Research Question: How does the soaking time (0,4,6 hours) and Temperature (25°C and 80°C) of Kombu (*Laminaria japonica*) affect the amount of iodine remaining in the Kombu before consumption using colorimetric determination with 1.0% starch indicator?

Introduction and Background Information

Seaweed has been increasingly lauded worldwide as a food with various health benefits, providing "a rich and sustainable source of macronutrients and micronutrients to the human diet" and helping "the alleviation of risk factors associated with noncommunicable diseases such as obesity, type 2 diabetes and cardiovascular disease" (Cherry et al. 1). As such, edible seaweed has been readily consumed by many in the form of snacks, salads, soups and more.

Iodine is a micronutrient that helps to ensure normal thyroid function, growth and development. According to the World Health Organization, the recommended daily iodine intake ranges from 90-250μg for people in different age groups and conditions: 90 μg for preschool children (0 to 59 months); 120 μg for schoolchildren (6 to 12 years); 150 μg for adolescents (above 12 years) and adults; 250 μg for pregnant and lactating women (World Health Organization). Food and water are major sources of iodine - seaweeds, seafood and dairy products all contribute to the daily intake of iodine for the average person.

Over the past decades, iodine deficiency disorder (IDD) has been categorised as a serious problem worldwide, it being "a significant health problem in 118 countries" (Kapil). Iodine is essential for producing hormones that the thyroid gland uses; excess or deficiency in iodine intake may be detrimental to one's health, "lead[ing] to a great number of symptoms" (Institute for Quality and Efficiency in Health Care [IQWiG]) such as goitre and hypothyroidism. The most severe cases of IDD may lead to "Brain damage and irreversible mental retardation" (Kapil 267). Furthermore, thyroid cancer has been linked to both under and overconsumption of iodine - "the prevalence of anaplastic thyroid cancer (ATC)... decreased in many countries with the introduction of iodized salt", however "papillary thyroid cancer (PTC) increased" (Lund and Wu). To combat the prevalent problem of iodine deficiency worldwide, "universally effective… salt iodization programmes" (Micronutrient Deficiencies) have been implemented to supplement citizens' diets. However, excessive intake of salt may itself lead to adverse effects, such as hypertension - hence, seaweed may be seen as a viable alternative to iodized salt, acting as a healthier option whilst providing a source of iodine. However, excessive intake of iodine may also have negative effects, such as "iodine-induced goitre due to persistent stimulation by thyroid-stimulating antibodies that… propagate lymphocytic infiltration" (Farebrother et al. 53).

In marine algae, "iodine exists as a combination of organic and inorganic forms", with "more than 60% of total soluble iodine [being] I^-" (Hou et. al 218) and low IO^-_3 values. However, in Kombu (*Laminaria japonica*), iodine is present in both inorganic and organic forms, with iodine "accumulated… in its reduced form, iodide" (Küpper et. al 6954). Furthermore, it was found that "Ninety-nine percent of total iodine is soluble in *Laminaria japonica*", with "more than 94%" (Hou et. al 218) of the water-soluble inorganic iodine being I^- coupled with low IO^-_3 values. The remaining insoluble residue would be considered as organic iodine. The very high percentage of water-soluble iodine present in Kombu signifies that when soaked in a liquid, such as in soup broth or hot pot, much of its iodine content would diffuse into the liquid. Therefore, unless the liquid is consumed, the benefits of the iodine would be largely lost, as it would have dissolved into the water.

In the metabolism of iodine in the body, "a series of stages involving the hypothalamus, pituitary, thyroid gland and blood" (Farhana and Ganie 13) are followed. The most important reactions from the metabolic process is the production of thyroxine (T4) and triiodothyronine (T3) thyroid hormones, which then "diffuse… into the blood stream" (Farhana and Ganie 14). Both T3 and T4 hormones are responsible for increasing the basal metabolic rate; this relies on a feedback mechanism. A fall in the levels of T3 or T4 would stimulate the pituitary gland's secretion of TSH, leading to the release of additional thyroid hormones from the thyroid gland into circulation.

Personal Engagement/ Significance

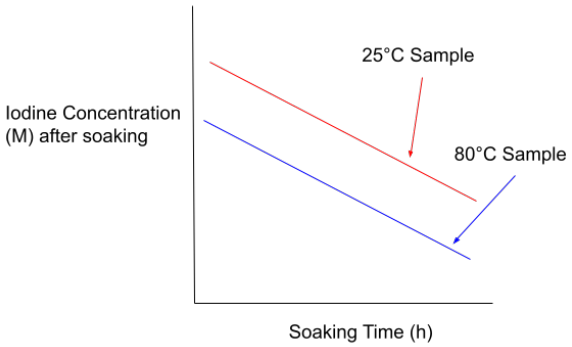
According to a study conducted by Hong Kong's Centre of Food Safety, "most seaweed and seaweed products contained very high iodine levels" (Dietary). Furthermore, "the mean level of dried kelp was 2 600 000 μg/kg" (Dietary), which translates to 26 grams/kg, a very high amount - even a small amount of kombu consumed per day would meet or exceed the recommended daily limits of iodine intake by the WHO. However,

