INTERNATIONAL BACCALAUREATE DIPLOMA PROGRAMME
CHEMISTRY INTERNAL ASSESSMENT

Title: The effect of pH on Maillard reaction
Research Question: *To what extent does the pH of coconut water affect its browning, measured as an absorbance, caused by the Maillard reaction between its chemical components, namely reducing sugars and amino groups on proteins, peptides and amino acids?*

1 Introduction:

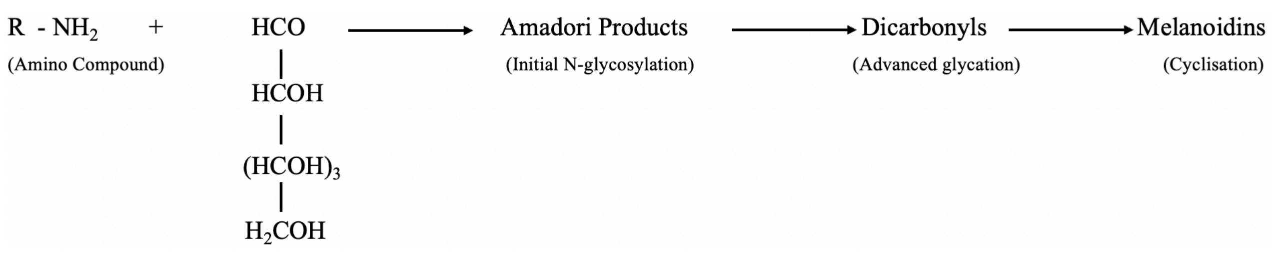
More than one and a half years ago, the government imposed the first lockdown to limit the spread of the novel coronavirus. It was at this time when bread baking became a heightened activity in many households, and my former biology teacher tasked our class with baking a loaf of bread. Although my first bread was far from perfect, since then, I have improved and experimented with multiple types and recipes, from ciabatta to Artesian bread. Nevertheless, I still struggle to achieve a dark brown crust comparable to the commercially available bread. While I was working on my Extended Essay, I explored the Maillard reaction that takes place between amino groups on proteins, peptides and amino acids, and reducing sugars. The Maillard reaction occurs during the thermal preparation of the food, and it is responsible for the observable browning of the food (de Oliveira et al., 2016).

Learning about Maillard reaction has sparked my interest in influencing the factors affecting this reaction, helping me to achieve a darker crust on bread. I found that besides temperature, increasing pH can decrease the activation energy of the Maillard reaction (Oh et al., 2006); hence, pH can affect browning in an inversely proportional manner. Since the coconut water contains all the reactants involved in the Maillard reaction, i.e., proteins (0.1 g) and sugars (3 g), I investigated its browning under different pH, with my research question formulated as follows: ***To what extent does the pH of coconut water affect its browning, measured as an absorbance, caused by the Maillard reaction between its chemical components, namely reducing sugars and amino groups on proteins, peptides and amino acids?***

2 Exploration:

2.1 Studied reaction:

Although coconut water contains multiple amino compounds and reducing sugars, all of them undergo Maillard reaction in the same manner. Therefore, the general and simplified scheme can be written as follows:



Following the initial N-glycosylation, Amadori products undergo a series of rearrangements. A sufficient supply of energy leads to the advanced glycation and formation of dicarbonyls, more specifically, advanced glycation end products. If the supply of energy continues, these products start forming polymeric substances–brown pigments known as melanoidins (de Oliveira et al., 2016).

2.2 Background:

Maillard reaction occurs mainly under conditions with high humidity (presence of water as a substrate) and high temperature. Since the concentration of the reactants in the Mallard reaction is not limiting, and it heavily relies on the external factors favouring the reaction, it can be classified as a zero-order reaction with a constant rate under constant conditions. Alongside temperature and water content, the acidity of the system in which the reaction takes place also determines the rate of Maillard reaction (Stamp & Labuza, 1983).

