

### Research Question

How does the degree of fatty acid unsaturation, quantified by their iodine value, of sunflower, canola, peanut, palm, and coconut oils affect the extent of the Maillard reaction in bread, as measured through the concentration of advanced-glycation end products, as measured through UV-Vis spectrophotometry?

### Introduction and Rationale

As an avid baker, I have tried my fair share of recipes and experimented with different substitutions for ingredients. White bread is one of my favourite things to bake; not only is it easy to prepare, it is also very versatile as a basic component for any meal. One thing I noticed, however, was how varied the crust looked in colour every time I prepared a batch with different substitutions; I soon discovered the golden-brown hue of bread crust was a result of the Maillard reaction. The Maillard reaction is a non-enzymatic browning process responsible for the unique flavour of certain food as a result of the glycation of proteins present in the food,<sup>1</sup> and its control is critical to cooks and bakers seeking to take advantage of the flavour compounds produced. In the context of baking in particular, bakers are interested in optimising this process in a bid to achieve an ideal aromatic and colour profile. However, the resulting products of the Maillard reaction also include advanced glycation endproducts (AGEs)<sup>2</sup> like melanoidins, formed at the later stages of the reaction. AGEs,<sup>3</sup> as proteins that have undergone glycation after interaction with sugars, have been found to play a role in the development of atherosclerosis, diabetes, and Alzheimer's disease.<sup>4</sup> These harmful products have led to a rising need to manage the extent of the Maillard reaction<sup>5</sup> in the food industry. Melanoidins are of interest due to their characteristic pigmentation that can be measured by means of spectrophotometry,<sup>6</sup> and therefore can be used to quantify the browning reaction intensity.<sup>7</sup>

Although there have been few reports on the exact mechanism of the effect of lipids on the Maillard reaction, it has been found that different intensities of browning were observable when oils of different degrees of unsaturation were used<sup>8</sup>. The iodine value of oils describes their degree of unsaturation, allowing the degree of unsaturation to be varied for the purposes of this investigation by using oils with a range of iodine values.

### Background Information and Literature Review

#### *Maillard Reaction Mechanism*

The Maillard reaction is a series of complex interactions beginning with a condensation between the free amino group of an amino acid and the free carbonyl group of a reducing sugar<sup>9</sup> to form an N-substituted glycosylamine, an unstable Schiff base, with a carbon-nitrogen double bond<sup>9</sup> where the nitrogen is not bonded to hydrogen. The Schiff base then undergoes Amadori rearrangement,<sup>5</sup> to form Amadori products. Glucose and lysine are the predominant sugar and amino acid found in bread, and this study will focus on their reaction; the Amadori product for the glucose-lysine Maillard reaction is fructoselysine. The Amadori products then undergo several different possible reaction pathways<sup>9</sup> in a series of oxidations, reductions, and hydrations, before finally forming melanoidins<sup>10</sup>.

#### *Relationship with Degree of Fatty Acid Saturation*

The effect of lipids on the formation of AGEs through the Maillard reaction is still a disputed topic, with most of the current literature focusing on Nε-carboxymethyllysine (CML), Nε-carboxyethyllysine (CEL) and pyrraline. Toyosaki et al.<sup>8</sup> claimed saturated fatty acids possibly acted to inhibit the Maillard reaction, but this is not agreed upon by others.

In a glucose-lysine reaction, lysine-derived AGEs are primarily formed from the degradation of the fructoselysine Amadori product.<sup>11</sup> However, it has recently also been found that a significant

proportion of the aforementioned AGEs formed also comes from the reaction between lysine and dicarbonyl compounds.<sup>11</sup> While the relationship between the presence of lipids and the degradation of fructoselysine is still unconfirmed, it has been noted that lipids are involved in the second pathway for AGE formation. Lipid oxidation allows for the formation of  $\alpha$ -dicarbonyl compounds, in particular glyoxal, which consequently promotes AGE formation.<sup>11</sup> Additionally, lipid oxidation produces hydroxyl radicals, which have been reported to promote the production of CML in particular. Since unsaturated fatty acids are more likely to undergo oxidation than their saturated counterparts<sup>12</sup>, it is proposed that unsaturated fatty acids promote AGE formation to a greater extent.

