Reaction Mechanism of Schiff Base Formation in the Maillard Reaction

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Abstract— This paper attempts to address the gaps in knowledge of how a chemical called acrylamide forms. Acrylamide, a possible carcinogen present in most fried, starchy foods, has been a subject of intense research for the past decade, and the most likely reactants in this reaction (asparagine and a reducing sugar) have already been found. However, there are still ambiguities regarding how exactly acrylamide and certain intermediates to it, such as the Schiff base, form. To determine on an atomic level how reducing sugars and asparagine can form the Schiff base intermediate and thus acrylamide, theoretical methods were used to find transition states in the reaction from asparagine to Schiff base, and these transition states provided insight into our conclusion: that the Schiff base intermediate formation was determined almost completely by the position of the hydroxyl groups on the reducing sugar. Knowing that the hydroxyl groups almost exclusively determine whether or not the Schiff base and thus acrylamide forms allows researchers to theorize and then test new ways to prevent acrylamide formation – for example, the addition of other molecules which more readily attract hydroxyl groups.

Index Terms— acrylamide, sparagine, Schiff Base, formation mechanism, Maillard Reaction, computational study, reducing sugar, glyceraldehyde

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1 INTRODUCTION

SINCE 2002, when Swedish scientists first discovered acrylamide in starchy foods, many studies have been done both about this molecule and its formation mechanisms. This monomer, based on studies with rodent models, is genotoxic, neuropathic, and possibly carcinogenic. It is metabolized to glycidamide, an epoxide also thought to be neuropathic. Thus, research on acrylamide has been stressed by national authorities in Europe and America and even the World Health Organization. It is necessary to further understand the implications of ingesting acrylamide, and the foods which acrylamide may be present in. Studies regarding the carcinogenic properties of acrylamide have not been completely conclusive, as more epidemiological studies are needed to solidify the link between acrylamide ingestion and human cancer risk. However, the presence of cancer in rodents which ingest acrylamide is sufficient motivation to begin studying the formation mechanisms of it. Studies over the past few years have ultimately concluded that, especially in starchy foods, the primary route to formation of acrylamide is through a reaction of asparagine and some reducing sugar. The discovery of this reactant has already helped scientists theorize and test methods for limiting acrylamide formation – for example, asparaginase, an enzyme that breaks down asparagine into aspartic acid, has been shown to reduce acrylamide formation by over 90%. However, the cost of asparaginase is too high to use practically to limit acrylamide formation. Thus, it is necessary to further research the formation mechanism of acrylamide in order to effectively discover other, cheaper ways to limit acrylamide formation in foods.

There are basically two hypothesized mechanisms for the creation of acrylamide from an asparagine molecule and a reducing sugar. After the carbonyl group on a reducing sugar bonds with the amide side of asparagine, a Schiff base is formed, which then decarboxylates into a decarboxylated Schiff base. From there, the decarboxylated Schiff base can hydrolyze to create 3 aminopropioamide that can degrade into

acrylamide via the elimination of ammonia. The decarboxylated Schiff base can also lose an imine to directly form acrylamide.

The formation of acrylamide from the decarboxylated Schiff base itself is well documented by prior studies. The formation of the Schiff base itself, however, is of particular interest because in current literature, the focus is general substances that may react with asparagine to form acrylamide. It is not well understood on an atomic level how the reducing sugars interact with asparagine to form the Schiff base, why some sugars react, why some do not, and even why two of the same sugars with different conformations react differently. There has not been sufficient research to determine a set of "rules" or common situations that allow for the formation of a Schiff base. This paper attempts to address this gap in research by using different conformations of a reducing sugar that reacts easily with asparagine, calculating which of these reactions are thermodynamically favorable using theoretical methods, and from these results obtaining a set of general conditions that are necessary for the Schiff base to form. Our hypothesis is that the position of the hydroxyl groups on the reducing sugars will be instrumental in determining whether or not the Schiff base will form. If the hydroxyl groups on the reducing sugar point towards the NH2 group of asparagine, then the reaction either will be unfavorable or will not go through.