Activation energy describes the amount of energy that successfully triggers a reaction. In the Maillard reaction, the condensation (N-glycosylation) occurs between the amino functional group (NH_2) on amino acid and the carbonyl functional group (CO) on the saccharide. This reaction, however, is energetically unfavourable as it requires overcoming hydrogen bonds between hydrogen and nitrogen in amines and a double bond between the carbon-oxygen bond of carbonyl. Therefore, the reaction requires a considerable supply of energy to stochastically increase the frequency of collisions between the particles possessing sufficient kinetic energy to activate the Maillard reaction. Still, the E_a for the Maillard reaction varies between 23 and 238 kJ/mol depending on the conditions such as the state of an amino compound (reduced/ oxidised), humidity and salinity. Hence, the energy in the form of heat is not the only decisive factor in the reaction.

The acidity of a solution is measured as pH, a value denoting the concentration of protons (H^+) in the aqueous solution on a logarithmic scale. pH value has a considerable effect on the solubility of proteins, macromolecules composed of amino acids linked by a peptide bond, in a solution. A protein has an isoelectric point (pI) equal to the pH value at which it has no net charge and so is least soluble. The previous research has shown a general trend implying that the solubility of a protein is directly proportional to the pH of its surroundings, i.e., decreasing pH decreases the solubility of a protein (Jiang et al., 2010). Solubility of a protein in the environment is an important prerequisite for the condensation of reducing sugars with the amino acids comprising proteins by N-glycosylation. The alkaline environment (pH>7) can induce deprotonation of amino compounds by decreasing the E_a for the Maillard reaction, during which the reducing saccharide reduces the amino compound (Lund & Ray, 2017). Therefore, the pH of the reaction environment affects the state of the reactants involved in the Maillard reaction, directly determining its rate (conversion of reactants to the products) and even occurrence.

Acidic environment:

$R\text{-}NH_2 + H^+ \longleftrightarrow R\text{-}NH_3^+$ amino compound is reduced $\longrightarrow$ further reduction by reducing saccharide- high E_a

Alkaline environment:

$R\text{-}NH_2 \longleftrightarrow R\text{-}NH + H^+$ amino compound is oxidised $\longrightarrow$ reduction by reducing saccharide- low E_a

Figure 1 The reduction and oxidation of an amino compound in terms of hydrogen gain/loss and its effect on the E_a of Maillard reaction (reduction)

3 Method

3.1 Model Reaction System

Since the Maillard reaction occurs mainly during the thermal preparation of *food*, I chose coconut water as a model system for the Maillard reaction. According to the information provided by the producer, 100 cm^3 of coconut water contains 0.1 grams of proteins and 3 grams of sugars. Together with its edibility/potability, it makes coconut water suitable not only for the acquisition of quantitative data (absorbance, laboratory experiment) but also qualitative data (browning during baking).

3.2 Variables

3.2.1 Independent Variable:

The concentration of H^+ in the solution affects the oxidation state of the amine functional group, and therefore, pH can determine the browning caused by the Maillard reaction (*Figure 1*). I chose 5 distinct values of pH, which should be a sufficient number to construct a reliable trendline demonstrating its effect on the browning. Moreover, to counter possible errors in measurements, I evaluated the intensity of browning for every pH value in triplicate. The pH values tested were 3, 4, 6, 7, 8. Since the natural pH of the coconut water is 4 (acidic), I chose one point below and then only above until I reached the value of 8. To decrease and increase the pH of coconut water citric acid and sodium bicarbonate were used, respectively. The food with values on an interval between 3 and 8 should not be harmful to people and correspond to the pH of the food. For a similar reason, only weak acid and weak base with common culinary applications were used.

3.2.2 Dependent Variable:

Coconut water is mostly transparent liquid with low absorbance. Since the Maillard reaction is known as non-enzymatic browning, its products can affect the absorbance of the substance measured in absorbance units (AU). There are three stages of Maillard reaction, with the last being specific for the formation of melanoidins, brown and poorly soluble polymers. In preliminary trials, measuring absorbance at 420 nm proved to produce the most consistent result and was in line with the published literature (Karseno et al., 2017; Stamp & Labuza, 1983). Therefore, absorbance can reliably show the different stages of Maillard reaction reached in a directly proportional manner, i.e., higher the absorbance higher the concentration of melanoidins and hence more advanced stage of the reaction.

