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Short Communication

Formation of Acrylamide in Browning Model Systems under Mild Cooking Conditions

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Abstract

Acrylamide formed in an L-asparagine/D-glucose Maillard model system treated under mild simulated cooking conditions (at 100°C and various pH) was measured. The amount of acrylamide formed ranged from 0.04 ± 0.02 μg/g at pH 3 to 17.4 ± 1.2 μg/g at pH 9. In the case an initial pH 3, acrylamide formation did not occur significantly even after 25h heating (1.9 ± 0.6 μg/g). In the case of initial pH 5, acrylamide formation increased suddenly to 6.1 ± 1.2 μg/g after 15 h heating and leveled off thereafter. In the case of pH 8 and 9, a steady, semi-linear increase of acrylamide was observed. Acrylamide formation was not observed when the initial pH was 8 or 9 until heated for 20 min in this Maillard reaction system. In these cases, the formation of acrylamide increased steadily up to 15h (12.4 ± 0.8 μg/g) and up to 20 h (22.5 ± 1.7 μg/g), respectively, then leveled of overall, the higher initial pH the more acrylamide formation was observed. The present study demonstrates that acrylamide forms under mild cooking conditions.

Keywords: *Acrylamide; GC/MS; Maillard Reaction; L-Asparagine; Mild Cooking Condition*

Introduction

Since toxic acrylamide was found in a Maillard reaction system in 2002 [1], it has been analyzed in various heat-processed foods, in particular starch rich-foods such as potato chips and French-fries [2]. The U.S. Food and Drug Administration (USDA) reported the levels of acrylamide in 286 commercial food products, which had been treated at high temperatures of over 170°C [3].

Among hypotheses advanced concerning acrylamide formation in heat-processed foods, a Maillard browning reaction consisting of a sugar and an amino acid has received a great deal of attention as the most rational formation mechanisms [4] and many studies on acrylamide formation were conducted using Maillard reaction systems. For example, when asparagine, which has the same amide moiety as acrylamide, was heated alone at 180°C for 30 minutes, 0.99 μg/g of acrylamide was formed, whereas the addition of D-glucose to asparagine increased its formation to 1200 μg/g [5]. In addition, acrylamide formation increased from 117 μg/g to 9270 μg/g by addition of D-glucose in a system consisting of asparagine, potato starch, and water [6]. These results suggest that asparagine and D-glucose play an important role in acrylamide formation in Maillard reaction systems [5,7].

The pH of a Maillard solution decreases after reaction due to formation of acids [8].

The initial reaction step of a Maillard reaction is an interaction between an amino group of an amino acid and a carbonyl group of sugar to form imine compounds, such as Amadori compounds; and the formation of these imine compounds is strongly associated with the pH of the reaction solutions [9]. The optimum pH at which these imine compounds are formed is generally near 5, and formation drops at higher and lower pH's [10]. Therefore, initial pH must play an important role in the formation of Maillard products, including acrylamide.

There are virtually no reports on acrylamide formation in foods prepared or stored under mild conditions. The preparation methods for some foods, such as beef stews and all kind of soups, require a long period of cooking at relatively low temperatures (approximately 100°C). Moreover, in addition to the cooking method, initial pH of the reaction solution may be important for the formation level of acryl-

amide. Therefore, in the present study, acrylamide formation in an asparagine/D-glucose Maillard model system simulating the mild cooking condition of 100°C and with various initial pH values was investigated.

Materials and Methods

Chemicals and Reagents

L-Asparagine, *D*-glucose, and 2-methylacrylamide were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Authentic acrylamide was bought from Bio-Rad Laboratories (Hercules, CA, USA). ^{13}C3-Acrylamide was purchased from Cambridge Isotope Laboratories (Andover, MA, USA). All solvents were from VWR International (Brisbane, CA, USA).

Stock solutions of acrylamide (1000 µg/ml) and 2-methylacrylamide (1000 µg/ml) were prepared by dissolving 100 mg of acrylamide and 100 mg of 2-methylacrylamide in 100 ml of deionized water. Acrylamide standard solutions were diluted to 0.1 mg/ml, 0.5 mg/ml, 1.0 mg/ml, 5 mg/ml, 10, mg/ml, and 20 mg/ml with deionized water to prepare a standard curve for quantitation of acrylamide in the samples.

Sample Preparations

Aqueous solutions (15 ml) containing 0.1 M each of *L*-asparagine and *D*-glucose were heated in a 20 ml-vial with a teflon sealed cap at 100°C and various pHs for different times in an oven. All samples were prepared in triplicate.