#### *Iodine Value*

The iodine value of an oil is a measure of the amount of iodine, in grams, consumed by 100g of the substance, used to determine the degree of unsaturation of the oil. The double carbon-carbon bond in an unsaturated fatty acid reacts with the iodine, such that a higher iodine value corresponds with a greater number of double bonds and therefore a higher degree of unsaturation. Sunflower, canola, peanut, palm, and coconut oil are chosen for their wide range of iodine values, provided by the literature.<sup>13</sup> However, the iodine value of oils may vary from brand to brand; therefore, I seek to determine the iodine values of the specific samples I used in my investigation to attain a more accurate control over the independent variable.

Oil	Iodine Value (Approximate range)
Sunflower	110-143
Canola	105-126
Peanut	86-107
Palm	50-55
Coconut	6-19

Figure 1: Table of literature iodine values for investigated oils.<sup>13</sup>

#### *Wijs' Method and its Iodometric Alternative*

The traditional method for determining the iodine value of oils is the Wijs' method, where iodine monochloride dissolved in anhydrous ethanoic acid in titration against sodium thiosulfate solution.<sup>14</sup> However, Wijs' reagent is expensive, the carbon tetrachloride required is environmentally unfriendly, poses significant safety risks if handled improperly, and is therefore inappropriate for use in this investigation. Instead, this work uses an alternative method proposed and experimentally validated by Shimamoto et al.<sup>15</sup> The oil sample is dissolved in ethanol and stirred before ethanolic iodine solution is added. Titration with sodium thiosulfate solution is then carried out where the iodine value can be calculated as:

$$\text{iodine value} = \frac{(B-A) \times C \times 12.69}{m}$$

where  $B$  and  $A$  are the volumes, in  $\text{cm}^3$ , of sodium thiosulfate used in the titration of the blank and sample respectively,  $C$  is the concentration, in  $\text{mol dm}^{-3}$  of sodium thiosulfate solution, and  $m$  is the mass of the oil sample used in grams.

#### *UV-Vis Spectrophotometry for Measuring Melanoidins*

The Beer-Lambert law describes the linear relationship between the absorbance of a sample and its concentration, as shown:

$$A = \varepsilon lC$$

where  $A$  is the absorbance of the sample,  $\varepsilon$  is its molar absorptivity,  $l$  is the path length through the medium, and  $C$  is the concentration of the sample.<sup>6</sup>

Unfortunately, it is especially difficult to prepare samples of synthetic melanoidin to adjust their concentrations: they require the ultrafiltration and refluxing of a reducing sugar-amino acid solution over a long period of time,<sup>16,17</sup> both of which are techniques unavailable in my lab. Thus, it is not possible to plot a calibration curve from scratch to determine the concentrations of samples. However, since it has been found that the molar absorptivity of melanoidins is independent of environmental factors such as pH, temperature, and reaction time<sup>2</sup>, I instead use an estimated literature value<sup>18</sup>,  $0.571 \pm 0.01 \text{ mmol}^{-1} \text{ cm}^{-1}$ , for the reaction between glucose and lysine, the main sugar and amino acid responsible for the Maillard reaction in bread<sup>19</sup>.

### Hypothesis

This investigation hypothesises that as the degree of saturation increases (iodine value decreases), the amount of AGEs produced during the Maillard reaction decreases.

### Chemicals and Apparatus

In the iodometric titration of the oils,  $1100 \text{ cm}^3$  of ethanol, 17 g of iodine,  $4000 \text{ cm}^3$  deionised water, and 40 g of hydrated sodium thiosulfate, and a few drops of starch solution were used. Apparatus used include an electronic weighing balance ( $\pm 0.001 \text{ g}$ ), beakers, a glass stirring rod, a  $100 \text{ cm}^3$  measuring cylinder ( $\pm 0.5 \text{ cm}^3$ ),  $20 \text{ cm}^3$  pipette ( $\pm 0.03 \text{ cm}^3$ ), pipette filler, burette ( $\pm 0.1 \text{ cm}^3$ ), retort stand, and conical flasks.