3.2.3 Controlled Variables:

1. <u>Time and Temperature:</u> The supply of heat to the reaction system affects the conversion of reactants to the products. Thermal preparation of food usually happens at temperatures over 333.15 K (60 °C). Hence, the maximal reachable temperature of 375.5 K (102 °C) was maintained for 8 minutes and 30 seconds. This ensured that the difference in absorbance between samples with different acidity would not be caused by qualitatively and quantitively unequal supply of energy in the form of heat.
2. <u>Instruments:</u> The shape of the beaker with the sample was maintained consistently as the different beakers have a non-identical area of contact with the source of heat and can interfere with the heating of a sample.
3. <u>Volume:</u> Water is the major component of coconut water. The inconsistent volume of heated samples could interfere with the time of heating as a sample with a higher volume would require more energy to reach the fixed temperature. Hence to avoid the

inconsistent time of heating before the experimental temperature was reached, an equal volume of 15 cm^3 was always heated.

3.2.4 Measured Variables:

1. Volume difference: Since the evaporation of water might have contributed to the overall absorbance, I measured this variable for later evaluation of its effect on absorbance.

3.3 Apparatus

1. Graduated cylinder (± 0.1 cm^3)
2. Spatula
3. pH meter (± 0.01)
4. Magnetic stirrer
5. Beaker 25 cm^3
6. Tripod
7. Gauze mat
8. Bunsen burner
9. Thermometer (± 0.1 °C)
10. Cuvette
11. Colourimeter (± 0.01 AU)
12. Stopwatch (± 1s)

3.4 Experimental Procedure

1. 15 cm^3 of well-shook coconut water was measured in the graduated cylinder and poured into the 25 cm^3 beaker.
2. The beaker was placed on the magnetic stirrer (medium speed), and the pH meter was set up for a continuous reading mode with a correction to 298.15 K (25 °C)
3. Sample preparation:
   a. Natural- pH was not altered, note the natural acidity (Tab. 2- NA)
   b. Acidified- a small amount of citric acid using a spatula was added until a pH of about 3 was reached
   c. Alkalinised-
      i. With a magnetic stirrer on, a small amount of sodium bicarbonate using a spatula was being added every 15 seconds until the pH of about 6 was reached
      ii. With a magnetic stirrer on, a small amount of sodium bicarbonate using a spatula was being added every 15 seconds until the pH of about 7 was reached
      iii. With a magnetic stirrer on, a small amount of sodium bicarbonate using a spatula was being added every 15 seconds until the pH of about 8 was reached
4. The beaker was placed on the gauze mat on a tripod over a Bunsen burner above the tip of the primary flame
5. A holder was used to place a thermometer into the beaker so that it would not touch the bottom of the beaker
6. When the temperature reached 375.5 K (102 °C), 8 minutes and 30 seconds (time of heating) commenced being counted
7. Immediately after 8 minutes and 30 seconds the beaker was moved to the container with cold water
8. The coconut water was let to cool down to 298.15 K (25 °C)
9. The change in volume was determined using a graduated cylinder
10. Using Pasteur pipette the cuvette was filled with the coconut water to the mark
11. The colourimeter was re-calibrated
12. The absorbance was measured
13. Steps 1-11 were repeated with 3 samples for every pH value

3.5 Risk Assessment

1. <u>Safety considerations:</u>
   Sodium bicarbonate is generally deemed as a safe chemical, however, it may cause eye, skin and respiratory tract irritation (*Sodium Bicarbonate- Material Safety Data Sheet*, 2008).
   Citric acid may cause severe eye irritation (*Citric Acid- Safety Data Sheet*, 2014).
   To address these safety issues, personal protective equipment (latex gloves, laboratory coat and shoes, goggles and FFP2 face mask) was worn during the whole experiment. While working with the Bunsen burner all precautions (absence of combustible materials, flame supervision and heat-resistant gloves) preventing fire, explosion and harm were taken.
2. <u>Ethical considerations:</u>
   There were no ethical aspects of this experiment to be considered.
3. <u>Environmental considerations:</u>
   The waste was disposed of in compliance with the environmental standards stated in the safety cards of used chemicals (*Citric Acid- Safety Data Sheet*, 2014; *Sodium Bicarbonate- Material Safety Data Sheet*, 2008).