2 PROCEDURE

2.1 Materials/Methods

The program that was used for our calculations was Gaussian 09. Glyceraldehyde was used as our sugar molecule because it was computationally inexpensive and known to produce acrylamide. Unfortunately, because of limited resources to use for the computations, the entire asparagine molecule could not be used. Instead, ammonia was substituted for asparagine in order to simulate the NH2 group on the amide side of the as-

paragine.

The ten most stable conformations of glyceraldehyde were found for use in the calculations [3], and optimized to their lowest energy state (torsion values in Table 1, atom numbers in Fig. 1)*.

TABLE 1
FOUR TORSION ANGLES IN DEGREES DETERMINED BY 6-31 G* OPTIMIZATION.

Conformation	$\varphi1^a$	$\varphi2^b$	$\varphi3^c$	$\varphi4^d$
1	-8.9	16.1	60.2	58.2
2	-0.5	1.3	180.2	-55.7
3	131.7	-85.6	64.1	-78.1
4	-1.3	2.6	-61.9	181.7
5	-2.7	-5.7	53.6	173.5
6	-3.2	5.3	-61.6	-83.6
7	172.3	-83.6	69.1	180.2
8	156.4	186.1	177.9	185.6
9	150.7	161.8	-67.4	64.7
10	145.8	-75.8	-64.2	65.2

(a)	O4–C1–		C2–O5 torsion angle.
(b)	C1–C2–		O5–H8 torsion angle.
(c)	C1–C2–C3–		O6 torsion angle.
(d)	C2–C3–		O6–H9 torsion angle.

*Bond lengths will optimize to the same bond lengths used in this paper as long as correct torsion angles and methods are used.
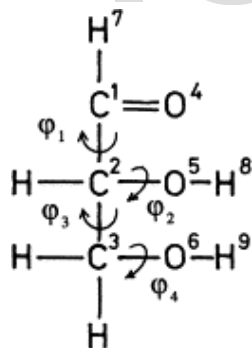

Fig. 1. Atom numbers used to set torsion angles for optimized glyceraldehyde molecule, to be used with Table 1.

Afterwards, ammonia was reacted with each stable glyceraldehyde conformation. The ammonia group was placed with the nitrogen facing the carbon on the aldehyde group of the glyceraldehyde, and the entire system was then optimized* to the lowest energy state. From the results of this optimization, the most reactive conformations were determined by seeing if the ammonia molecule aligned correctly with the glyceraldehyde molecule. If the dipole moments between the ammonia nitrogen and the aldehyde carbon, and between the ammonia hydrogen and the aldehyde oxygen, then the reaction would likely not go forth with those conformations. After recording which of the conformations seemed more likely to react, based on the dipole moments and positioning of the ammonia molecule in relation to the glyceraldehyde, a QST2* transition state was run using the completed Schiff base as the product complex and the glyceraldehyde-ammonia system as the reactant complex. After running the QST2 methods on each of the reactive conformations, a transition state guess for each of the conformations was obtained. The transition state guesses were used for QST3 methods on each of the reactive glyceraldehyde conformations, with the same reactant and product complexes as the QST2 calculations. The QST3 method helped refine the transition states for each of the glyceraldehyde conformations. Because the QST2 and 3 methods did not work for these reactive conformations, water molecules had to be added to the system to lower the energy barrier for the reaction and make it possible for the QST2 and 3 methods to do the correct calculations. Ultimately four sets of calculations were done with the reactive conformations: QST2 with one water molecule, QST3 with one water molecule, QST2 with two water molecules, and QST3 with two water molecules.

3 RESULTS

The most reactive conformations were found to be glyceraldehyde conformations 1, 2, 5, and 10. The energy levels for these conformations, in comparison to the energy levels for the other conformations, were far more favorable for a reaction to occur.