it was found that “around 59% of the population [in Hong Kong] intakes below 50μg [of iodine]/day”, which falls under the recommended daily intake of the WHO. This means that there is “the existence of mild iodine deficiency [in Hong Kong], which can result in clinical IDD” (Kung et. al 419).
Many people in Hong Kong eat hot pot regularly, and I noticed that they would often order seaweeds, in particular Kombu (*Laminaria japonica*) to put into the soup to enhance the flavour. However, it is a common conception that drinking hotpot soup may be unhealthy due to the high amounts of sodium, fats and oils involved, therefore many people would opt not to consume the hotpot soup. As a result, much of the iodine content from the kombu would be left in the soup. I am interested in finding how much iodine is left in Kombu after soaking in a liquid, and whether the amount left would be enough to sustain a healthy diet with sufficient iodine content.

Hypothesis

***Null Hypothesis - (H_0)*:** There is no significant difference in the means of values obtained as Iodine concentration after different soaking times of seaweed.
***Alternate Hypothesis - (H_A)*:** There is a significant difference in the means of values obtained as Iodine concentration after different soaking times of seaweed.

To test the veracity of these claims and to find whether the hypothesis should be accepted, multiple T-tests will be conducted to verify significant differences in iodine after different soaking conditions (time and temperature).



Graph 1: Hypothesis for experiment

Trends Expected

As the soaking time increases, a greater amount of iodine within the sample (solubles) will leave the seaweed, therefore resulting in a lower amount within the seaweed extract, resulting in a decreased value of absorbance measured using the colorimeter. The increase in temperature of the soaking water from 25°C to 80°C will increase the particle kinetic energy within the sample resulting in a larger amount of iodine leaving the sample. The expected results are demonstrated in Graph 1.

Pre-testing

To test the methodology, pre-testing was conducted to check its validity. Based on a study regarding Iodide Determination using spectroscopy, “the maximum absorption band… to identify iodide based on the formation of an insoluble blue iodine-starch complex… is obtained at 615 nm” (Sulistyarti et al. 45). Therefore, this investigation will aim to be based on the 615nm wavelength (orange region).

However, it was found that the Vernier colorimeter used for the experiments was unable to be set directly to 615nm. Hence, the 635nm wavelength (red region) was chosen to conduct testing, as it was the closest alternative to the optimal value; the slightly altered wavelength would not significantly disrupt or skew the results gained from the experiment (assumption).

The original mixture of chemicals - potassium iodide and starch solution, did not produce the desired product iodine, signalled by the lack of a blue-black colour appearing in the mixture with the addition of starch indicator. Furthermore, the viscosity of the solution was low, suggesting that the chemicals had not produced a reaction together. Therefore, accurate results could not be obtained. Consequently, a new method was derived where the chemicals sulfuric acid and hydrogen peroxide were added to the original mixture - this proved to be successful, as then the solutions turned a blue-black colour with a high viscosity, indicating that the presence of iodine by the starch indicator had been detected.

The sheets of kombu did not have a consistent shape and size, as multiple batches of kombu were used over the course of the experimentation. To minimize the variances between sizes and shape, the samples were sorted and cut into identical squares using scissors and rulers, weighed so they were 20 grams each utilising an electronic scale with 3 d.p, then cut into 5 separate pieces using a scissor. This ensured that the size and shape of the tested kombu are more or less the same.

Independent Variables:

Independent Variable	Method of monitoring	Purpose
Temperature of samples (25℃ and 80℃)	Water baths will be set to 25℃ and 80℃, A thermometer will be put into each water-bath to accurately measure the temperature of the water and ensure consistency between multiple trials. Ample time will be given for the water bath to reach the specified temperature.	Simulating room temperature and hotpot simmering conditions respectively, testing if different amounts of iodine are released at different temperatures.
Amount of time soaked (0 hrs, 4 hrs, 6 hrs)	A stopwatch will be used to keep records of how long the samples have been left in the water baths. For the "0 hrs" samples, the samples were placed into the water-bath for 5 minutes measured by a stopwatch to simulate a short time of soaking.	To measure whether different amounts of iodine are released by the Kombu into the liquid in different time conditions.

Dependent variables:

Dependent Variable	Method of monitoring	Purpose
Iodine dissolved by the seaweed samples over a certain period, measured by the concentration of iodine (mol dm^{-3}) in the kombu water	A colorimeter will be used to measure light absorbance readings following the 635nm red-colour wavelength averaging the results over 10 seconds to increase reliability.	A lower absorbance reading (measured using A.U) indicates that there is lower iodine content in the kombu liquid after treatment. This can be observed by an increase in the intensity of the blue-black colour of the solution when the kombu liquid is in the cuvette, ready to be tested.