For the preparation of the bread samples, 20 g each of sunflower, canola, peanut, palm, and coconut oil were used, along with 1000 g flour, 15 g salt, 15 g yeast, 30 g sugar, and 600 g water. An electronic weighing scale was used for the measurement of ingredient masses, a kitchen oven was used for baking, and a blender was used to crush the crust.

The preparation of samples for analysis required 5 g disodium hydrogen phosphate, 1 g sodium dihydrogen phosphate, and a few drops of hydrochloric acid. Apparatus included a  $10 \text{ cm}^3$  pipette, electronic pH probe, centrifuge, filter paper, and a filter funnel. Finally, for the analysis of the samples, a UV-Vis spectrophotometer ( $\pm 0.005 \text{ A}$ ) was used along with cuvettes.

### Variables

Independent variable: iodine value, and therefore degree of saturation of oil

Dependent variable: absorbance of crust sample, and therefore concentration of melanoidins produced in the reaction

Controlled Variable	Method of control	Purpose
Fermentation environment (humidity, temperature)	All dough samples were fermented and baked together	Eliminating any difference in environmental conditions ensures the extent of AGE production is reliant only on the degree of oil unsaturation
Fermentation and baking time	All dough samples were fermented and baked for the same amount of time	
Buffer solution pH	pH was monitored using a pH probe and adjusted by adding hydrochloric acid	To ensure crust from each sample is equally dissolved before spectrophotometric analysis

Figure 2: Table of controlled variables

### Preliminary Trials

Prior to the experiment, several preliminary trials were conducted, especially for the process of determining the iodine values of the oils. Initially, following the values used in the methodology of

Shimamoto et al. resulted in a very large volume of sodium thiosulfate required in titration for any colour change to be observed, as a result of the very small amount of oil used. Hence, the amount of oil used was increased, and this change was reflected in the formula used in calculations of iodine value.

During the measurement of browning intensity, upon scanning for the wavelength of maximum absorbance using the darkest sunflower oil sample, it was observed that the absorbance value was too high, at  $> 3A$ . Hence, all samples were diluted in a 1:4 ratio such that the absorbance was  $< 1A$ . The wavelength of maximum absorbance was found to be 309 nm, which aligns with the literature estimate of 280 nm - 360 nm<sup>17</sup>.

## Procedure

### Iodine Value Determination

For a more accurate control of the independent variable, the iodine value of the oils used in the baking of samples was experimentally determined instead of relying on their literature range. To reduce the cost, time investment, safety risk, and environmental impact of handling Wijs' reagent and carbon tetrachloride, the titrimetric method described by Shimamoto et al. was adapted to suit the available apparatus.<sup>20</sup>

1. 0.10 g of sunflower oil was dissolved thoroughly with 15 cm<sup>3</sup> of 95% ethanol in a 500 cm<sup>3</sup> beaker, under vigorous stirring with a magnetic stirrer.
2. 20 cm<sup>3</sup> of the 0.1 mol dm<sup>-3</sup> iodine solution was added with a pipette, and stirred again.
3. After 5 minutes, 100 cm<sup>3</sup> of cold distilled water was added with a measuring cylinder.
4. 20 cm<sup>3</sup> of the solution was pipetted for titration with the 0.1M sodium thiosulfate solution. The conical flask was swirled until the yellow-brown faded to a pale straw colour.
5. A few drops of starch indicator was added for a blue colour to develop. Titration continued until the blue colour disappeared.
6. Steps 4 and 5 were repeated for five replicates, and steps 1-6 were repeated for a blank, canola, peanut, palm, and coconut oil.