In running the transition state calculations, none of the transition state calculations went through except for G-2. This means that the energy barrier for the reactions with G-1, G-5, G-10 and acrylamide are far too prohibitive for the reaction to naturally move forward, hence the necessity of adding water molecules to the reaction. The torsion angles for these conformations after optimization with ammonia are in Table 2 (using Fig. 1 as a reference for atom numbers)

TABLE 1
FOUR TORSION ANGLES FOR MOST REACTIVE CONFORMATIONS IN DEGREES DETERMINED BY 6-31 G* OPTIMIZATION WITH AMMONIA

Conformation	$\phi1^a$	$\phi2^b$	$\phi3^c$	$\phi4^d$
1	-10.1	16.8	52.2	58.5
2	43.8	-59.4	-178.7	-44.5
5	16.7	-55.9	51.4	-179.8
10	102.1	-70.4	-64.2	109.1

(a)	O4–C1–C2–O5 torsion angle.
(b)	C1–C2–O5–H8 torsion angle.
(c)	C1–C2–C3–O6 torsion angle.
(d)	C2–C3–O6–H9 torsion angle.

*Bond lengths will optimize to the same bond lengths used in this paper as long as correct torsion angles and methods are used.

In general, what we have found is that there are two stages of reaction that occur.

After adding one water molecule to the product and reactant complexes, the transition state calculations went through. The pictures detailing the transition state results before Schiff base formation are displayed below. (Fig 2)
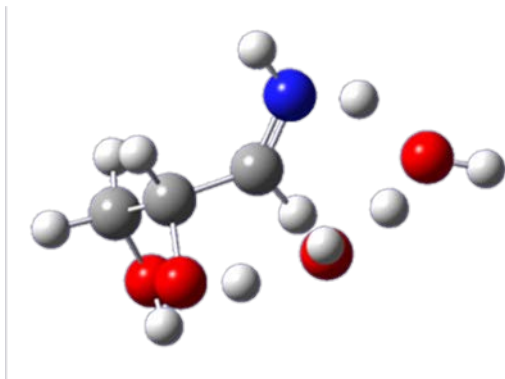

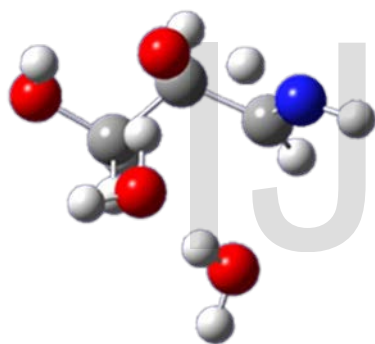Fig. 2a. Glyceraldehyde-1 transition state with one water mole-

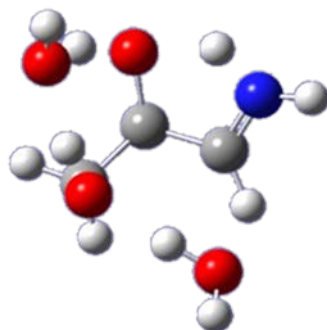Fig. 2b. Glyceraldehyde-2 transition state with one water molecule

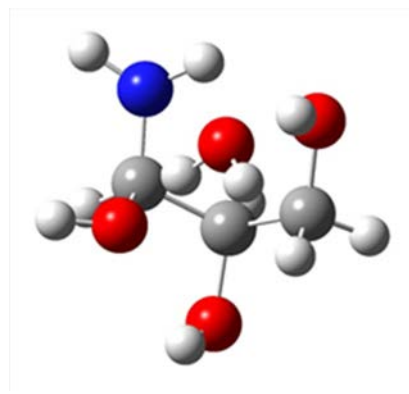Fig. 2c. Glyceraldehyde-5 transition state with one water molecule

