Why Food Browns: Understanding the Maillard Reaction

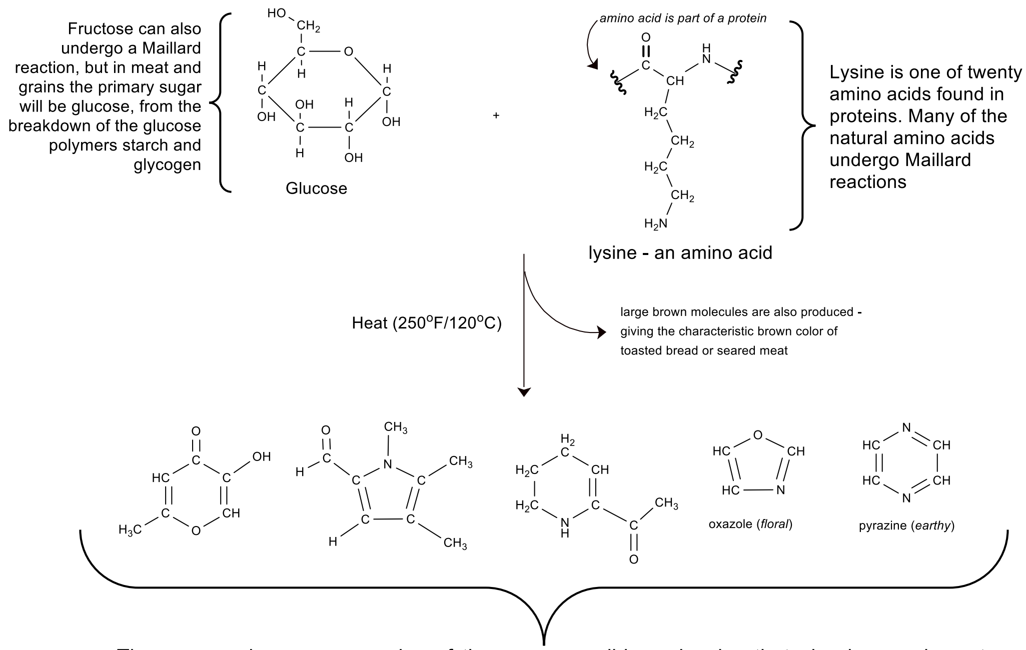
Introduction:

We have all cooked food and most of us have cooked often enough to understand that the cooking process does more than simply heat the food. The cooking process induces chemical reactions that change both the flavor and appearance of the food. When it comes to cooking meats, heating the food causes the proteins, especially those nearest the surface of the meat, to denature. These denatured proteins become more chemically reactive to other molecules in their environment.

One of the primary reactions that occur when browning meats and many other foods is called the Maillard Reaction. The Maillard Reaction is a non-enzymatic chemical reaction between amino acids and reducing sugars that commonly give brown foods their desired flavor. As with many chemical reactions, the outcome of the reaction will depend upon the amino acids and sugars present, but also on the amount of heat and other elements of the reaction environment such at pH.

Background:

The Maillard Reaction is commonly referred to as one of the most important flavor producing reactions in all of cooking. This reaction is also commonly referred to as the browning reaction due to the fact that the products of the chemical reactions have a brown color. As the chemical reaction proceeds, a series of chemical ring structures are formed that reflect brown light, thus providing the color. Of course it is not the change in color that causes the change in flavor, but as you will see in the following experiments, we can take advantage of the color change to evaluate and quantify this reaction. The Maillard reactions literally can produce hundreds to thousands of different chemical compound in small quantities that all contribute to the color and flavor of the food. These compounds include an array of ring structures and the products can be classified based upon these structure. In this series of experiments we will look at the Maillard Reaction and how it impacts the color and flavor of foods.



These are only some examples of the many possible molecules that give browned meat and toasted bread their complex flavors. The products of the Maillard reactions with different amino acids yield flavors ranging from floral and leafy to earthy and meaty. Aroma molecules made from the Maillard reactions include nitrogen atoms and sulfur atoms (not shown) from the amino acids.