### Bread Sample Preparation

Commercially available ingredients were used in order to replicate the conditions of typical home baking. With reference to the process used by Toyosaki et al.,<sup>11</sup> this method similarly used oil instead of butter to bring the total added lipid content to 10% by mass with reference to flour, in accordance with bakers' percentages. Fermentation and baking times also followed the aforementioned work's procedure, but the rest of the ingredient ratios were adapted to better mimic those used by bakers; most white bread typically has a hydration of around 60%, so the water-to-flour ratio was modified accordingly.

1. 200g of flour, 3g of salt, 3g of instant yeast, and 6g of sugar was weighed out with an electronic kitchen scale and mixed together in a bowl. 120g of water and 20g of sunflower oil were added and mixed thoroughly by kneading.
2. The dough was fermented for 90 minutes at 28-30°C. The dough was then shaped and left to ferment for another 60 minutes. The dough was baked for 25 minutes in an oven preheated to 200°C.

### Phosphate Buffer Preparation

Some previous studies used a Tris-HCl buffer to maintain a 7.4 pH. However, tris hydrochloride is expensive and not easily available. Thus, similar to Toyosaki et al.<sup>8</sup>, a phosphate buffer is used instead. Ratios are adapted from AAT Bioquest.<sup>20</sup>

1. 160 cm<sup>3</sup> of deionised water was prepared in a 200 cm<sup>3</sup> beaker.

2. 4.043 g of disodium hydrogen phosphate and 0.6787 g of sodium dihydrogen phosphate were added to the deionised water and stirred thoroughly.
3. pH was measured with an electronic pH probe, and pH was adjusted to 7.4 with a few drops of hydrochloric acid as the pH initially recorded was too high.
4. Deionised water was added until  $200\text{ cm}^3$  is reached.

#### *Browning Intensity Measurement*

This study adopted a similar method used by Takeuchi et al.<sup>21</sup> in their analysis of the concentrations of various AGEs present in commercially available foods, whereby the solid food is crushed and a buffer is added before the sample is centrifuged.

1. 1 g of the finely crushed crust of the bread baked with sunflower oil was added  $10\text{ cm}^3$  of the buffer. The mixture was centrifuged at 3500 rpm for 10 minutes and filtered. This was repeated for the samples baked using canola oil, peanut oil, palm oil, and coconut oil.
2. One of the cuvettes was filled with the buffer to serve as a blank. Another cuvette was filled with the first sample and scanned over wavelengths 200 nm to 400 nm to determine the wavelength of maximum absorbance, as the literature range for melanoidins is 280 nm - 360 nm<sup>17</sup>.
3. The absorbance of the samples was recorded at this wavelength.
4. Steps 1-5 were repeated for a total of five trials, preparing a new batch of samples for each trial.

#### *Risk Assessment*

##### Safety Considerations

Iodine is toxic upon inhalation and care was taken to minimise exposure when possible. Glassware was handled carefully to avoid breakage.

##### Ethical Considerations

There are no ethical risks involved in this investigation.

##### Environmental Considerations

All chemicals were disposed of appropriately and the sink washed as improper disposal of chemicals could have harmed the environment. Leftover bread was composted to reduce the impact of wastage on the environment.