Controlled Variables:

Controlled Variables	How is it controlled?	Purpose
Amount of chemical solutions used Concentration and Volume of solutions used (KI and H_2SO_4)	Multiple tools that allow for precise liquid collection (such as measuring cylinders, pipettes and syringes) will be used to measure amounts of solutions required.	The amounts of solutions used in each trial should be kept consistent to ensure that any change in absorbance readings is only caused by a change in iodine concentration.
Weight (amount) of seaweed used in each trial	For each trial, 20 grams of kombu are cut into squares and weighed using an electronic scale (3 d.p, ±0.005g)	By keeping the amount of seaweed used in each trial consistent, it would ensure that any variances in iodine concentration are caused by one of the independent variables (temperature or amount of time soaked)

Colorimeter settings	Throughout all trials, the same colorimeter unit, computer and software will be used to measure results. The absorbance wavelength will be kept at 635nm.	The 615nm wavelength (orange) was chosen because of its complementary nature to blue. However, due to constrictions, 615nm was not possible. Hence, 635nm wavelength (red) was chosen instead.
Amount of water added to each sample	Throughout all trials, 200mL of distilled water will be added to each trial (100mL before inserting into the water bath, and an additional 100mL after the crushing and blending process).	The amount of water is kept constant to ensure that any changes in iodine concentration are caused by the independent variables, not by dilution.

Uncontrollable Variables:

Uncontrollable Variables	**Why?**	**How are they rectified?**
Iodine content of different kombu sheets	Between different batches of kombu utilised in trials, the iodine content cannot be guaranteed to be equal in each sheet of kombu.	A control trial will be performed to measure iodine content released into a liquid for each new batch of kombu. Any outliers will be replaced.

Apparatus table

Materials		**Reagents**
Colorimeter (±0.0005 A.U.)	Syringes (1mL, 5mL, 10mL)	0.001M KI solution
Mortar and Pestle	Pipettes	2.0% H_2O_2 solution
Electronic blender	Cuvettes	1.0M H_2SO_4 solution
Electronic scale	Stopwatch(±0.05s)	1.0% Starch Solution
2 Water baths (25°C, 80°C)	Scissors	Distilled Water
Beakers (10mL, 50mL, 100mL, 500mL ±0.1mL)	Ruler	
Test tubes	Laptop (for logging/graphing software)	
Kombu sheets	Glass rod	
Measuring cylinder (10mL, 50mL, 100mL ±0.1mL)	Strainer	

Method

Segment One: Calibration Curve

1. Obtain 100.0 mL of stock 0.001M KI (aq) (potassium iodide) and observe the following:
   a. Observe colour of solution (take photos)
   b. Observe the viscosity of the solution
2. Perform dilution of the stock solution to produce the different concentrations required for the calibration curve using the following dilution table:

a. Use a series of measuring cylinders to obtain the intended concentration (100mL combined volume)

Intended Concentration (M)	Volume of Stock Solution (mL)	Volume of deionized water (mL)
0.0010	20.00	80.00
0.0020	40.00	60.00
0.0030	60.00	40.00
0.0040	80.00	20.0
0.0050	100.00	0.00

3. Obtain a colorimeter and cuvette.
   a. Connect the colorimeter to the laptop and initiate the data logging software
   b. Set the wavelength: 635 nm (red colour)
4. Transfer distilled water into a cuvette; use it to calibrate the colorimeter.
5. Transfer 0.5 mL 1.0M H_2SO_4(aq) + 0.5 mL H_2O_2 into a beaker
6. Transfer 1.0 mL of the 0.0002 M KI solution from Step 2b using a dropping pipette
7. Transfer 1.0 mL of Starch solution into the mixture using another dropping pipette
8. Insert the cuvette into the colorimeter and initiate data collection at 635 nm
9. Repeat Steps 5 - 8 two more times to get three trials of absorbance recording
10. Repeat Steps 3 - 8 , whilst changing the concentration of KI solution (0.0004, 0.0006, 0.0008 and 0.0010M)