Fig. 2d. Glyceraldehyde-10 transition state with one water molecule

After adding one water molecule, two was added to every reaction just to see if the second water molecule would interfere with the ammonia bonding to the glyceraldehyde, and to see if it would change any aspect of the transition states that we found with one water molecule. Due to time and resource constraints, this second type of calculation was only run on G-2. Based on the similarities between G-1, 2, 5, and 10 for the calculation with only one molecule, it was inferred that the results from the calculation with two water molecules could also be applied to the other glyceraldehyde conformations. The result of the G-2 calculation is below (fig. 3).
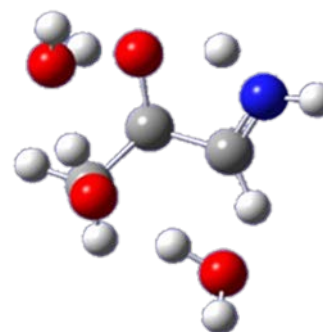

Fig. 3. Glyceraldehyde-2 transition state with two water molecules

The reaction process, as determined with QST2 and 3 calculations, is shown below. (Figure 3).

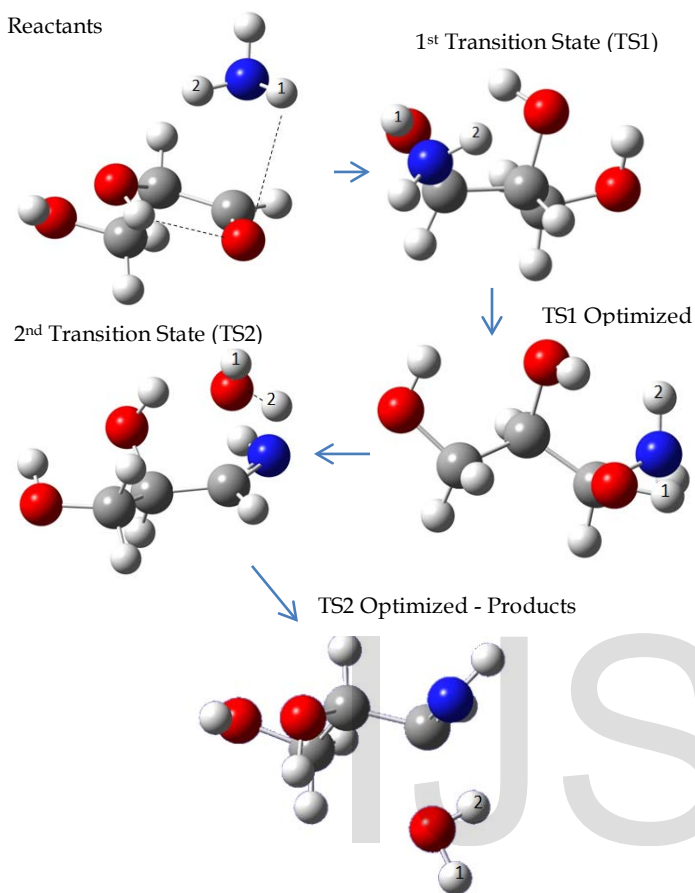termined with QST2 and 3 calculations, is shown below. (Figure 4)