4 Analysis

Table 1 Raw data- the effect of pH on absorbance including measurable controlled variables

pH [± 0.01]	Absorbance [± 0.01 AU]	Time until T reached [sec]	Volume [± 0.1 cm^3]	Volume after [± 0.1 cm^3]	Absorbance Mean [AU]	Absorbance StDev [AU]
3.33	0.5	69	15	8		
3.30	0.61	71	15	7	0.56	0.06
3.31	0.57	70	15	7.3		
4.44	1.1	96	15	8		
4.44	1.11	87	15	7.5	1.11	0.01
4.45	1.12	92	15	7.3		
6.27	1.42	78	15	7.8		
6.32	1.47	81	15	8.1	1.42	0.05
6.24	1.37	77	15	7.7		
7.27	1.52	73	15	8.4		
7.38	1.51	74	15	8.6	1.51	0.02
7.24	1.48	71	15	8.1		
7.98	1.52	67	15	7.8		
8.01	1.53	68	15	7.7	1.52	0.01
7.97	1.52	81	15	7.6		

Note. StDev- standard deviation, T= 375.5 K

Calculation of arithmetic mean (average):

$$Mean = \frac{\sum Absorbance\ of\ samples\ per\ pH\ value}{number\ of\ samples\ per\ pH\ value}$$

Example calculation of arithmetic mean (average):

$$Mean = \frac{0.5 + 0.61 + 0.57}{3}$$
$$Mean = 0.56$$

Calculation of sample standard deviation (StDev):

$$Standard\ deviation = \sqrt{\frac{\Sigma(observed\ value\ of\ a\ sample - the\ mean\ value\ of\ the\ observation)^2}{number\ of\ observations - 1}}$$

Example calculation of sample standard deviation:

$$Standard\ deviation = \sqrt{\frac{(0.5 - 0.56)^2 + (0.61 - 0.56)^2 + (0.57 - 0.56)^2}{3 - 1}}$$
$$Standard\ deviation = 0.556\ AU$$

4.1 Statistical Analysis of Processed Data

To explore the relationship between the pH and the progress of the Maillard reaction measured as an absorbance, I used the analysis of covariance (ANCOVA). ANCOVA is the only test allowing for the determination of the effect of IV under the effect of the controlled variables, namely the volume of the sample before and after heating (volume difference), and time of heating until a certain temperature was reached. The input values for pH were rounded to the nearest whole number to eliminate the error aggravated by the imprecise addition of the sodium bicarbonate and citric acid respectively. The input values, as well as intervention samples, are displayed in *Table 2*. For the statistical analysis, software Jamovi version 1.6 (Jonathon Love et al., 2021) was used.