Segment Two : Preparation and Experimentation of Seaweed

1. Measure 20.0g of dry seaweed using an electronic balance and weighing boat
   a. Make qualitative observations regarding the colour and distribution of the sample
2. Measure and transfer 100.0 mL of distilled water into the sample using a 100.0 mL measuring cylinder beaker.
3. Measure the amounts of seaweed (20g) and cut into 5 equal strips.
   a. Place seaweed samples inside the beaker with water.
4. Place the beaker in the pre-setup water bath at 25° C (Room Temperature; Control Setup) and allow it to calibrate for a period of 30s controlled using a stopwatch (±0.05s)
5. Leave the sample in the water bath for a period of 0 hours controlled using a stopwatch (IV1)
6. Repeat Steps 1 – 4 9 more times to obtain a total of 10 trials per conditions
7. Repeat Steps 1 – 5 for the remaining independent variable conditions (4, 6 hours)
8. Repeat Steps 1 – 6 whilst altering the temperature of the water bath to the different temperature condition of 80°C (simulate hotpot conditions)
9. Obtain the different samples and discard any liquid remaining within each sample.
10. Place the kombu sample into a mortar and crush the sample using a pestle (10 times) to ease the breakdown of cellular components and release the remaining iodine.
    a. Transfer the crushed sample into the blender
    b. Add a further 100 mL of water into the mixture
11. Blend the samples for a period 15 seconds controlled using a stopwatch.
    a. Transfer blended samples into separate beaker and label “Blended Seaweed”
12. Repeat Steps 9-12 for each sample

Segment Three: Data Collection

1. Obtain the blended sample from Part Two, labelled “Blended Seaweed”
2. Extract the liquid from the blended sample, using a strainer to separate the seaweed and liquid.
3. Obtain a dry cuvette and:

a. Transfer 0.5mL H_2SO_4 + 0.5mL H_2O_2
b. Transfer 2.0 mL of the liquid from the blended sample using a dropping pipette
c. Transfer 0.5 mL of the starch indicator into the sample using a dropping pipette

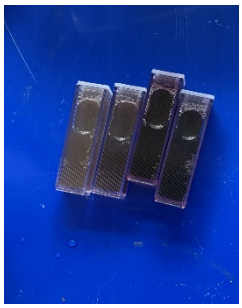
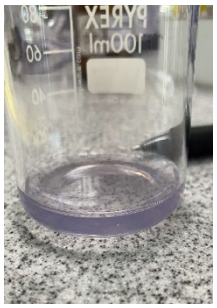
4. Insert the cuvette in the colorimeter and measure the absorbance at the wavelength of 635nm (red wavelength)
    a. Record the values found into the graphing software
5. Repeat Steps 1 - 4 for the water samples containing extracted iodine of 0 hours, 25°C sample (9 trials)
6. Repeat Steps 1 - 5 for the other soaking times under 25°C sample (4, 6 hours)
7. Repeat Steps 1 - 6 for the other temperature condition (80°C)

Safety and Environmental Concerns / Risk assessment

Concern	Prevention
Wasting seaweed	To achieve identical sizes and weights of seaweed samples, many irregularly shaped sheets of seaweed were trimmed. As a result, a lot of waste trimming and residue were created, which may be harmful to the environment as it may be considered as litter. Therefore, to minimize the potential environmental impacts caused by waste trimmings, any waste or extra seaweed would be collected and used to make soup stock.
Blender	There are multiple sharp blades in the blender, which may injure the user if inadvertently touched. Furthermore, sharp objects could be expelled from the blender when blending, which may cause injury to the eye. Therefore, protective goggles will be worn at all times when conducting experiments, and multiple checks will be performed to ensure that the blender is used under safe conditions.
Glassware and chemicals	Various glassware will be used during the experimentation process, which poses the risk of injury if equipment is damaged and broken. Furthermore, corrosive chemicals would be handled, such as H_2SO_4, which may cause injury if in contact with skin or eyes. Therefore, protective goggles would be worn at all times when conducting experiments, and all experiments would be performed standing up to ensure ample time to react if equipment is accidentally broken or chemicals spilt.

Data Collection and Analysis

Qualitative Observations

		
When discarding the existing Kombu liquid after taking samples out of the water bath, the liquid was a dark green colour, had high viscosity and had a distinct seaweed smell.	A sample iodine-starch test done with the Kombu liquid shows light blue-black colour, indicating light presence of iodine. This is from 4hrs, 25°C sample.	Different colour intensities of the blue-black colour present in different samples, demonstrating different iodine concentrations are exhibited here. The left two samples were from 0 hrs, 25°C samples, and the right two are from 6 hrs, 25°C.

Quantitative Data

Table 1: Raw Data Table showing the values obtained for the calibration curve (Absorbance ±0.0005 A.U.)

Trial No.	*One*		*Two*		*Three*	
Concentration of KI(aq) (M)	Transmittance (%)	Absorbance (A.U.)	Transmittance (%)	Absorbance (A.U.)	Transmittance (%)	Absorbance (A.U.)
0.001	1.1626	1.9346	1.1758	1.9478	1.1603	1.9323
0.002	0.9218	2.0354	0.9184	2.0319	0.9245	2.0381
0.003	0.8725	2.0593	0.8682	2.0550	0.8767	2.0635
0.004	0.8335	2.0791	0.8102	2.0558	0.8885	2.1341
0.005	0.8455	2.0729	0.8910	2.1184	0.9121	2.1395
NOTE: Only the absorbance value (A.U.) will be used to draw the calibration curve						

