The Impact of Thermal Processing Conditions on Acrylamide Formation in Foods

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Abstract: The objective of this investigation is to assess the influence of various thermal treatment parameters on the generation of acrylamide within food products, concentrating particularly on the interplay of four key variables: the degree of heat application, the duration of exposure to heat, the proportion of glucose relative to aspartic acid, and the level of water content present in the sample. A systematic analysis was conducted to understand how these factors independently and collectively affect the formation of acrylamide. The findings derived from the experiments suggest that both the intensity of the applied heat and the length of its application exert a substantial effect on the development of acrylamide. Notably, the scenario wherein the temperature is set at 180 degrees Celsius for a period of 20 minutes has been observed to result in the most pronounced increase in acrylamide levels. The proportion between glucose and aspartic acid, along with moisture content, are also crucial, with the maximum acrylamide formation occurring at a ratio of 1.2:1.0 and a moisture content of 35.2%. The findings of this study have significant practical application value in guiding the reduction of acrylamide formation during food processing, which helps to enhance food safety and protect public health.

Keywords: Acrylamide; Food; Thermal Processing; Model System

1. Introduction

Acrylamide (CAS: 79-06-1) is a colorless, crystalline compound that readily dissolves in a multitude of solvents including water, ethanol, methanol. Industrially, it is primarily synthesized through the catalytic hydration of acrylonitrile and is a key raw material in the field of fine chemicals [1]. As a monomer with a vinyl group, acrylamide readily undergoes polymerization to form polyacrylamide, resulting in a gelatinous substance. Since the 1950s, polyacrylamide has been widely used in various industries, including the paper industry, textile dyeing, and the purification of drinking water [2]. Although polyacrylamide itself is non-toxic, its monomer, acrylamide, can metabolize in the body to form glycidamide (GA), which has significant alkylating activity and can interact with important biomolecules in the human body, such as hemoglobin and DNA. Consequently, in the year 1994, the International Agency for Research on Cancer (IARC) designated acrylamide as a substance belonging to Group 2A, meaning it is "possibly carcinogenic to humans" [3-4].

In the month of April during the year 2002, researchers from Stockholm University collaborated with the Swedish National Food Administration(SNFA) jointly announced the detection of acrylamide (AA), which possesses neurotoxicity and potential carcinogenicity, in foods that are rich in starch and have undergone high-temperature processing (above 120°C) [5]. Subsequently, nations such as the United Kingdom, the United States, Canada, and Norway likewise performed examinations to ascertain the quantities of acrylamide present in various food items, subsequently disclosing their findings to the public. This discovery quickly attracted significant attention from the global scientific community. During the month of June in that very year, both the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) held an emergency expert seminar in Switzerland to discuss the potential impact of acrylamide in food on human health and to establish the International Acrylamide Information Network, aimed at promoting information sharing and research [2]. In 2005, WHO, FAO, and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) undertook a thorough evaluation of the safety implications associated with acrylamide in consumables and thereafter released a collaborative advisory notice to notify the populace regarding the potential hazards posed by acrylamide in edibles. They also suggested strategies aimed at diminishing the presence of this compound in food products and guaranteeing their overall security [6].

Edible items rich in carbohydrates, particularly those exposed to intense heat treatments like deep-frying and oven-baking, tend to generate acrylamide [7]. These foods include items like potato chips, bread, and biscuits [8-10]. The processing of these foods typically occurs in an environment of high temperature and low moisture, where the high temperature not only facilitates the cooking process but also allows for the control of energy transfer through the adjustment of heating temperature and duration [11]. Concurrently, the lower moisture content accelerates heat transfer, aiding in the even cooking of the food. Based on this, it can be hypothesized that the high-temperature and low-moisture processing environment may promote the formation of acrylamide. Although most studies focus on the independent examination of factors such as temperature, time, and moisture on the generation of acrylamide, in-depth studies on how the interaction of these factors affects the formation of acrylamide are relatively rare.