Before we start investigating the Maillard Reaction we need to recall a little basic chemistry. The essential chemical reaction in this process occurs between an amino acid and a reducing sugar. As you know, amino acids are biologically important organic molecules containing both an amine ($-NH_2$) and a carboxyl group ($-COOH$) along with specific side chains that make each of the 20 common amino acids distinct. Amino acids are the monomers used as building blocks to produce proteins. As proteins denature during heating, these amino acids become accessible to chemical reactions. A reducing sugar is any sugar that either has an aldehyde group or can form this group when in solution. Monosaccharides that contain an aldehyde group are known as aldoses. These include the most common monosaccharides glucose and fructose. Some disaccharides such as lactose and maltose are also reducing sugars. The complex chemistries associated with the Maillard Reaction are covered in Chapter 6 of the textbook.

In this experiment we will do two simple activities. First, we will run the Maillard Reaction with a series of amino acids and sugars to determine their reactivity and gain a greater understanding of how the reaction works. In the second exercise, we will apply this knowledge to cooking, by dipping foods into various solutions and evaluate them on the length of time it takes for the food to brown. This exercise we will also allow us to evaluate the impact of pH in the browning process.

Pre-Laboratory Questions and Concepts:

Calculations and Questions:

1. What does it mean to have a 1 M solution?

2. How many moles of solute are in a 50 mM solution?

3. Calculate the amount of Sodium Bicarbonate ($NaHCO_3$) required to make 1 L of 0.02 M solution.

   $NaHCO_3$ molecular weight = 84.01 g / mol

   Grams Solute = 0.02 mole $NaHCO_3$ / Liter x MW (g/mole) x 1 Liters = X g of solute

4. Calculate the amount of amino acid required to prepare 50 mL of the following 50 mM amino acid solutions.

Amino Acid Number	**Amino Acid**	**Molecular Weight**	**Total g Solute / 50 mL**
1	Lysine	146.19	
2	Leucine	131.17	
3	Glutamic Acid	147.13	
4	Glycine	75.07	

   Grams Solute = 0.050 mole / Liter x MW (g/mole) x 0.05 Liters = X g of solute

5. Calculate the amount of sugar required to prepare 50 mL of the following 50 mM sugar solutions.

Sugar Letter	**Sugar**	**Molecular Weight**	**Total g Solute / 50 mL**
A	Arabinose	150.13	
B	Fructose	180.16	
C	Xylose	150.13	
D	Ribose	150.13	
E	Glucose	180.16	
F	Sucrose	342.30	

6. Diagram the structure of each of the amino acids given in question 4. How are they structurally similar? How are they structurally unique?

7. Diagram the structure of each of the sugars given in question 5. How are they structurally similar? How are they structurally unique? Which of the sugars are reducing sugars?

Process of Sciences:

This entire laboratory exercise is investigative in nature. You will be evaluating the browning of carbohydrates, amino acids, and food items due to the Maillard Reaction. The questions you have just answered should help you think about the five different experiments you will be doing in this laboratory exercise. At this time, just as you have seen in the previous laboratory exercises you should create:

1. A key question being investigated in each of the exercises below.

2. A hypothesis or proposed answer to the question asked.

3. A prediction for the outcome of the experiment based upon your hypotheses you developed.

   The prediction should written as an if/then statement and be specific to the measurements being made.