Fig 3. G-2 Reaction Process (1 = hydrogen involved in first stage of the reaction, 2 = hydrogen involved in second stage).
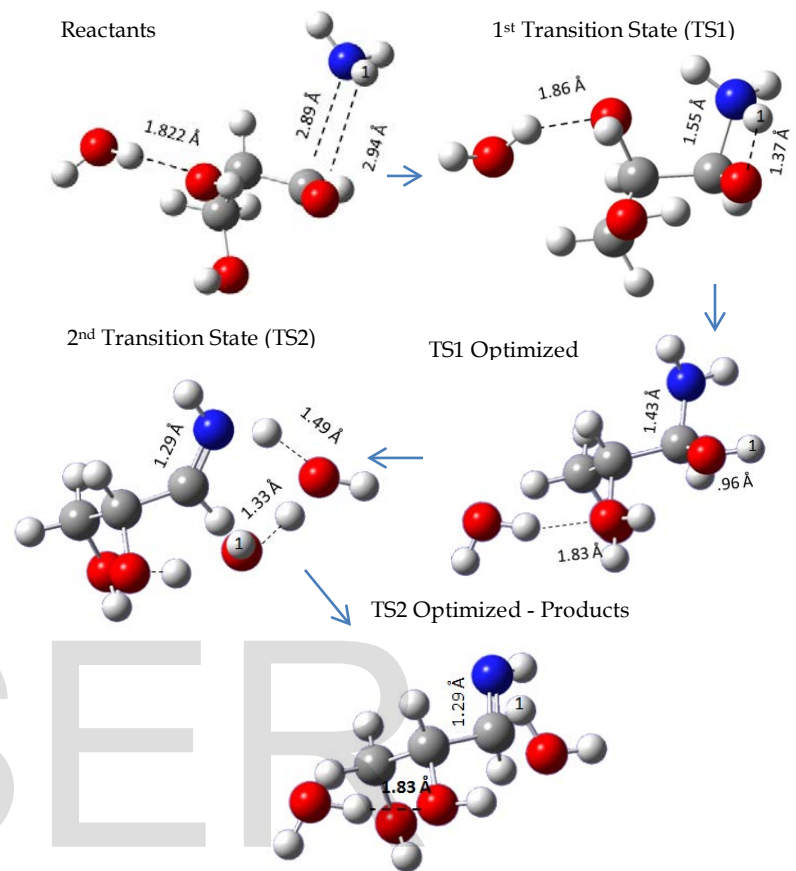

Fig 4. G-2 Reaction Process with one water molecule added. (1 = hydrogen donated by ammonia).

The reaction process with one water molecule added, as de-

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DISCUSSION

4.1 Standard Reaction

The placement of the ammonia molecule after the optimization calculations was largely influenced by the hydroxyl groups present on the glyceraldehyde molecule. The hydrogen atoms on the ammonia would actively seek out the oxygen atoms in the hydroxyl groups and form hydrogen bonds with them. Due to this tendency, only one conformation of glyceraldehyde allowed the ammonia molecule to stay in place to go under an effective nucleophilic attack.

The figure below shows the position of ammonia in the resulting reactions with the selected conformations of glyceraldehyde. Taking a look at G-1, the ammonia molecule is completely dragged away by the center hydroxyl group. The hydroxyl hydrogen is loosely bonded with the carbonyl hydrogen, which leaves an electron pair in the open to attract the

ammonia.

For G-5, the ammonia is pulled away much like in G-1, but for completely different reasons. In this case, the end hydroxyl's hydrogen atom is out of the picture by being pointed opposite from the rest of the molecule. This leaves the right side of the oxygen fully exposed for hydrogen bonding, which the ammonia immediately takes advantage of by bonding with it. At the same time, its rightmost hydrogen atom seeks out a loosely held attraction with the carbonyl oxygen. This whole system is held in place by the middle hydroxyl hydrogen bonding with the ammonia nitrogen.