In exploring the complexity of acrylamide formation in foods, research faces challenges due to the diversity of food components and the extensive range of influencing factors. At present, investigators commonly utilize streamlined chemical frameworks, including the interaction between reducing sugars and asparagine, to mimic the pathway through which acrylamide is generated. These models, due to their simple composition, reduce interfering factors, thereby simplifying the complexity of the research. Given that the main reducing sugar in foods is glucose and that some non-reducing carbohydrates can also decompose to form glucose at high temperatures, this study adopts the system of glucose and asparagine as a model. To achieve a more profound comprehension of the processes leading to acrylamide generation within environments characterized by elevated temperatures and reduced humidity levels, this research comprehensively examined the influences of various factors—including the degree of thermal treatment, duration of heating, proportion of glucose to asparagine, and water content—on acrylamide synthesis within a simulated experimental setting.

2. Experimental Materials and Equipment

Experimental Materials: D-(+)-Glucose and L-Asparagine were purchased from Aladdin Industrial Corporation; N-Propylethylenediamine (PSA) used in this study was sourced from Shanghai Anpu Experimental Technology Co., Ltd.; [$^{13}C_3$]-Acrylamide isotope (1 mg/mL) was sourced from Cambridge Isotope Laboratories.

Instrumental Equipment: High-Performance Liquid Chromatography (HPLC) system (Model 1525, Waters), Single Quadrupole Mass Spectrometer (Model ZQ 2000, Waters), Chromatographic Column (Venusil C18 MP, Sigmas), Digital Precision Balance (Sartorius Scientific Instruments Co., Ltd.), Low-Speed Automatic Balancing Centrifuge (Shanghai Yiheng Scientific Instrument Co., Ltd.), and a Magnetic Stirrer with a Heating Mantle (Model DF-101S, Gongyi Yuhua Instrument Co., Ltd.).

3. Experimental Methods

3.1 The Effect of Temperature

In the reaction vessel, precisely measured 1.5614g of a mixture containing equal molar amounts of glucose and asparagine. The mixture was subjected to heating treatment using an oil bath with a fixed duration of 10 min. A range of heating temperatures was set, specifically 140°C, 160°C, 180°C, 200°C, and 220°C, to investigate how changes in temperature affect acrylamide formation. After each temperature point was reached, the experimental container underwent swift chilling within an icy aqueous medium, effectively halting the ongoing chemical transformation.

3.2 The Effect of Heating Duration

Similarly, in the reaction vessel, an equimolar mixture of 1.5614g of glucose and asparagine was weighed. The oil bath was consistently regulated at a constant temperature of 180 degrees Celsius, while the periods of exposure to heat were selectively adjusted to encompass intervals of 10, 15, 20, 25, and 30 minutes, respectively. This approach was adopted to investigate the effect of varying heat application times on the generation of acrylamide. Once the chemical process had concluded, the resultant concoction was swiftly subjected to a chilling procedure utilizing a bath filled with crushed ice, thereby arresting any further progression of the reaction.

3.3 The Effect of Reactant Ratios

Different molar ratios of glucose to asparagine mixtures were accurately weighed in the reaction vessel, specifically 2.0:1.0, 1.5:1.0, 1.0:1.0, 1.0:1.5, and 1.0:2.0, with corresponding masses of 2.2220 g, 1.8918 g, 1.5614 g, 2.0018 g, and 2.4622 g, respectively. All experiments were carried out using an oil bath set to 180°C for 20 minutes to evaluate how varying addition ratios influence acrylamide production. After the reaction was completed, the process was rapidly halted by cooling in an ice bath.

3.4 The Effect of Moisture Conten

In the reaction vessel, an accurately weighed mixture of glucose and asparagine, totaling 2.0018 g, was prepared in a ratio of 1.2 to 1.0. Subsequently, the mixture was supplemented with various amounts of distilled water, namely 100 μL, 200 μL, 300 μL, 400 μL, 500 μL, and 600 μL, to investigate the influence of different moisture contents on the synthesis of acrylamide. Afterward, the mixture was brought to 180°C in an oil bath and held at that temperature for 20 minutes. Once the heating was completed, the reaction system was rapidly chilled using an ice bath to quickly terminate the reaction.

3.5 Quantitative Analysis of Acrylamide

Two milliliters of a [$^{13}C_3$]-acrylamide isotope standard solution (5 μg/mL) were introduced into the vessel containing the reactants, then diluted with pure water and transferred to a 100 mL volumetric flask, filling up to the mark. Three milliliters of the solution was taken, and 0.2 g of PSA was added for purification treatment. After vortexing and centrifugation, the solution was filtered using a 0.22 μm filter membrane suitable for aqueous solutions, in preparation for analysis by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS).