Table 2 Input data for the statistical analysis including intervention overview

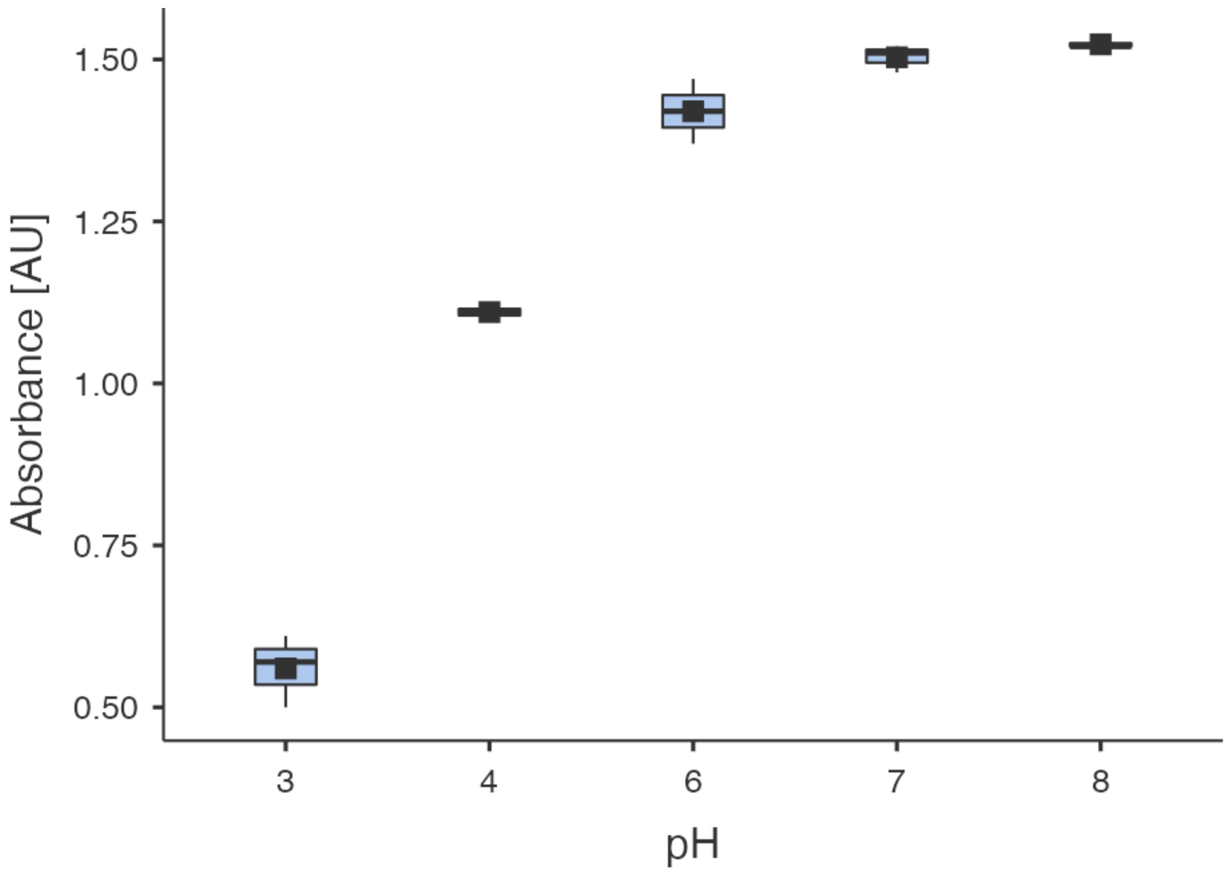
pH altering substance	pH	Absorbance ± 0.01 AU	Absorbance Mean	Absorbance StDev	Volume difference [cm^3]
	3	0.5			-7
Citric acid	3	0.61	0.56	0.06	-8
	3	0.57			-7.7
	4	1.1			-7
NA	4	1.11	1.11	0.01	-7.5
	4	1.12			-7.7
	6	1.42			-7.2
	6	1.47	1.42	0.05	-6.9
	6	1.37			-7.3
	7	1.52			-6.6
Sodium bicarbonate	7	1.51	1.51	0.02	-6.4
	7	1.48			-6.9
	8	1.52			-7.2
	8	1.53	1.52	0.01	-7.3
	8	1.52			7.4

Table 3 ANCOVA based on the input data (Jamovi)

	Sum of Squares	df	Mean Square	F	p
pH	1.802	4	0.450	304.89	<0.001***
Volume difference	6.48×10^{-5}	1	6.48×10^{-5}	0.0438	0.839
Time of heating	4.29×10^{-4}	1	4.29×10^{-4}	0.2906	0.605
Residuals	0.012	8	0.0014		

The values of *p<0.05, **p<0.01 and ***p<0.001 were considered statistically significant.

Graph 1 Graph displaying the relationship between pH and absorbance [AU]



The values are displayed as mean with standard error mean (Jonathon Love et al., 2021).