## Results

### Determination of Iodine Value of Oils (Titration)

#### Raw Data

Sample	Trial Number	Volume of sodium thiosulfate used in titration ( $\pm 0.10 \text{ cm}^3$ )		
		Initial	Final	Used
Sunflower	1	0.00	28.40	28.40
	2	0.00	28.60	28.60
	3	0.00	28.35	28.35
	4	0.00	28.50	28.50
	5	0.00	28.65	28.65
	Average	0.00	28.50	28.50
Canola	1	0.00	30.65	30.65
	2	0.00	30.75	30.75
	3	0.00	30.75	30.75
	4	0.00	31.00	31.00
	5	0.00	30.75	30.75
	Average	0.00	30.78	30.78
Peanut	1	0.00	32.70	32.70
	2	0.00	32.60	32.60
	3	0.00	32.90	32.90
	4	0.00	33.00	33.00
	5	0.00	32.95	32.95
	Average	0.00	32.83	32.83
Palm	1	0.00	38.60	38.60
	2	0.00	38.50	38.50
	3	0.00	39.10	39.10
	4	0.00	38.90	38.90
	5	0.00	39.00	39.00
	Average	0.00	38.82	38.82
Coconut	1	0.00	38.60	38.60
	2	0.00	38.50	38.50
	3	0.00	39.10	39.10
	4	0.00	38.90	38.90
	5	0.00	39.00	39.00
	Average	0.00	38.82	38.82

Figure 3: Table of Raw Titration Data

#### Sample Derivations

Iodine Value Calculation	Error Propagation
$\text{iodine value} = \frac{(40.00 - 28.50) \times (0.1) \times 12.69}{0.1}$ $= 145.934$ $\approx 146 \text{ (0 dp)}$	$\text{percentage uncertainty} = \left[ \frac{0.001}{0.1} + \frac{0.5}{15} + \frac{0.5}{100} + \frac{0.03}{20} + \frac{0.03}{20} + \frac{0.1}{28.50} \right] (100\%)$ $= 5.4842\% \text{ (5sf)}$ $\text{absolute uncertainty} = (5.4842\%)(145.935)$ $= 8.0034 \text{ (5sf)}$ $= 8 \text{ (1sf)}$

Figure 4: Sample Derivations and Error Propagation for Iodine Value Determination

Refer to Figure 8 below for the complete table of processed data. It is not possible to find the percentage error. Due to the highly variable nature of the oils' iodine values, the literature provides a range of possible values instead of a specific number. However, with reference to the ranges found in Figure 1, most of my calculated values lie on the higher end within the ranges stated, with only sunflower and palm oil slightly higher than the literature range.

#### *Measurement of Absorbance*

#### Qualitative Observations

After the samples had been prepared, it was visually observed that the sunflower sample was significantly darker in colour than the others, and the coconut sample was significantly lighter. However, the canola, peanut, and palm samples were similar in appearance.



Figure 5: Appearance of bread just after baking (top), Appearance of filtered melanoidin solutions of bread crust baked using (left to right) sunflower, canola, peanut, palm, and coconut oils (bottom).

### Raw Data

Sample	Absorbance ( $\pm 0.005A$ )						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Average
Sunflower	0.961	0.959	0.958	0.960	0.961	0.961	0.960
Canola	0.832	0.823	0.830	0.834	0.829	0.828	0.830
Peanut	0.733	0.721	0.729	0.727	0.724	0.722	0.726
Palm	0.673	0.593	0.614	0.632	0.666	0.566	0.624
Coconut	0.532	0.576	0.565	0.528	0.585	0.529	0.553

Figure 6: Table of Raw Absorbance Data

### Sample Derivations

Concentration of Melanoidins	Error Propagation of Concentration of Melanoidins
$\text{concentration} = \frac{0.960}{0.571 \times 1}$ $= 1.6813$	<p>Taking into account the <math>\frac{0.01}{0.571}</math> uncertainty from the literature value of molar absorptivity,</p> $\text{percentage uncertainty uncertainty} = \left[ \frac{0.005}{0.960} + \frac{0.01}{0.571} \right] (100\%)$ $= 2.2721\% \quad (5sf)$ $\text{absolute uncertainty} = (2.2721\%)(1.6813)$ $= 0.038201 \quad (5sf)$ $= 0.04 \quad (1sf)$

Figure 7: Sample Derivations and Error Propagation of the Calculation of the Concentration of Melanoidins