The analytical conditions were set as follows: A Venusil MP C18 chromatographic column was used; the injection volume was set to 20 μL; the mobile phase consisted of a blend of methanol and water (volume ratio 1:9); The flow rate was held steady at 0.8 mL per minute; the analysis time was set to 10 min; the ionization source used was positive ion mode (ESI+); the selected reaction monitoring mode (SIR) was employed; the monitoring ion mass for acrylamide was set to 72, while the monitoring ion mass for the [$^{13}C_3$]-acrylamide isotope was 75; the capillary voltage was adjusted to 3.0 kV, and the cone voltage was set to 20 V.

3.6 Data Processing

Each experimental procedure was repeated three times to ensure reliable results. Statistical analysis of the experimental data was completed using SPSS software version 19.0, while the plotting of charts and graphs was carried out with Microsoft Excel software version 2021.

4. Results and Discussion

4.1 The Impact of Heating Temperature on Acrylamide Yield

Within the glucose and asparagine reaction system, heating temperature is a pivotal factor that directly affects the quantity of acrylamide produced. According to reports in the literature [12], the formation of acrylamide requires the food to reach temperatures exceeding 120°C during the heat treatment process. For this study, we maintained a 1:1 ratio of glucose to asparagine and applied a heating time of 10 minutes to investigate the variation in acrylamide formation per unit of asparagine across different heating temperatures: 140°C, 160°C, 180°C, 200°C, and 220°C.

The experimental findings will be analyzed to establish the ideal temperature conducive to maximizing acrylamide generation, recognizing that reaction kinetics and the progression of the temperature-sensitive Maillard reaction play pivotal roles. The data will be presented in graphical form to illustrate the relationship between temperature and acrylamide yield clearly. The statistical significance of the differences observed at various temperatures will be assessed using appropriate tests available in SPSS, ensuring a robust interpretation of the experimental outcomes.

As depicted in Figure 1, as the heating temperature increased, the production of acrylamide per unit of asparagine exhibited an initial rise followed by a decline, reaching its maximum at 180°C. According to the perspective of Robert et al. [13], the movement of the reactant molecules is crucial in facilitating the generation of acrylamide. This implies that in a solid state, the reaction between glucose and asparagine requires melting of both to proceed effectively. If the heating temperature is set too low, glucose and asparagine cannot melt, leading to an insufficient reaction. However, as the heating temperature is raised and the reactants begin to melt, the extent of the reaction increases. Nevertheless, if the temperature continues to rise, acrylamide molecules, due to their instability at high temperatures, are prone to decomposition and polymerization. This not only promotes the formation of acrylamide but also leads to its degradation. Therefore, at excessively high heating temperatures, the rate of acrylamide degradation may exceed its synthesis rate, resulting in a reduced content of the final product, acrylamide.

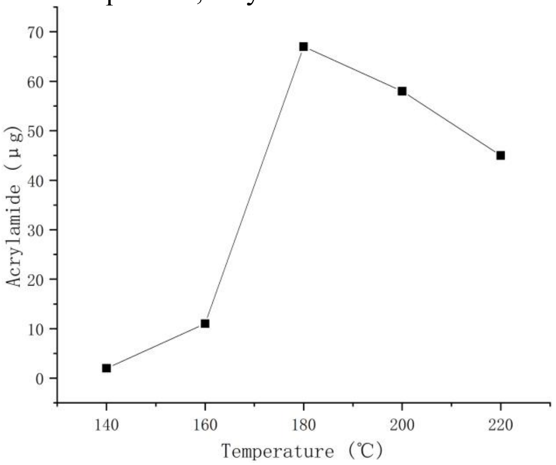

Figure 1. Influence of Heating Temperature on Acrylamide Formation

4.2 The Effect of Heating Duration on Acrylamide Yield

In the reaction system composed of glucose and asparagine, the duration of heating also significantly affects the yield of acrylamide. Generally speaking, a longer heating time helps to advance the chemical reaction between glucose and asparagine. However, if the heating time exceeds the optimal range, it may lead to further decomposition or polymerization of the already formed acrylamide, thereby reducing the final yield [14]. In the experiment, the ratio of glucose to asparagine was maintained at 1:1, and the heating was consistently applied at 180°C for durations of 10 min, 15 min, 20 min, 25 min, and 30 min. Based on the findings in Figure 2, as the duration of heating increases, the amount of acrylamide generated per unit of asparagine shows an increasing and then decreasing pattern, with the highest yield of acrylamide occurring at the 20-minute heating time point.