Table 4 Results of the statistical analysis (ANCOVA) and their interpretation with respect to hypothesis and confounding effect

pH x Absorbance
H_E: Alteration of the acidity of coconut water with sodium bicarbonate or citric acid will affect its browning, measured as absorbance.
H_0: Alteration of the acidity of coconut water with sodium bicarbonate or citric acid will not affect its browning, measured as absorbance.
p= 0.001* **
Result: Null hypothesis can be rejected.
Covariate- Volume difference
p= 0.839
Result: No statistically significant effect of volume difference on pH x absorbance was recorded, hence volume difference is not a confounding variable.
Covariate- Time until the desired temperature was reached
p= 0.605
Result: No statistically significant effect of time of heating until the desired temperature was reached on pH x absorbance was recorded, hence the time of heating is not a confounding variable.

The statistical analysis revealed a significant effect of pH on absorbance measured in AU [$F(4)= 304.87$, $p < 0.001$]. These results imply that the probability of the results obtained by the experiment due to chance is less than 0.1 %. More specifically, the results imply that the effect of pH on the Maillard reaction measured as absorbance [AU] is significant. Additionally, neither the volume of water evaporated nor the time of heating until the desired temperature was reached [$F(1) = 0.291$, $p=0.605$] exhibit a statistically significant effect on the overall absorbance, and therefore, they do not confound the cause and effect relationship between pH and Maillard reaction.

Nevertheless, as can be seen in *Graph 1*, the data follow the logarithmic trend and the gradient of change in absorbance between pH 7 and 8 is lower than in other segments. Therefore, the rise in pH does not affect the Maillard reaction in indefinitely proportional manners.

4.2 Qualitative Observations

1. With increasing pH, the coconut water was visibly browner.
2. After 8.5 minutes of heating at 375.5 K, the coconut water had a pervasive smell.
3. After the end of the heating and at pH 6 and higher, there were dark brown sediments on the beaker's wall.
4. During washing of the beaker, I noticed that the dark brown sediments dissolved in water without any need for soap.

4.3 Apparatus Uncertainties

To work out the percentage uncertainties, the following formula was used:

$$Percentage\ uncertainty = \frac{uncertainty}{measured\ value} \times 100$$

Example calculation of percentage apparatus uncertainty:

$$Percentage\ uncertainty = \frac{0.1\ cm^3}{15\ cm^3} \times 100$$

$$Percentage\ uncertainty = 0.667\%$$

Table 5 Apparatus uncertainties

Apparatus	Uncertainty	Percentage Uncertainty
Graduated cylinder	± 0.1 cm^3	± 0.667 %
pH meter (pH=4)	± 0.01	± 0.003 %
pH meter (pH=6)	± 0.01	± 0.002 %
pH meter (pH=8)	± 0.01	± 0.001 %
Thermometer	± 0.1 °C	± 0.098 %
Stopwatch	± 1 s	± 0.002 %
Mean		± 0.129%

5 Evaluation

5.1 Conclusion

Since the apparatus used in this experiment was relatively simple and the numeric values obtained from the colourimetry were used for comparison, not a direct detection of a fixed value, the results of this experiment provide pertinent evidence supporting the answer to the research question. The reliability of the data acquisition is demonstrated by a low percentage of apparatus uncertainty (± 0.129%), and a major variable capable of interfering with the absorbance -water evaporation- did not have a significant effect on absorbance.

Based on the results of this experiment, the pH of a solution significantly affects the browning induced by the Maillard condensation reaction between reducing sugars and amino groups on proteins, peptides and amino acids in the coconut water. Quantitative data demonstrate a strong effect of the pH on the browning, and both increase proportionately, e.g., in higher pH the absorbance is higher than in the acidic environment over a fixed time. These results are supported also by the qualitative data showing the difference in the colour of the coconut water and colour change induced by heating.
In the context of the scientific literature published on this subject, the results of this experiment are in line with Karseno et al. (2017), who reported a significant effect of pH on the Maillard reaction as well as the directly proportional relationship between these two variables. Likewise, the results of this experiment correspond to the theory proposed in section 2.*2 Background* of this paper. Hence, it is possible to conclude that pH has indeed an effect on Maillard reaction, most likely via protonation and deprotonation of the compound, which affects the activation energy required for the reaction to commence.