4. An explanation of your reasoning for each of your hypotheses and predictions.

Procedures:

<u>Solution Preparation</u>

1. 1 Liter 0.02 M Sodium Bicarbonate Buffer, pH = 9.5
   A. Place 500 mL distilled or deionized water into a 1 liter beaker.
   B. Add Sodium Bicarbonate to the water slowly while mixing.
      In Question and Calculations #3 above you calculated the amount of sodium bicarbonate you needed to add to make 1 liter of 0.02 M solution.
   C. Using a pH meter, adjust the pH to 9.5 by adding NaOH.
   D. Transfer solution to volumetric flask and add water to final volume of 1 L

2. 0.5 Liter Acetic Acid Buffer, pH = 5.5
    A. Place 250 mL distilled or deionized water into a 500 mL beaker.
    B. Using a pH meter to measure pH, add vinegar unto you reach pH 5.5.
    C. Transfer solution to volumetric flask and add water to final volume of 500 mL
    D. Transfer the solution to a container appropriately labeled for each amino acid

3. 50 mM Amino Acid Solutions in Water.
    A. You will be preparing amino acid solutions in water for the amino acids lysine, leucine, glutamic acid and glycine.
    B. In the Calculation and Questions #4 you calculated the quantity of amino acid needed to make 50 mL of 50 mM amino acid solution.
    C. You will make a separate solution for each amino acid listed.
    D. Place 25 mL distilled or deionized water into a 50 mL beaker.
    E. Slowly add the determined amount of amino acid while mixing gently.
    F. Transfer solution to volumetric flask and add water to final volume of 50 mL.
    G. Mix thoroughly.
    H. Transfer solution to a container appropriately labeled for each amino acid solution.

4. 50 mM Carbohydrate Solutions in Water.
    A. You will be preparing sugar solutions in water for the sugars arabinose, fructose xylose, ribose, glucose, sucrose.
    B. In the Calculation and Questions #5 you calculated the quantity of amino acid needed to make 50 mL of 50 mM amino acid solution.
    C. You will make a separate solution for each sugar listed.
    D. Place 25 mL distilled or deionized water into a 50 mL beaker.
    E. Slowly add the determined amount of sugar while mixing gently.
    F. Transfer solution to volumetric flask and add water to final volume of 50 mL.
    G. Mix thoroughly.
    H. Transfer solution to a container appropriately labeled for each sugar solution.

5. 50 mM Amino Acid Solutions in Bicarbonate Buffer.
    A. You will be preparing amino acid solutions in bicarbonate buffer for the amino acids lysine and glutamic acid.
    B. In the Calculation and Questions #4 you calculated the quantity of amino acid needed to make 50 mL of 50 mM amino acid solution.
    C. You will make a separate solution for each amino acid.

D. Place 25 mL bicarbonate buffer into a 50 mL beaker.
E. Slowly add the determined amount of amino acid while mixing gently.
F. Transfer solution to volumetric flask and add bicarbonate buffer to final volume of 50 mL.
G. Mix thoroughly.
H. Transfer solution to a container appropriately labeled for each amino acid solution in bicarbonate buffer.

6. 50 mM Carbohydrate Solutions in Bicarbonate Buffer.
   A. You will be preparing sugar solutions in bicarbonate buffer for the sugars arabinose and ribose.
   B. In the Calculation and Questions #5 you calculated the quantity of amino acid needed to make 50 mL of 50 mM amino acid solution.
   C. You will make a separate solution for each sugar.
   D. Place 25 mL bicarbonate buffer into a 50 mL beaker.
   E. Slowly add the determined amount of sugar while mixing gently.
   F. Transfer solution to volumetric flask and add bicarbonate buffer to final volume of 50 mL.
   G. Mix thoroughly.
   H. Transfer solution to a container appropriately labeled for each sugar solution.

Exercise 5.1: The Maillard Reactions with Simple Amino Acids and Carbohydrates

In this exercise we will be comparing the level of browning due to the Maillard Reaction in solutions of carbohydrates and amino acids alone and in combination. The amino acids and carbohydrates being used are given in the table below.