Data Processing: Calculating Mean and Standard Deviation

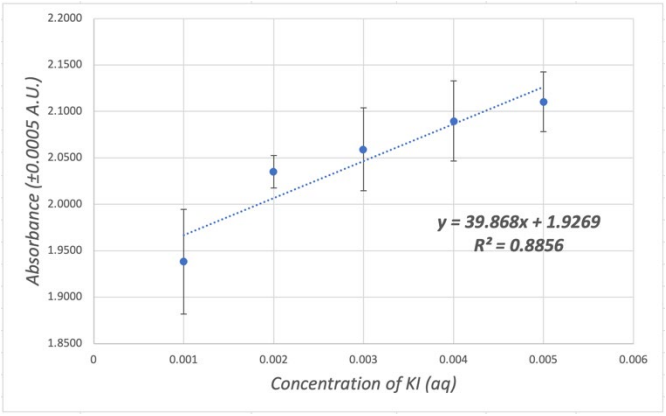
Table 2: Processed Calibration Curve Data table (Absorbance ±0.0005 A.U.)

	Absorbance (A.U.)	
Concentration of KI(aq) (M)	Mean	Standard Deviation
0.001	1.9382	0.0563
0.002	2.0351	0.0173
0.003	2.0592	0.0445
0.004	2.0897	0.0432
0.005	2.1103	0.0322

Calibration Curve Equation:

Y = 39.868x + 1.929

Graph 2: Calibration Curve



The mean and standard deviation of the absorbance is plotted against the concentration of potassium iodide (KI) to create the calibration curve.

Table 3: Raw Data of Absorbance at 635nm for the Kombu samples under different temperature and soaking time stress

Temperature (±0.5°C)		25°C			80°C		
Soaking Time (Hrs)		0	4	6	0	4	6
Trial No.	T1	2.0674	2.0831	2.0720	2.1950	2.0557	1.9323
	T2	2.2870	2.1567	1.9876	2.4612	1.9980	1.9711
	T3	2.1878	2.1434	2.0512	2.1956	2.1181	1.9399
	T4	2.3923	2.0175	1.9752	2.1734	2.0378	2.0730
	T5	2.3543	2.0453	1.9689	2.2055	1.9956	2.0260
	T6	2.4232	2.1348	1.9932	2.3280	1.9523	1.9360
	T7	2.0542	2.1667	1.9642	2.4041	2.0063	1.9713
	T8	2.2870	2.0887	2.0033	2.3980	2.0226	1.9392
	T9	2.1836	2.1766	2.0895	2.1523	2.0920	1.9926
	T10	2.3427	1.9844	1.9950	2.1434	1.9384	2.0245

Data Processing

Conversion of absorbance values to concentration of I_2 (M)

Calibration Curve:

Y = 39.868x + 1.929

Sample Calculation: 25°C 0 Hours Soaking Time (Trial One)

Absorbance = 2.0674

2.0674 - 1.929 = 39.868X (Concentration of Iodine; M)

X = 0.00347M

Table 4: Processed Data Table (Concentration of I- Values)

Temperature (±0.5°C)		25°C			80°C		
Soaking Time (Hrs)		0	4	6	0	4	6
Trial No.	T1	0.00347	0.00387	0.00359	0.00667	0.00318	0.00008
	T2	0.00898	0.00571	0.00147	0.01335	0.00173	0.00106
	T3	0.00649	0.00538	0.00307	0.00669	0.00474	0.00027
	T4	0.01162	0.00222	0.00116	0.00613	0.00273	0.00361
	T5	0.01067	0.00292	0.00100	0.00694	0.00167	0.00243
	T6	0.01240	0.00516	0.00161	0.01001	0.00059	0.00018
	T7	0.00314	0.00596	0.00088	0.01192	0.00194	0.00106
	T8	0.00898	0.00401	0.00186	0.01176	0.00235	0.00026
	T9	0.00639	0.00621	0.00403	0.00560	0.00409	0.00160
	T10	0.01038	0.00139	0.00166	0.00538	0.00024	0.00240

Data Processing

Mean Concentration of Iodine

$$Mean\ Concentration\ of\ Iodine\left(\frac{mol}{dm^3}\right) = \frac{SUMMATION\ OF\ TRIALS\left(\frac{mol}{dm^3}\right)}{10}$$

$$\frac{0.00347+0.00898+0.00649+0.01162+0.01067+0.01240+0.00314+0.00898+0.00639+0.01038}{10} = 0.00825 \text{ mol dm}^{-3}$$

Standard Deviation Formula: $S = \sqrt{\frac{\sum|x-\bar{x}|^2}{n-1}}$

$$S = \sqrt{\frac{|0.00347-0.00825|^2 + |0.00898-0.00825|^2 + |0.00649-0.00825|^2 + |0.01162-0.00825|^2 + |0.0167-0.00825|^2 + |0.01240-0.00825|^2 + |0.00314-0.00825|^2 + |0.00898-0.00825|^2 + |0.00639-0.00825|^2 + |0.01038-0.00825|^2}{10-1}}$$