G-10 is unique from any of the other conformations in that the middle hydroxyl is completely kept out of the reaction process. In this conformation, the end hydroxyl forms a semi-hexagonal ring formation with the carbonyl, with a weak hydrogen bond holding the shape together. Without the center hydroxyl to provide some stability, the ammonia jumps to the next available stable position, which is a hydrogen bond with the end hydroxyl. Due to the weak attractive nature of the carbonyl, the ammonia is very easily motivated by other polar forces. A visual example of these descriptions is provided (Fig. 5).
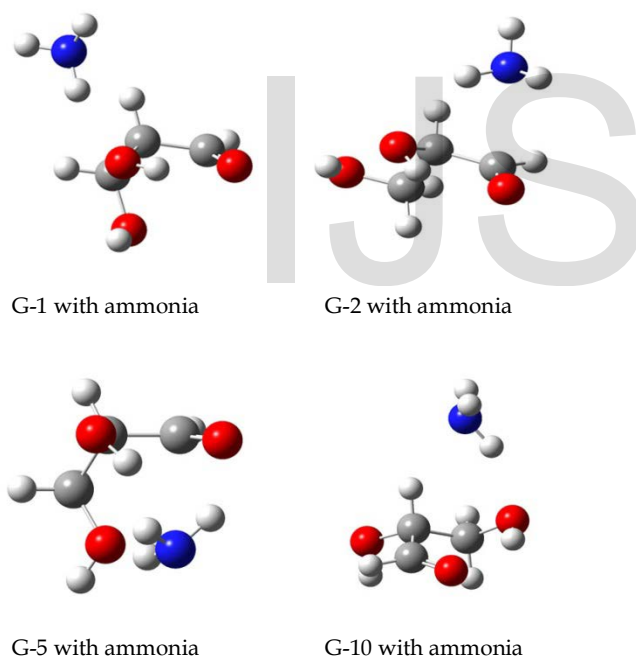

Fig. 5. Visual example of above descriptions, showing what orientations that the glyceraldehyde and ammonia take

G-2 produces the only successful result, since the hydrogen atom on the end hydroxyl group is pointed slightly towards the oxygen atom in the center hydroxyl. The pull delivered by the electron pair is distributed between this hydrogen and the one on the ammonia. Thus, the attractive force on the ammonia is not strong enough to pull it away from its position above the carbonyl, which allows it to undergo the aforementioned nucleic attack and eventually rearrange into the Schiff

base. The QST2 and QST3 calculations confirmed that the reaction was viable, and the frequency calculations demonstrated the detachment of the carbonyl oxygen to create water with another detached hydrogen atom from the ammonia (Fig 6).
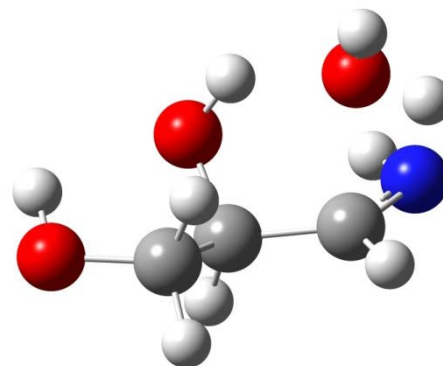

Fig. 6. The transition state before the Schiff base is completed. The lone hydrogen will bond with the unstable OH to form water.

4.2 Standard Reaction With 1 Water Molecule

The main purpose water served within the reaction was to neutralize the attractive forces of the hydroxyls. With the addition of water, every conformation of glyceraldehyde proved to be successful in converting into the Schiff base. There were two positions water could take, either between the center hydroxyl oxygen and carbonyl oxygen or between the end hydroxyl oxygen and the center hydroxyl oxygen. In these positions, the water is able to hydrogen bond with the hydroxyl groups and essentially complete the cyclic formation. Ammonia is relatively untouched in its position above the carbonyl, allowing for easy bonding with the carbon.

Curiously enough, the transition state calculation for the Schiff base did not turn out as expected for any of the conformations. The theory always ran into a fault when we attempted to remove hydrogen directly from the ammonia and bond it with the carbonyl oxygen to create water. We hypothesized that due to the stable nature of the cyclic formation created by the water molecule, it was impossible for the carbonyl bond to be cut off naturally. Therefore, the only way we were going to get the hydrogen ion onto the OH was through proton hopping, aka the Grotthuss mechanism, where an excess hydrogen is diffused through a network of hydrogen bonded molecules. Using this mechanism, we were able to obtain a conclusive transition state for the Schiff base. (Fig. 6).
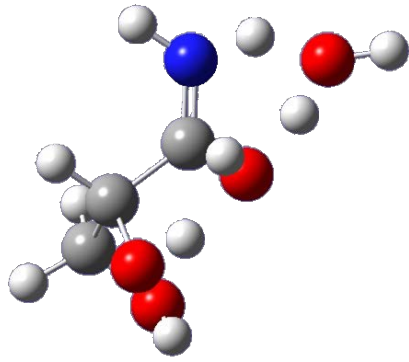
Figure 2.3: The transition state to the Schiff base for G-5. Here you can see the Grotthuss mechanism in action.