Amino Acid Number	**Amino Acid**	**Molecular Weight**	**Sugar Letter**	**Sugar**	**Molecular Weight**
1	Lysine	146.19	A	Arabinose	150.13
2	Leucine	131.17	B	Fructose	180.16
3	Glutamic Acid	147.13	C	Xylose	150.13
4	Glycine	75.07	D	Ribose	150.13
			E	Glucose	180.16
			F	Sucrose	342.30

Procedure 5.1.1. Browning of Amino Acids and Simple Sugars in Water

1. In this exercise we will observe the browning of amino acids and sugars alone and in combination in water.
2. Obtain 34 test tubes and cuvettes.
3. Label the test tubes as follows:
    A. 1, 2, 3, 4, for the amino acids alone
    B. A, B, C, D, E, F for the sugars alone
    C. 1A through 1F for the amino acid lysine with the six different sugars
    D. 2A through 2F for the amino acid leucine with the six different sugars
    E. 3A through 3F for the amino acid glutamic acid with the six different sugars
    F. 4 A through 4F for the amino acid glycine with the six different sugars
4. For test tubes 1, 2, 3, 4 add 2 mL of the appropriate amino acid solution.
5. For test tubes A, B, C, D, E, F add 2 mL of the appropriate sugar solution.
6. For the remaining test tubes add 1 mL of the appropriate amino acid solution and 1 mL of the appropriate sugar solution as labeled on tubes.
7. Mix the solutions thoroughly.
8. Prepare two water baths each large enough to hold 34 test tubes.
    A. A 100 °C water bath
    B. An ice water bath
9. Place the test tubes into the hot water bath for 30 minutes.
10. Transfer the test tubes from the hot water bath to the ice water bath to stop the chemical reaction.
11. Transfer an appropriate volume of each of the solutions into a cuvette.
12. Read each cuvette in a spectrophotometer at 360 nm.
13. Record the absorbance in Data Table 5.1.1.

Procedure 5.1.2. Browning of Amino Acids and Simple Sugars in Bicarbonate Buffer.

1. In this exercise we will observe the browning of the amino acids lysine and glutamic acid and sugars arabinose and ribose alone and in combination in bicarbonate buffer.
2. Obtain 8 test tubes and cuvettes.
3. Label the test tubes as follows:
    A. 1 and 3 for the amino acids alone
    B. A and D for the sugars alone
    C. 1A and 1D for the amino acid lysine with the two different sugars
    D. 3A and 3D for the amino acid glutamic acid with the two different sugars
4. For test tubes 1 and 3 add 2 mL of the appropriate amino acid solution.
5. For test tubes A and D add 2 mL of the appropriate sugar solution.

6. For the remaining test tubes add 1 mL of the appropriate amino acid solution and 1 mL of the appropriate sugar solution as labeled on tubes.
7. Mix the solutions thoroughly.
8. Check your two water baths that they are still in the appropriate temperature range.
9. A 100 °C water bath
10. An ice water bath
11. Place the test tubes into the hot water bath for 30 minutes.
12. Transfer the test tubes from the hot water bath to the ice water bath to stop the chemical reaction.
13. Transfer an appropriate volume of each of the solutions into a cuvette.
14. Read each cuvette in a spectrophotometer at 360 nm.
15. Record the absorbance in Data Table 5.1.2.

Exercise 5.2: The Maillard Reaction in Action

<u>Procedure 5.2.1. Browning Times of Foods Treated at Different pHs.</u>

1. Obtain potatoes, carrots, onions and chicken
2. Cut or slice the food items into 1 inch pieces.
3. Obtain three container and label them Distilled water, Acetic Acid Buffer, and Sodium Bicarbonate Buffer.
4. You will be cooking three different samples of each of the four foods listed and recording the amount of time it takes the majority of the food items in the pan to brown.
5. Dip one food item per solution at a time and cook immediately after each piece of that item is dipped.
6. Evenly coat a frying pan with canola oil and heat to 325°F as you are dipping each food item.
7. The frying pan will need to be cleaned between each cooking event.
8. Preparing food samples and browning.
    A. Distilled Water Dip
        a. Dip several (8 – 10) pieces of potato into distilled water
        b. Add to the warmed frying pan
        c. Brown the potatoes stirring regularly
        d. Record the amount of time from when the potatoes are added to the frying pan to when the majority of the potato pieces are browned
        e. Repeat the cooking exercise for the carrots, onions and chicken
        f. Record you data in Data Table 5.2.1.