After 1 ml of internal standard ^{13}C3-acrylamide (200 ng/ml in water) and 3 ml deionized water were added to 1 ml of reaction mixture, the reaction solution was purified on an OASIS-HLB solid phase extraction column (Waters Corporation, Milford, MA) by a previously reported method [5]. The column was washed with 3.5 ml methanol followed by 3.5 ml water prior to use. The sample solution (1.5 ml) was allowed to pass through the column. The column was then eluted with 0.5 ml water and the eluate was discarded; subsequently, the column was eluted with 1.5 ml water and the eluate was subjected to LC/MS/MS.

Analysis of Acrylamide

Acrylamide analysis was conducted with a Hewlett Packard 1100 liquid chromatograph interfaced to an Applied Biosystems API 2000 MS/MS via an atmospheric pressure chemical ionization (APCI) source operating in the positive ion mode at 475^{0}C with nitrogen gas. The mass spectrometer was operated in selective ion monitoring mode (SIM) to observe the transition of m/z 72 to m/z 55 for acrylamide and m/z 75 to m/z 58 for ^{13}C3-acrylamide. Chromatographic separation was accomplished with a 50 × 2.1 mm Hypercarb column (Thermo, San Jose, CA) with a 5 µm particle size. The mobile phase condition was isocratic at 96/4% aqueous acetic acid (0.1%)/methanol with a flow rate of 400 µl/min.

Under these conditions, acrylamide eluted at 1.4 min. Triplicate samples were prepared for all experiments.

Results and Discussion

Non-enzymatic browning reactions produce alkyl acids and subsequently a reaction mixture becomes acidic. Therefore, pH must play an important role in acrylamide formation in aqueous food systems, in particular under mild conditions. Figure 1 shows the relationship between initial pH and final pH of an aqueous browning reaction system consisting of L-asparagine/D-glucose heated at 100°C for 15h. Values are mean ± SD (n = 3). When initial pH was lower than 5, pH did not change significantly (final pH = 5.0 ± 0.12). When the initial pH was increased to above 7, the final pH decreased to below 6, suggesting that the browning reaction progressed faster at pH > 7 than at pH < 7. When the initial pH was over 10, the final pH was not consistent (error value was over 40% at pH 12). These results indicate that a Maillard solution becomes acidic as the reaction progresses.

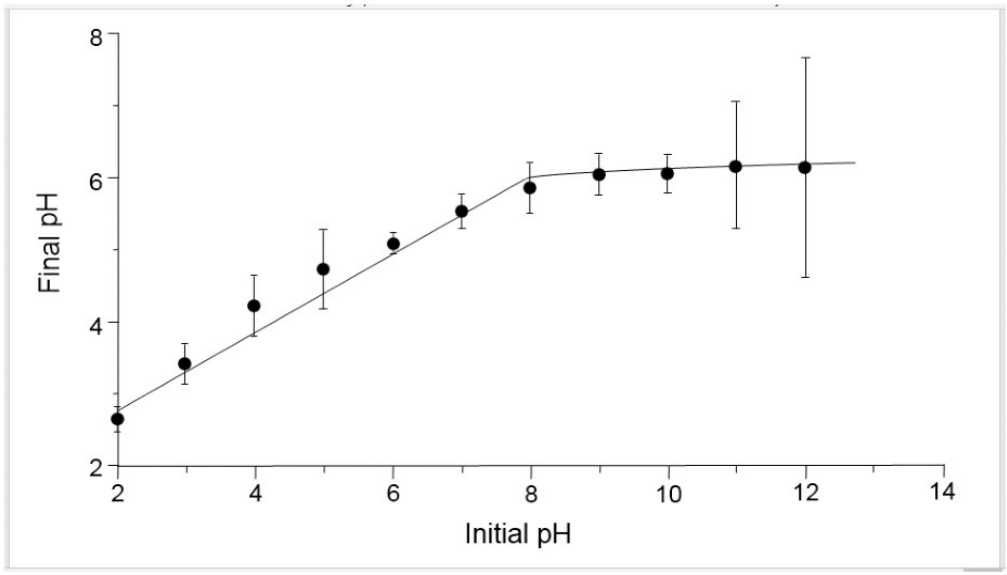

Figure 1: *Relationship between initial pH and final pH of an aqueous browning reaction system consisting of L-asparagine/D-glucose heated at 100 C for 15h.*

Figure 2 shows the amounts of acrylamide formed from an L-asparagine/D-glucose browning model system heated at various initial pHs and held at 100°C for 12h. Values are mean ± SD (n = 3). The amount of acrylamide formed ranged from 0.04 ± 0.02 μg/g at pH 3 to 17.4 ± 1.2 μg/g at pH 9. The formation of acrylamide increased linearly (R^2 = 0.983) up to pH 9 and then leveled off. However, as mentioned above, when initial pH increased to over 10, the final pH was inconsistent.

