalate in presence of oxalic acid creates soluble compounds which can easily leach from the vegetable. In cell walls of plants there are covalent bonds that are affected by high temperatures. Since calcium is a compound responsible for maintaining the stability of cell walls in plants (WHITE), increasing the temperature will facilitate calcium leaching, and the destruction of cell walls as a result. This theory is supported by the *Observations* section, in which more mushy and soft properties of carrots processed at high temperatures were mentioned. Loss of rigidity and turgor of carrots was caused by increased permeability of carrots's membrane, resulting from low amount of calcium in carrots' content (Wiley and McCulloch).

4. Conclusions

This experiment aimed to investigate the effect of subjecting *Daucus carota* to thermal processing at different temperatures, T, (ºC) (25ºC, 40ºC, 55ºC, 70ºC, 85ºC) on its calcium content. The hypothesis with two different assumptions has been put forward. First assumption suggested that with the increase of temperature in which carrots have been thermally processed, there will be a visible decreasing trend in calcium content left in those carrots. This part of the hypothesis has been proven (*Interpretation of the results.*). The second part of the hypothesis assumed a significant increase of Ca^{2+} leached into the water, hence, in the EDTA solution used, between trials conducted in temperatures lower or equal 55ºC and higher or equal 70ºC. However, as shown in *Interpretation of the result, Table 5* and *Table 7,* this part of the hypothesis has been rejected, since the highest increase occurs between trials conducted at 40ºC and 55ºC (~36% difference). There was only about a 10% difference in Ca^{2+} leached between 55ºC and 70ºC. All in all, the hypothesis has been only partially confirmed.

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Conflicts of Interest

The authors declare no conflict of interest.

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