4.3 Standard Reaction With 2 Water Molecules

For the calculations involving two water molecules, both cyclic formations were created, but the reaction processes were identical to those involving one water. The second cyclic formation had no theoretical effect on the formation of the transition states or the reaction itself.

5 CONCLUSION

Through our research on the reaction between glyceraldehyde and ammonia, we were able to discover the mechanisms of this reaction on the atomic level. Furthermore, we are able to apply this reaction mechanism of ammonia with virtually any reducing sugar, since previous works have noted that asparagine's alpha-amino group reacts with the carbonyl group of reducing sugars to initiate that process that results in the formation of acrylamide. This is a step beyond what was researched in previous experiments involving the creation of acrylamide through reaction with amino acid asparagine, which focused mainly on pinpointing acrylamide formation in various food products.

Our work answers the question of the roles of individual atoms in Schiff base formation within the Maillard reaction involving asparagine. Although we do not use the asparagine molecule itself due to computational limitations, the substitution with ammonia is good enough to gain significant results, since the amine-group is the primary focus of this reaction. Through our results, we are able to conclude that the formation of the Schiff base involves two significant steps. The first step, which is the binding of the nitrogen atom to the carbon atom, is heavily influenced by the attractive pull of the hydroxyl groups. In fact, the position of these hydroxyl groups completely determines the success of the reaction. When water molecules are put into the reaction, the hydroxyl groups tend to bond with them, leaving the ammonia free of distractions, which allows a reaction to occur with every conformation of glyceraldehyde. In the second step, which is the release of a hydrogen atom and the breaking of the carbon double bond to create water, we have found substantial evidence of proton hopping when water molecules are present.

Although no work has been done previously on the specifics of Schiff base formation, our results are favored by the literature that discusses the Maillard reaction. The Schiff base produced in our calculations matches exactly what is illustrated in previous works. Furthermore, our results comply with current chemical theory, and are experimentally sound. The basis of our study relies on the fact that the amino group of asparagine reacts with the carbonyl group of reducing sugars during the Maillard reaction. However, this assumption has been confirmed in so many various studies that we can essentially accept this as a fact. Because of the absolute nature of calculation and the various methods we utilized, there are no alternative explanations to our work, unless the previous statement mentioned is wrong, which is highly unlikely.

In the future, actual lab experiments need to be carried out in order to confirm our theoretical conclusions. These experiments should start off with using our original molecules, ammonia and glyceraldehyde, but later expand to reacting asparagine with different reducing sugars. It is important that these experiments trace the movements of key atoms, such as the nitrogen and the released hydrogen, so that our reaction pathway can be confirmed.

Due to limitations in technological resources and time, we were not able to confirm our theoretical results by performing the same calculations again with asparagine instead of ammonia. We also would have gone on to using higher levels of theory in our calculations, which would make our results more reliable and energy calculations more exact. Furthermore, given more time, we would have eventually gone on to look at what compounds could be added into the reaction to interfere with the formation of the Schiff base, which is really the goal of acrylamide research.

The problem of acrylamide in food has only been one of recent interest, so there is still much research that needs to be done in order for scientists to find a valid solution. On the molecular level, we have yet to find out all the possible chemicals that asparagine can react with to form acrylamide. Furthermore, this research project only focused on the formation of the Schiff base, which is merely the initial step in the Maillard reaction. Studies still need to be done on the rest of the reaction leading up to acrylamide, so that we can gain a clear-cut understanding of what exactly is involved in the creation of potential dangers of our food. The conclusions from this project are just the beginning in a large scale molecular analysis of the Maillard reaction.

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