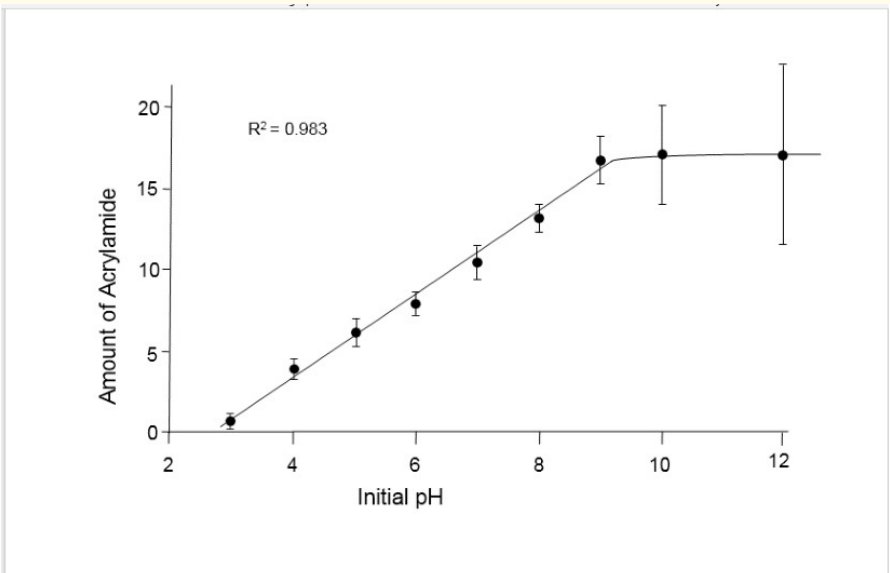

Figure 2: *Amount of acrylamide formed from an L-asparagine/D-glucose browning model system heated at various initial pHs and 100°C for 12h.*

Figure 3 shows the amounts of acrylamide formed from an L-asparagine/D-glucose browning model system heated at 100°C for various periods at initial pH 3 and 5 (A) and 8 and 9 (B). Formation of acrylamide was not observed at initial pH 3 and 5 until 5h heating time had passed, suggesting that at strongly acidic conditions it does not form within 5h cooking time. In the case of initial pH 3, acrylamide formation did not occur significantly at 100°C even after 25h heating (1.9 ± 0.6 μg/g). In the case of initial pH 5, acrylamide formation increased suddenly to 6.1 ± 1.2 μg/g after 15 h heating, then leveled off.