5.2 Strengths

The low dispersion of data from the mean denoted by the standard deviations of the individual absorbances was close to zero, pointing to the consistency of the data acquired in this experiment. Moreover, measuring the volume of the sample before and after the heating proved to be a major strength of this experiment as the possible effect of the volume difference could be eliminated by the statistical analysis (ANCOVA). Additionally, the statistical test revealed that the probability of the results being obtained randomly (by chance) is less than 0.1%, which supports the significance of the effect that pH has on the Maillard reaction. The low apparatus uncertainties also increase the reliability of the data collection and outline appropriate construction of the apparatus. Similarly, since the browning of the samples was apparent, also the selection of coconut water appears to be the appropriate selection for the chosen study.
The results of this experiment are in line with the proposed theory and previously published data (Karseno et al., 2017; Lund & Ray, 2017; Stamp & Labuza, 1983). The qualitative evaluation enhances the quantitative interpretation of the results and hence provides pertinent evidence for the role of pH in the Maillard reaction.

5.3 Limitations

Alongside the strengths, my experiment also has several limitations.
First, and perhaps most notable limitation, is the lack of direct measurements for the products of the Maillard reaction. More specifically, although the Maillard reaction certainly occurred, some of the browning of the coconut water might have been caused by the caramelisation. This seems to be at least partially the case as, after the heating, the brown water-insoluble precipitate was present floating on the surface of the water (probably advanced glycation end products), but the brown solid substance on the walls of the beaker was well-soluble in water. Caramel is water-soluble and coconut water contains a considerable amount of sugar (3 grams). Hence, there is a chance that alongside the Maillard reaction the change in the absorbance induced by browning could be to some extent caused by the caramelisation which can also be described as a systematic error. Therefore, it should be consistent across all samples.

Second, the samples were heated using a Bunsen burner whose flame results in an unequal distribution of heat. Similarly, I lit the Bunsen burner individually for every sample and during the four hours of work, the temperature of the surroundings might not have been constant. As a result, several possible inconsistencies in the heating of the individual samples might have occurred. Nevertheless, these were not quantified, and therefore I cannot determine the extent of their effect on the final absorbance.

The third limitation concerns the colourimeter. The values obtained by the colourimeter were probably not absolutely precise. More specifically, upon the start and every re-calibration, the colourimeter showed absorbance 0.02 AU, which should not be possible in the absence of cuvette and sample. Hence, the values could have been imprecise. Nevertheless, since I was comparing the Maillard reaction measured as an absorbance under different acidity of an environment, I was interested in the differences between the values rather than in them explicitly. Therefore, I conclude that this technological error of the device should not interfere with the interpretation of the results but deserves to be brought up for future considerations.

5.4 Extensions and Future Outlook

With the limitations of this experiment in mind, I consider the direct detection of advanced glycation end products to be crucial for the most precise detection of the effect of the pH on the Maillard reaction. This could be done by the measurement of furosine and lactulosyllysine with liquid chromatography and mass spectrophotometry. Further methods include the determination of available lysine by 1-fluoro-2,4-dinitrobenzene (FDNB) or O-ptahalaldehyde (OPA). Nevertheless, both of these methods require thorough safety consideration given the toxicity of the reagents used.

The inconsistency in the temperature can be addressed by using an oven, which should at least expose the sample to the heat with at least partial equality. Moreover, the oven is a closed system mostly independent of external temperature and humidity.

If I were to conduct this experiment again, I would choose a slightly different reaction system- probably a solution of pure amino acid and sugar so that I can control the concentration of both and be able to model reactions with specific reactants and products. Though, this could be considered as an overly artificial setup.

At last, but not least, measuring the rate of a reaction under different pH could also provide us with a deeper understanding of the kinetics of the Maillard reaction. More specifically, measuring and absorbance of the sample heated for several points in time for every pH value (acidic, neutral, alkaline). The gradient of the lines between certain points could then be compared and different rates of change (reaction) could be compared.

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