### Processed Data

Sample	Iodine Value	Absorbance	Concentration of Melanoidins
Sunflower Oil	$146 \pm 8$	$0.96 \pm 0.02$	$1.68 \pm 0.04$
Canola Oil	$117 \pm 7$	$0.83 \pm 0.02$	$1.46 \pm 0.06$
Peanut Oil	$91 \pm 5$	$0.73 \pm 0.02$	$1.27 \pm 0.05$
Palm Oil	$59 \pm 3$	$0.62 \pm 0.02$	$1.09 \pm 0.04$
Coconut Oil	$15 \pm 0.8$	$0.55 \pm 0.01$	$0.97 \pm 0.04$

Figure 8: Table of Processed Data

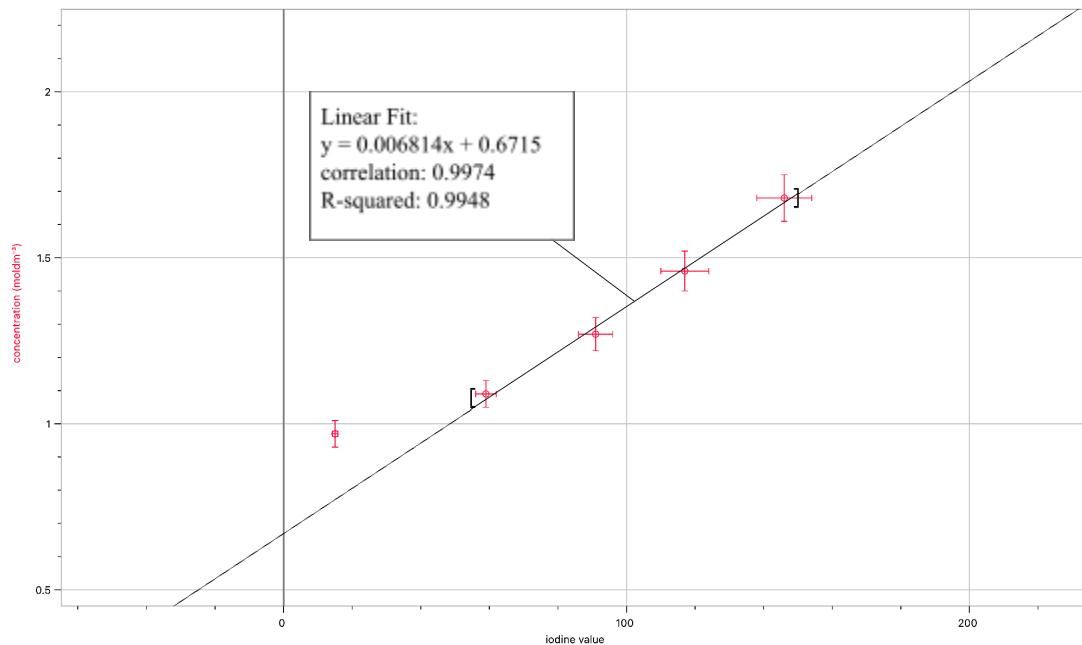


Figure 9: Graph of Iodine Value against Concentration, plotted in Loggerpro

### Discussion and Analysis

There was a relatively low uncertainty of both the iodine value and the concentration of melanoidins calculated, with the percentage uncertainty of each falling below 10%. This is indicative of the high precision of the instruments used, and is validated by the fact that the iodine value determined approximately lies within the range of literature values provided. The relatively low maximum standard deviation in absorbance of 0.049A also indicates low random error.

As seen in Figure 8, as the iodine value of the oils decreases from 146 to 15, the concentration of melanoidins produced at the end of the Maillard reaction decreases from 1.68 to 0.97. Although at first glance the relationship appears to be exponential, upon closer inspection, the highest four data points closely follow a linear graph. When the two possible graphs were plotted, the linear graph excluding the first data point showed a better correlation of 0.9974. The first data point is taken to be an anomaly. One possibility for this was that due to availability, the coconut oil purchased was from a different brand. This could have resulted in the introduction of impurities, which could have been involved in the Maillard reaction, allowing for a higher concentration of AGEs produced than anticipated. Taking the outlier into account, the linear trend confirms the hypothesis that as the degree of saturation increases, the amount of AGEs produced through the Maillard reaction increases, in line with the trend suggested by the literature. Notably, the graph does not intersect the vertical axis at the origin but above it, indicating that even at full lipid saturation, the Maillard reaction would still progress a notable amount, providing evidence against the suggestion made by Toyosaki et al.<sup>8</sup> that saturated fatty acids inhibit AGE production.