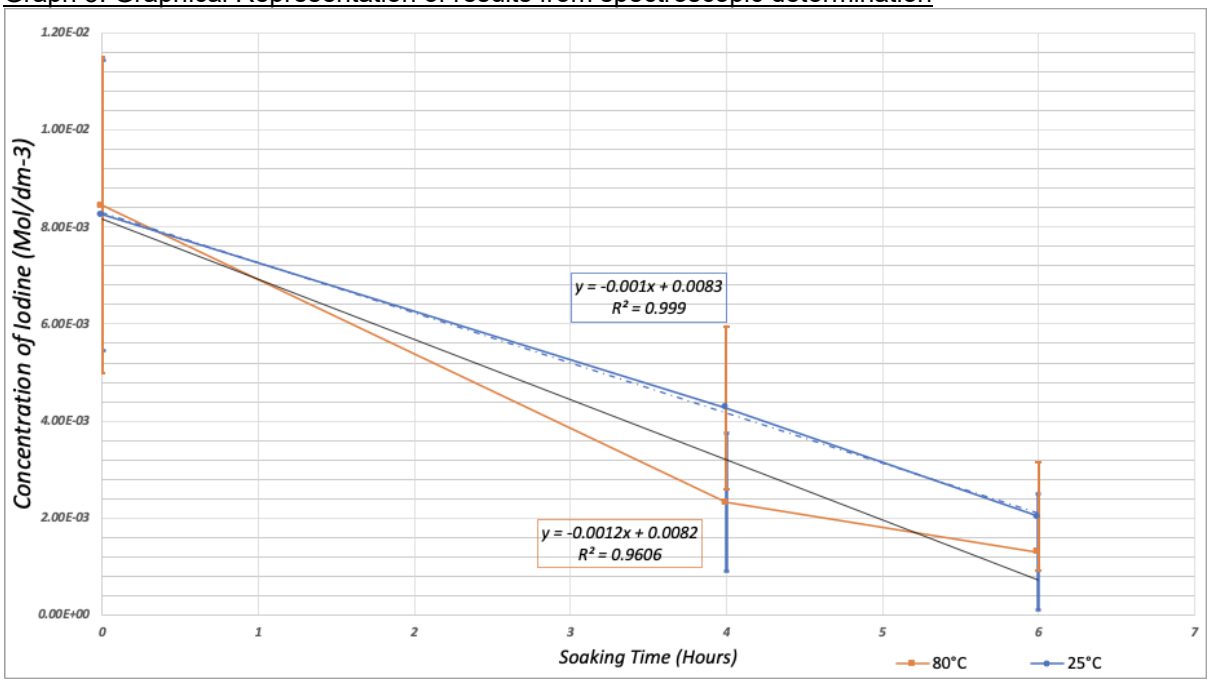
$$S = 0.00326$$

Table 5: Final Processed Data Table

Soaking Temperature		25°C			80°C		
Soaking Time		0	4	6	0	4	6
Concentration of Iodine (M)	Mean	0.00825	0.00428	0.00203	0.00844	0.00232	0.00129
	Standard Deviation	0.00326	0.00168	0.00112	0.00300	0.00142	0.00120

Graphical Representation

Graph 3: Graphical Representation of results from spectroscopic determination



Data Interpretation

Graph 3 shows the relationship between the soaking time of seaweed and the concentration of iodine calculated after spectroscopic determination. It is evident that there is a negative correlation between the independent and dependent variable irrespective of the temperature of the soaking water. As the soaking time increases the concentration of Iodine within the seaweed decreases under both temperature conditions. The linear trends lines used to model the data can be taken with a significant degree of confidence due to the high r-squared values obtained (0.999 and 0.9606) for each of the temperature conditions. (°C) An r-squared value closer to 1 indicates a high *goodness of fit,* which is the linear trend line that passes through the data points at different soaking times closely and evenly.

The gradients of the two lines verify the negative correlation with negative values of (-0.001 and -0.0012) respectively. The highest concentration of Iodine is obtained for the non-soaking conditions with values of 0.00825 and 0.00844 mol dm^{-3} respectively whilst the lowest values of iodine concentration was obtained for the 6 hours soaking time with values of 0.00203 and 0.00129 mol dm^{-3}. However, one of the marked observations is to understand the influence of temperature on the cleavage of iodine from the Kombu. The data points for each soaking time at the two respective temperatures are quite similar, which decreases the confidence of comparison and the influence of temperature in changing the iodine concentrations.
The primary observation of the data points indicates that with respect to temperature that is a significant decrease in the Iodine content between 0 to 4 hours in the 80°C (0.00844 to 0.00232M) sample in comparison to the sample at 25°C (0.00825 to 0.00428M).