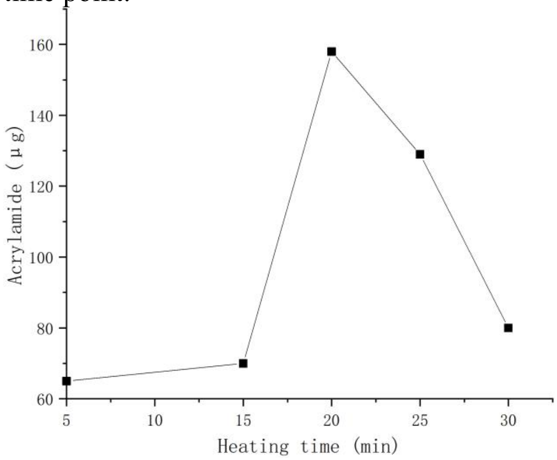

Figure 2. Influence of Heating Time on Acrylamide Formation

This section of the study delves into the detailed impact of temperature and time, key factors affecting acrylamide formation through the Maillard reaction. The findings underscore the importance of optimizing these parameters to maximize acrylamide yield while minimizing potential degradation, offering valuable insights for food processing industries aiming to control acrylamide formation.

4.3 The Impact of Glucose to Asparagine Ratio on Acrylamide Yield

In this experiment, employing a heating condition of 180°C for a duration of 20 minutes, the influence of glucose and asparagine mixtures at different molar ratios (2.0:1.0, 1.2:1.0, 1.0:1.0, 1.0:1.5, and 1.0:2.0) on the yield of acrylamide was investigated. The masses of the mixtures used were 2.2220 g, 1.8918 g, 1.5614 g, 2.0018 g, and 2.4622 g, respectively, to explore the impact of varying ratios on acrylamide formation. Observing Figure 3, it can be observed that as the proportion of glucose decreases relative to asparagine, the amount of acrylamide produced per unit of asparagine first increases and then decreases, with the highest yield of acrylamide occurring at a glucose to asparagine ratio of 1.2:1.0. Based on how acrylamide forms, it primarily arises from the interaction of asparagine with glucose or similar α-hydroxy carbonyl compounds in a chemical process that involves the formation of Schiff bases as a crucial intermediate step. Therefore, only when the ratio of glucose to asparagine is appropriate can the production of Schiff base be maximized, and an excess of either glucose or asparagine may inhibit the formation of Schiff base.

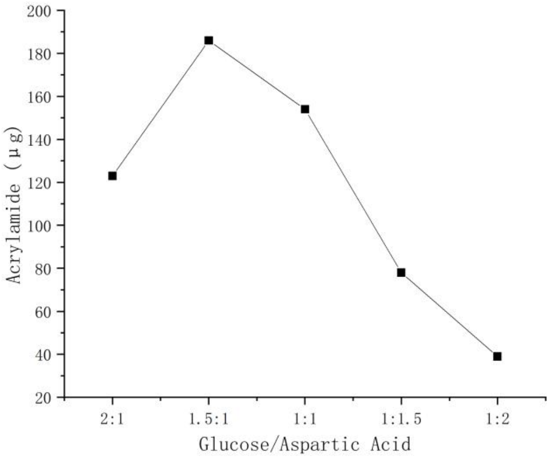

Figure 3. Influence of Glucose / Asparagine Addition Proportion on Acrylamide Production

This section of the study elucidates the importance of stoichiometric balance between glucose and asparagine in the formation of acrylamide. The findings highlight the necessity for an optimal ratio to facilitate the Maillard reaction and Schiff base formation, which are critical for maximizing acrylamide yield. These insights are crucial for the food industry to understand and control the acrylamide formation process during thermal processing.