The results support the notion that lipid characteristics are an important determinant of the progress of the Maillard reaction, despite not being one of the main reactants. The trend described above corroborates the proposal of the high dependence of the formation of Maillard reaction products on the prevalence of lipid oxidation, which is in turn affected by the saturation of double bonds in the fatty acid hydrocarbon chain. The oxidation of fatty acids occurs simultaneously with the other mechanisms of the Maillard reaction with mutual influence on each other. The presence of the Amadori product, fructoselysine, supports lipid oxidation. Fructoselysine then degrades into the dicarbonyl compounds

glyoxal, methylglyoxal, or 3-deoxyglucosone, which react with lysine to form CML, CEL or pyrraline. Alternatively, glyoxal, methylglyoxal, and 3-deoxyglucosone are formed from lipid oxidation reactions. Furthermore, the free radicals liberated from the lipid oxidation reactions have been proposed to promote the conversion of the dicarbonyls to AGEs, a suggestion supported by the results.

## Conclusion

In conclusion, the data obtained in this study strongly supports the proposed Maillard reaction mechanism in which the presence of lipids play a crucial role in determining the extent of the production of AGEs. With the more oxidation-susceptible unsaturated fatty acids facilitating several processes in different pathways for CML, CEL, and pyrraline formation, as the degree of saturation of oils increases, the concentration of AGEs produced decreases.

## Evaluation

Being able to determine the iodine value of the specific oils used was another strength of this study. Although it was originally planned to just use literature iodine values, most studies only provided a range of values as the degree of saturation of each oil is highly variable. Hence, simply taking the median of each range provided would have been very inaccurate, especially since it was found that these oils in particular had iodine values that lay in the higher end of the range.

Another strength of this investigation was the choice of oils used. Although the differences in iodine value between each oil are not incremental, the wide range of iodine values studied accounts for a large majority of the oils typically used for cooking and baking. This allows for the trend to be generalised and made applicable to most contexts involving different oils.

A critical limitation of this investigation was its reliance on the literature value for the molar absorptivity coefficient of melanoidins, due to the unavailability of appropriate apparatus to prepare a standardised synthetic melanoidin solution to plot a calibration curve. However, the general trend of absorbance remains unaffected and allows for a sufficiently accurate estimate of the trend.

A weakness of this investigation is the process of investigating the Maillard reaction through the process of bread baking. Although this methodology is advantageous in replicating typical baking conditions and making the study relevant to the average home baker, the use of other ingredients in the bread could have resulted in inconsistencies with the introduction of impurities that may have either facilitated or inhibited the Maillard reaction. The iodine value used to quantify the independent variable does not account for these contributors to melanoidin formation, causing some degree of random error.

## Suggestions for Improvement and Future Work

To address the main limitation of this study of being unable to plot a calibration curve, future works should consider purchasing or preparing synthetic melanoidin solutions of known concentrations, allowing for the molar absorptivity coefficient of the particular sample to be determined to a greater degree of certainty, allowing for the unknown concentrations of AGEs formed to be more accurately calculated.

Additionally, this work focused mainly on the reaction between glucose and lysine. Although these are the most prominent of the sugars and amino acids present in bread, there are several other Maillard reactions taking place simultaneously with others, potentially with different mechanisms as the intermediate products may have different interactions with lipids. For a more comprehensive understanding of the Maillard reaction in bread that is applicable to real-life baking, it is worth considering the investigation of these secondary reactions in the presence of lipids. This can be examined most effectively by undergoing procedures similar to Takeuchi et al.<sup>21</sup> to identify different types of AGEs present in the product.

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