Errors and Uncertainties

The investigation was planned and executed in a manner to decrease the overall percentage uncertainty of the investigation. The use of spectroscopic analysis to determine iodine concentration decreases the overall % uncertainty of the investigation with accuracy of the absorbance reading at ±0.0005 A.U.. Additionally, the use of pipettes and droppers with high accuracy contribute to the overall decreased % uncertainty of the investigation.

The error bars in Graph 3 are plotted using Microsoft Excel and represent the variance of the data plotted as Mean Concentration of Iodine (mol dm^{-3}). The error bars represent 1 SD, meaning that 68% of the data points collected for that combination lies within the boundaries of the error bars. Furthermore, the error bars are quite large in magnitude which was expected due to the biological uncertainty brought about by the testing of a real-world species such as Kombu.

The highest standard deviation is obtained for the 0 hours soaking conditions (0.00326 and 0.0030 mol dm^{-3}) indicating that the initial amount of iodine present prior to treatment also varies significantly.

Overlap Analysis

Soaking Temperature		25°C			80°C		
Soaking Time		0	4	6	0	4	6
Concentration of Iodine (M)	Mean	0.00825	0.00428	0.00203	0.00844	0.00232	0.00129
	Standard Deviation	0.00326	0.00168	0.00112	0.00300	0.00142	0.00120

25°C Condition

0 Hours: 0.00825 ± 0.00326 (Range: 0.00499 - 0.01151)
4 Hours: 0.00428 ± 0.00168 (Range: 0.0026 - 0.00596)
6 Hours: 0.00203 ± 0.00112 (Range: 0.00091 - 0.00315)

Overlap between the two conditions indicated in **bold** necessitates the use of statistical tests such as a two-way t-test to verify if the data points are significantly different from each other, demonstrating that a combination of temperature and soaking time results in different amounts of iodine leaving the seaweed.

80°C Condition

0 Hours: 0.00844 ± 0.003 (Range: 0.00544 - 0.01144)
4 Hours: 0.00232 ± 0.00142 (Range: 0.0009 - 0.00374)
6 Hours: 0.00129 ± 0.0012 (Range: 0.00009 - 0.00249)

Significant overlap in all the data points collected for the particular soaking conditions indicates that multiple t-tests will be required to verify if there is a significant difference in the iodine concentrations obtained for the different soaking time conditions.

T-test

A student's t-test (statistical test) tests if two experiment groups (soaking time) differ in their concentration of iodine, achieved by comparing the two samples drawn from the two groups. The significance behind this statistical test is that if two soaking times of Kombu differ, we can conclude that the two populations from which the samples are drawn differ from each other. On the contrary, the two samples tested in this investigation are rather similar. Therefore, the Null Hypothesis (H_0) cannot be rejected, as the evidence in the collected data is insufficient to conclude that the two populations where the samples are drawn from differ from one another. Multiple t-tests are performed to determine significance for the variables mentioned in the overlap analysis.

***Null Hypothesis - (H_0)*:** There is no significant difference in the means of values obtained as Iodine concentration after different soaking times of seaweed.
***Alternate Hypothesis - (H_A)*:** There is a significant difference in the means of values obtained as Iodine concentration after different soaking times of seaweed.
Alpha: 0.05 (**95% Significance**)
Degrees of Freedom: $N_1 + N_2 - 2 = 10 + 10 - 2 = 18$
T-critical Value (Read from T-distribution table – 18 & v = 0.95): **1.734** (NIST/SEMATECH).

T-test Results

Table 6: T-test results

Soaking Temperature	Comparison (Soaking Times)	T-calculated value	Inference
25°C	0 and 4 Hours	***3.42559***	T-calculated value is significantly greater than T-critical value at 0.05 (95% Confidence). **The alternate hypothesis is accepted**
	4 and 6 hours	***3.52953***	
80°C	0 and 4 hours	***5.83282***	
	4 and 6 Hours	***1.75543***	T-calculated value is less than t-critical value at 0.05 (95% Confidence). **The Null hypothesis is accepted.**

Three of the 4 t-tests performed with the actual data returned values in which the t-calculated value was greater than the t-critical value at 95% confidence, providing the basis for rejecting the Null Hypothesis. Therefore, the Alternate Hypothesis is accepted. There is a significant difference in the means of data collected as iodine concentration for different soaking hours except for the soaking time comparison of 4 and 6 hours in which the Null Hypothesis is accepted (T-calculated value < T-critical Value).

Conclusion and Scientific Reasoning

The experiment was conducted to evaluate the use of seaweed in cooking methods such as hot pot and how it will affect the availability of Iodine within the diet. It was hypothesised in the exploration section that increasing soaking time and temperature of seaweed samples in water will cause a decrease in the amount of iodine present within the sample. The data obtained in the investigation supports the hypothesis that increasing soaking time decreases the amount of iodine present within the sample (Independent variable One), however is not able to accurately verify if the changes in temperature can affect the amount of iodine present within the sample (overlapping data, temperature not significant).