B. Acetic Acid Dip
   a. Dip several (8 – 10) pieces of potato into the acetic acid solution
   b. Add to the warmed frying pan
   c. Brown the potatoes stirring regularly
   d. Record the amount of time from when the potatoes are added to the frying pan to when the majority of the potato pieces are browned
   e. Repeat the cooking exercise for the carrots, onions and chicken
   f. Record you data in Data Table 5.2.1.

C. Bicarbonate Buffer Dip
   a. Dip several (8 – 10) pieces of potato into bicarbonate buffer
   b. Add to the warmed frying pan
   c. Brown the potatoes stirring regularly
   d. Record the amount of time from when the potatoes are added to the frying pan to when the majority of the potato pieces are browned
   e. Repeat the cooking exercise for the carrots, onions and chicken
   f. Record you data in Data Table 5.2.1.

<u>Procedure 5.2.2. Browning Times of Foods Dipped in Different Amino Acid and Carbohydrate Solutions in Water.</u>

1. Obtain potatoes and onions
2. Cut or slice the food items into 1 inch pieces.
3. Prepare labeled containers with 50 mM solutions of Leucine, Lysine, Glycine, Sucrose, Ribose and Arabinose for dipping foods.
4. You will be cooking six different samples of each of the two foods listed and recording the amount of time it takes the majority of the food items in the pan to brown.
5. Dip one food item per solution at a time and cook immediately after each piece of that item is dipped.
6. Evenly coat a frying pan with canola oil and heat to 325°F as you are dipping each food item.
7. The frying pan will need to be cleaned between each cooking event.
8. Preparing food samples and browning.
   A. Dip several (8 – 10) pieces of potato into one of the amino acid or carbohydrate solutions
   B. Add to the warmed frying pan
   C. Brown the potatoes stirring regularly
   D. Record the amount of time from when the potatoes are added to the frying pan to when the majority of the potato pieces are browned

   E. Repeat the cooking exercise for the onions
   F. Record your data in Data Table 5.2.2.
9. Repeat steps 4A through 4F for each of the different amino and carbohydrate solutions.

<u>Procedure 5.2.3. Browning Times of Foods Dipped in Combinations of Amino Acid and Carbohydrate Solutions in Water.</u>

1. Obtain potatoes and onions
2. Cut or slice the food items into 1 inch pieces.
3. Label three container and prepare each of the following solutions by combining 5 mL of 50 mM amino acid solution with 5 mL of the 50 mM carbohydrate solution:
   A. Ribose and Leucine
   B. Ribose and Lysine
   C. Ribose and Glycine
4. You will be cooking three different samples of each of the two foods listed and recording the amount of time it takes the majority of the food items in the pan to brown.
5. Dip one food item per solution at a time and cook immediately after each piece of that item is dipped.
6. Evenly coat a frying pan with canola oil and heat to 325°F as you are dipping each food item.
7. The frying pan will need to be cleaned between each cooking event.
8. Preparing food samples and browning.
   a. Dip several (8 – 10) pieces of potato into one of the combined amino acid and carbohydrate solutions
   b. Add to the warmed frying pan
   c. Brown the potatoes stirring regularly
   d. Record the amount of time from when the potatoes are added to the frying pan to when the majority of the potato pieces are browned
   e. Repeat the cooking exercise for the onions
   f. Record your data in Data Table 5.2.3.
9. Repeat steps 4A through 4F for each of the different combined amino and carbohydrate solutions.