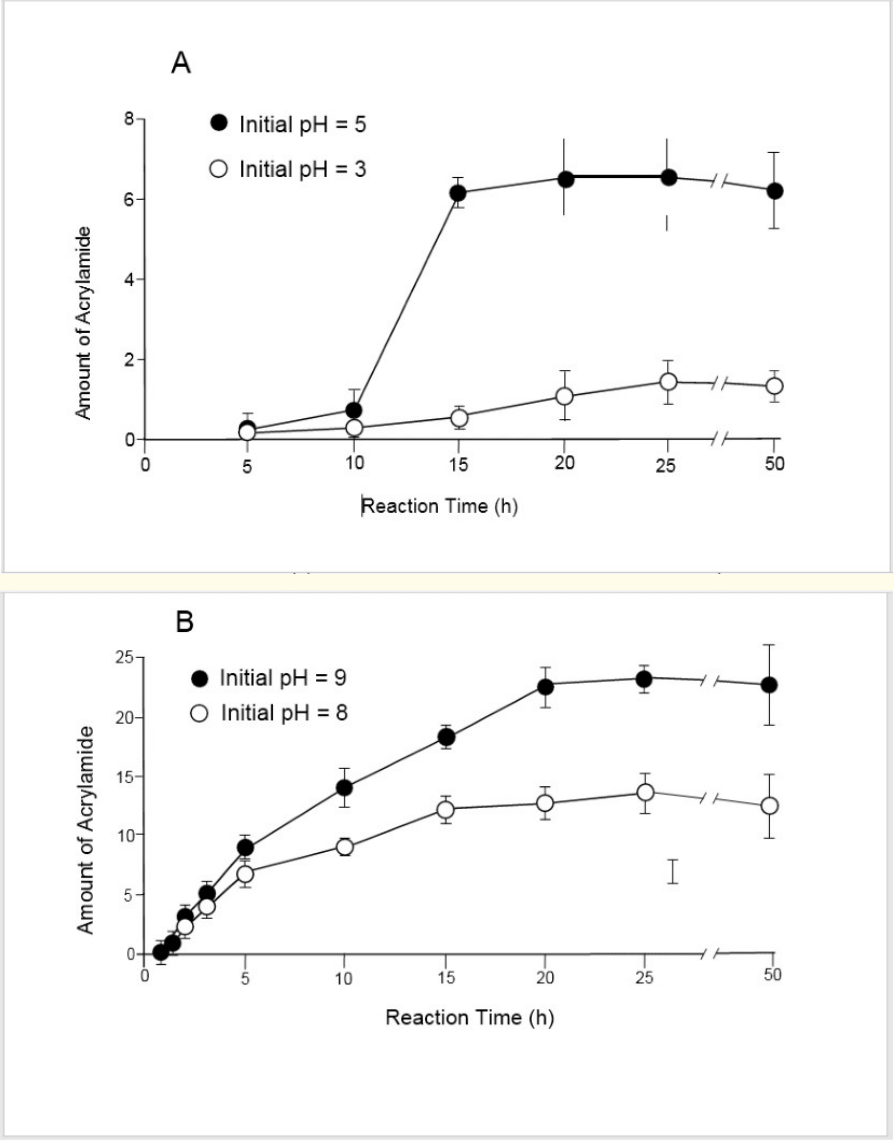

Figure 3: *Amount of acrylamide formed from an L-asparagine/D-glucose browning model system heated at 100 °C for various periods at initial pH 3 and 5 (A), and 8 and (B).*

In the case of pH 8 and 9, a steady semi-linear increase of acrylamide was observed.

Acrylamide formation was not observed at initial pH 8 or 9 until after 20 minutes in this Maillard reaction system. The formation of acrylamide increased steadily up to 15 h (12.4 ± 0.8 µg/g) and up to 20 h (22.5 ± 1.7 µg/g), respectively, then and leveled off. Overall, the higher pH the more acrylamide formation was observed.

Conclusion

Amounts of acrylamide found in various foods treated by relatively high temperatures were 0.17 - 3.70 µg/g in potato chips, 0.20 - 12.0 µg/g in French-fried, 1.27 µg/g in deep-fried potato and 1.18 µg/g in onion soup [11]. The results from the present study are comparable to these reports. Complex food systems and a simple Maillard system may not be entirely comparable. However, when L-asparagine and D-glucose was heated at 180ºC for 30 minutes, an average of 1.98 µg/g of acrylamide was formed [1]. The present study demonstrated that the amounts of acrylamide formed under a mild cooking condition (100ºC) for a prolonged time (over 20h) were comparable to those formed under high temperatures (180ºC) in a short time. In general, initial pH played a role in acrylamide formation in the present study.

Conflict of Interest

The authors declare no conflict of interest.

Bibliography

1. Stadler RH., *et al*. "Acrylamide from Maillard reaction products". *Nature* 419.6906 (2002): 449-450.

2. Arvanitoyannis IS and Dionisopoulou N. "Acrylamide: Formation, occurrence in food products, detection methods, and legislation". *Critical Reviews in Food Science and Nutrition* 54.6 (2014): 708-733.

3. U. S. Food and Drug Administration. "Exploratory data on acrylamide in food" (2018).

4. Mottram DS., *et al*. "Acrylamide is formed in the Maillard reaction". *Nature* 419.6906 (2002): 448-449.

5. Yasuhara A., *et al*. "Gas chromatographic investigation of acrylamide formation in browning model systems". *Journal of Agricultural and Food Chemistry* 51.14 (2003): 3999-4003.

6. Zyzak DV., *et al*. "Acrylamide formation mechanism in heated foods". *Journal of Agricultural and Food Chemistry* 51.16 (2003): 4782-4787.

7. Yaylayan VA., *et al*. "Why asparagine needs carbohydrates to generate acrylamide". *Journal of Agricultural Food Chemistry* 51.6 (2003): 1753-1757.

8. Davídek T., *et al*. "Sugar fragmentation in the Maillard reaction cascade: Formation of short-chain carboxylic acids by a new oxidative a-dicarbonyl cleavage pathway". *Journal of Agricultural and Food Chemistry* 54.18 (2006): 6677-6684.

9. Locas CP and Yaylayan VA "Further insight into thermally and pH-induced generation of acrylamide from glucose/asparagine model systems". *Journal of Agricultural and Food Chemistry* 56.15 (2008): 6060-6074.

10. Zalkin H and Sprinson DB "An investigation of imine formation in the isocitrate dehydrogenase reaction". *Journal of Biological Chemistry* 241.5 (1966): 1067-1071.

11. Friedman M. "Chemistry, biochemistry, and safety of acrylamide. A review". *Journal of Agricultural and Food Chemistry* 51.16 (2003): 4504-4526.

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