Evaluation

Absorbance readings of the seaweed solutions are collected with 4 d.p. (decimal places), ensuring that results are collected with a high degree of accuracy. Furthermore, the Vernier colorimeter utilised throughout the investigation had a high degree of accuracy (±0.0005 A.U), increasing the reliability of results as they are utilised when analysing and interpreting the results. Additionally, the percentage uncertainty of the absorbance readings is 0.025%, which is quite a low value. This is derived by dividing the uncertainty of the device by the lowest mean absorbance and multiplying by 100 (0.0005/1.9828) x 100 = 0.025%.

A calibration curve was used to convert absorbance values to concentrations, therefore the change in concentration in the standardized units of mol dm^{-3} is facilitated. As a result, the results of the investigation are able to be translated to more real-world applications. Moreover, by performing 10 trials per condition throughout the investigation, the reliability of the data can be improved, and it allows the opportunity to perform multiple statistical tests as an additional way to prove the veracity of the collected data.

Limitations in Approach

The vernier colorimeter has an absorbance range of 0 to 3.0 A.U. which falls within the range of data collected in the investigation (Lowest: 1.9323 and Highest: 2.4612), the online specification listed indicates that the useful range of recording is between 0 to 1 A.U. (Colorimeter Specifications). which means that the data collected contains a high degree of inaccuracy. This is a systemic issue with the device used for data collection, therefore in order to minimize errors; the concentrations of solutions used for the calibration curve can be diluted by a significant factor to reduce the absorbance readings. Additionally, the seaweed juice extracted from the different independent variable conditions can be diluted by a significant factor to produce more dilute solutions which will give absorbance readings in the range of 0 to 1.0 A.U.

Weaknesses

Weakness	Significance	Improvements
One: Inconsistency and loss of sample content	Transferring the crushed seaweed to the beakers to be put into the water baths, there would be a lot of seaweed lost through the transferring process. This may influence the veracity of results, as less amount of seaweed may artificially reduce the amount of iodine present in the seaweed juice.	Utilise a blender to blend and break apart each seaweed sample. This would minimize the procedural steps where the crushing process using the Mortar and pestle will be eliminated from the experimental procedure.
Two: Variance in times between sample testing	The blender could only blend one sample of seaweed at a time, which meant that when taking trials of a condition out from the water bath, some samples would have a greater delay than the others. This may have allowed more iodine to be released into the seaweed juice, disproportionately affecting the results of the experiment.	Using multiple blenders to prepare more seaweed samples along with using multiple colorimeters to test absorbance simultaneously. Streamline the process of preparing samples and inserting them into the cuvette. Increase the speed setting of the blender which will decrease the time needed to blend the samples for the different independent variable conditions. Additionally, a time limit for the preparation time between two samples can be introduced, to decrease any added variances between different samples.
Three: Difference in iodine content between batches of seaweed	Between different batches of seaweed used in the investigation, their iodine content would differ slightly due to genetic and metabolic differences. It was assumed that the differences in iodine content would be marginal.	Use a variety of different seaweed batches when testing each scenario (for example, mixing different seaweed batches when testing a scenario for 10 trials). This way all trials would be affected by the same magnitude in all trials and conditions. On the contrary, an iodine concentration test can be performed prior to experimentation (different soaking time and temperature). These values as iodine concentration can be listed and therefore the percentage decrease in Iodine concentration can be calculated.

Four: Evaporation of water content from seaweed samples	When placing the seaweed samples into the water baths, there was no cover applied to decrease the impact of evaporation. Especially with a high temperature (e.g 80°C), then the rate of evaporation (loss of water) would be higher increasing the concentration of iodine within the testing sample.	Place a cover over the water bath to reduce the rate of evaporation for the seaweed samples. Place cling film over the openings of each beaker to stop any water from evaporating when put in the water baths. Any amount of water evaporated will recondense back into the beaker.

Extension

Regarding the extensibility of this investigation, the water temperature of the water baths could be raised to better simulate hot pot conditions (90-100°C); it was unable to be done due to school safety restrictions. Furthermore, more types of seaweed could be tested, as Kombu is a very specific type of seaweed, and there often are many different types of seaweed used when eating hot pot. Additionally, the same test could be conducted on different foodstuffs high in iodine content, such as cod, where their iodine content "ranged from 29.8 µg to 512.8 µg" (Dahl et al. 5), with different cooking conditions such as pan-frying, baking and steaming to determine the differences in iodine content lost.

Furthermore, a wider range of temperatures may be tested, and the amount of iodine lost compared, to tell me how best to cook seaweed to maintain its taste and iodine content at the same time, creating healthier food while eating hot pot.

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