Results:

Exercise 5.1: The Maillard Reactions with Simple Amino Acids and Carbohydrates

Data Table 5.1.1. Maillard Absorbance in Simple Amino Acids and Carbohydrate Solutions in Water

Sol	Abs	Sol	Abs	Sol	Abs	Sol	Abs	Sol	Abs	Sol	Abs
1		A		1A		2A		3A		4A	
2		B		1B		2B		3B		4B	
3		C		1C		2C		3C		4C	
4		D		1D		2D		3D		4D	
		E		1E		2E		3E		4E	
		F		1F		2F		3F		4F	

Data Table 5.1.2. Maillard Absorbance in Simple Amino Acids and Carbohydrate Solutions in Bicarbonate Buffer

Sol	Abs	Sol	Abs	Sol	Abs	Sol	Abs
1		A		1A		3A	
3		D		1D		3D	

Exercise 5.2: The Maillard Reaction in Action

Data Table 5.2.1. Browning Time of Foods in Different pH Solutions

	Water Time	Acetic Acid Time	Bicarbonate Time
Potato			
Carrot			
Onion			
Chicken			

Data Table 5.2.2. Browning Time of Foods in Different Amino Acid and Sugar Solutions

	Leucine	Lysine	Glycine	Sucrose	Ribose	Arabinose
Potato						
Onion						

Data Table 5.2.3. Browning Time of Foods in Different Combined Amino Acid and Sugar Solutions

	Ribose and Leucine Time	Ribose and Lysine Time	Ribose and Glycine Time
Potato			
Onion			

Conclusions and Discussion:

1. In Exercise 5.1.1 we compared the level of browning due the Maillard Reaction in different amino acid and carbohydrate solutions in water alone and in combination.
    A. Which single carbohydrate gave the greatest level of browning?
    B. Which single amino acid gave the greatest level of browning?
    C. Which combination of carbohydrate and amino acid gave the greatest level of browning?

2. In Exercise 5.1.2 we compared the level of browning due the Maillard Reaction in different amino acid and carbohydrate solutions in bicarbonate buffer alone and in combination.
    A. What is the key difference between the experiments in Exercise 5.1.2 compared to 5.1.1?
    B. Which single carbohydrate gave the greatest level of browning?
    C. Which single amino acid gave the greatest level of browning?
    D. Which combination of carbohydrate and amino acid gave the greatest level of browning?
    E. How did the level of browning for these combinations compare to the results in section 5.1.1?

3. In section 5.2.1 food was browned after dipping in water, acetic acid buffer, or bicarbonate buffer.
    A. What was the main difference between these three solutions?
    B. How did the browning times compare for the different food items in each of the three solutions?
    C. For each of the different food items, which solution gave the fastest browning time? Which gave the slowest browning time?
    D. Using your data, how does changing pH impact browning times for the different food items?

4. In section 5.2.2 food was browned after dipping in different carbohydrate and amino acid solutions.
    A. How did the browning times compare for the different food items in each of the six solutions?
    B. For each of the different food items, which solution gave the fastest browning time? Which gave the slowest browning time?
    C. Compare the browning times of the food items dipped in carbohydrates and amino acids to the level of browning of the carbohydrates and amino acids from section 5.1.1.
        i. Did the patterns of browning for the carbohydrates alone match the patterns of foods dipped in carbohydrate solution?
        ii. Did the patterns of browning for the amino acids alone match the patterns of foods dipped in amino acid solution?

5. In section 5.2.3 food was browned after dipping in solutions of ribose combined with different amino acid.
    A. How did the browning times compare for the different food items in each of the three solutions?
    B. For each of the different food items, which solution gave the fastest browning time? Which gave the slowest browning time?
    C. Compare the browning times of the food items dipped in ribose and amino acids to the level of browning of the ribose and amino acids from section 5.1.1.

Process of Science Questions and Conclusions:

Earlier you created a key questions, hypotheses, predictions, and explanations for this prediction for each of the experiments in this laboratory exercise.

Based upon your data and the questions you have answered related to this exercise you should be able to complete the process of science questions and conclusions.

Answer the following questions.
1. Did your data support or falsify your hypothesis?
2. How did you come to this conclusion?
3. Did these results change your thinking about this topic? How?
4. What changes would you make to your hypothesis based on this new data?
5. What changes would you make to the experiments to better clarify your results?