4.4 The Impact of Moisture on Acrylamide Yield

During the Maillard reaction process, the role of moisture is a critical factor, typically measured by moisture content or water activity, and a relationship between the two can be established through the sorption isotherm at a specific temperature. Prior to investigating how moisture content affects the generation of acrylamide in the glucose/asparagine system, this study initially established the sorption isotherm of the system at 25°C. According to the data presented in Figure 4, for water activities below 0.8, the moisture level in the glucose/asparagine system is approximately less than 3%; however, when the water activity exceeds 0.8, the moisture content in the system increases sharply. In food processing practice, the initial moisture content of food raw materials is generally above 20%, which results in a corresponding water activity in the glucose/asparagine model that is maintained within a narrow range of 0.9 to 1.0. Furthermore, Mestdagh et al. [15], in their investigation of the potato model system, it was highlighted that moisture content has a more significant influence on acrylamide formation compared to water activity. Therefore, this experiment focuses on analyzing how moisture content influences acrylamide formation in the glucose/asparagine model.

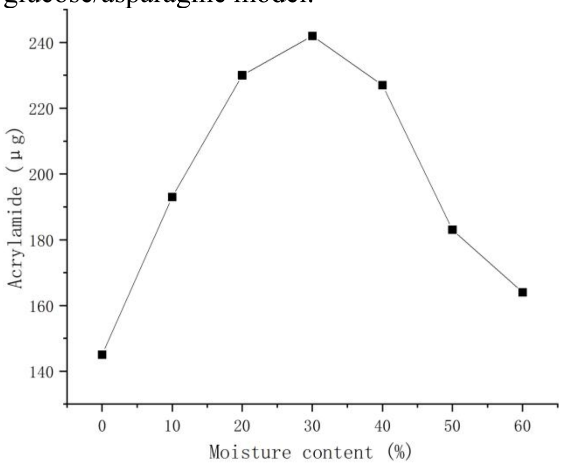

Figure 4. Influence of Adding Amount of Water on Acrylamide Formation

This section of the study emphasizes the significance of moisture in the Maillard reaction and its influence on acrylamide production. By examining the sorption isotherm and understanding how water availability relates to moisture levels, the research provides a basis for controlling the moisture levels in food processing to minimize acrylamide formation. The findings contribute to the body of knowledge on the role of moisture in food chemistry and the mitigation strategies for acrylamide in thermally processed foods.

Observation of Figure 4 reveals that as moisture content gradually increases, the production of acrylamide per unit of asparagine shows an initial rise followed by a decline, reaching its peak at a moisture content of 30%. This phenomenon may be due to an optimal amount of moisture enhancing the mobility and frequency of contact between reactant molecules, thereby promoting the reaction. However, when the moisture content exceeds a certain critical value, the excess moisture begins to dilute the reaction system, reducing the contact opportunities between reactants, leading to decreased reaction efficiency and, consequently, a reduction in the amount of acrylamide produced.

5. Conclusions

The research thoroughly examined how food processing variables affect acrylamide formation, specifically analyzing four key factors: temperature, duration of heating, glucose-to-asparagine ratio, and moisture content. By precisely controlling experimental conditions and combining single-factor experiments with response surface methodology, the study systematically analyzed the influence of each factor on the generation of acrylamide. It was found that heating temperature and duration significantly affect the formation of acrylamide, with the maximum yield occurring at 180°C for 20 min. Additionally, the molar ratio of glucose to asparagine and moisture content also played crucial roles in the formation of acrylamide. Notably, the maximum acrylamide yield was observed with a glucose-to-asparagine ratio of 1.2:1.0 and a moisture level of 35.2%. These findings provide important theoretical support for controlling the production of acrylamide in food processing. By optimizing processing conditions, the content of acrylamide in food can be effectively reduced, which is significant for ensuring food safety and public health. Future research can delve into how various food types and processing methods affect acrylamide formation, providing more extensive and practical guidance for the food industry.

This conclusion summarizes the study's findings and underscores the significance of the research in guiding the food industry to mitigate acrylamide formation. The insights gained from this study contribute to the broader understanding of the chemical processes involved in food processing and the development of strategies to enhance food safety.

Acknowledgments

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