EFFECT OF VARIOUS FACTORS ON THE FORMATION OF AMINOREDUCTONE DURING THE MAILLARD REACTION

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ABSTRACT

To create a basis for the production of aminoreductone (AR) followed by the use of it as a functional ingredient in food supplements, the effects of heating temperature, heating time, buffer concentration and reducing sugar/amino compound ratio on the formation of aminoreductone (AR), a product formed during the initial stage of the Maillard reaction,were investigated. In the model solution of lactose (262 mM) and butylamine (1.16 M) in 1.28 M phosphate buffer (pH 7.0), AR formation increased during the prolongation of the heating time at heating temperature less than 100^{o}C. In the heated model solution at 100 ^{o}C, the maximum amount of AR was obtained as soon after 10 min of heating time. After that, the decreases of the AR formation was observed by the progress of the advanced reactions of AR. The rates of AR formation increased with the increasing phosphate buffer concentration. Moreover, the increasing in AR formation incorporated in the increase of the butylamine/lactose ratio from 0.1 to 5. Our results might give more insight in the discovery of Maillard reactions in food because it suggested tendency of the AR formation and to control the formation of AR during food processing and model system base on the control of initial constituents and reaction conditions.

Keywords: Maillard reaction, aminoreductone, heating conditions.

1. INTRODUCTION

Maillard reaction, a common reaction between reducing sugars and amino compounds, occurs in the processing and storage of food. Maillard reaction is extremely important to create the desirable and unique flavors for food products [1, 2]. The Maillard reaction also causes problems due to the generation of loss nutrient or producing some disadvantage substance for human health. Since the Maillard reaction directly effects on the quality of food products, research on Maillard reaction is always one of the most concern of the food scientists in the world.

The formation of aminoreductone (1-[N^{ε}-(N^{α}-acetyllysinyl)]-1,2-dehydro-4-deoxy-3-hexulose) in the initial stage of the Maillard reaction was first reported by Pischetsrieder et al. (1998) in a heating solution of lactose and N^{α}-acetyllysine [3]. This group also emphasized an anti-hypertensive and antioxidant activity of AR [4]. Ukeda et al. proposed an assay for determining an ability of milk to reduce a tetrazolium salt XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2*H*-tetrezolium- 5-carboxanilide) (XTT) as a method of evaluating the extent of the Maillard reaction [5]. It was also shown that AR, which was formed during the Maillard reaction in a model solution of lactose and butylamine, was mainly responsible for the reducibility of XTT [4]. Compared to the previously reported such as the quantification of hydromethylfufural, lysine etc, the XTT assay is a rapid and convenient method and this method was quickly applied in Japanese milk factory. Since the formation of AR was obtained as an indicator for the extent of the Maillard reaction, the discovery about the role and characteristic of AR should be an interesting topic for food scientists. So far, an antioxidant activity [3], and an antimicrobial activity against *Helicobacter pylori*, a Gram-negative bacilli of AR were reported [6].

In the field of food technology, food scientists and producers always consider many factors that can contribute to a good and health beneficial products. Since foods containing AR might be valuable sources as antioxidative, antihypertensive ingredients [3, 7], and the protective ability of AR on riboflavin photo-degradation, this is the suggestion to concern about using AR as a functional ingredient in food and to control the formation of AR in food product. Study the effects of factors such as heating temperature and time, buffer concentration and the ratio of lactose/amino compound concentration on the formation of AR in the model solution has been conducted.

2. MATERIALS AND METHODS

2.1. Reagents

XTT was purchased from Sigma Chemical Co. (St. Louis, MO. USA). Lactose monohydrate, butylamine, potastium hydrophosphate and potastium dihydrophosphate were from Nacalai tesque, Inc. (Kyoto, Japan). All other reagents were of the highest grade commercially available. Milli-Q water was used in all procedures.

2.2. Establishing the model solution of reducing sugar and amino compound

The solutions containing lactose and butylamine were used as a model system of the Maillard reaction of lactose (model solution). The solutions were prepared according to the previous report [4]. Briefly, lactose monohydrate (127 mM) and butylamine (0 - 635 mM) were dissolved in 1.28 M phosphate buffer (pH 7.0). The concentration of sugar was set to the value based on that of lactose in milk [5]. Also, the model solution containing lactose monohydrate (127 mM) and butylamine (15 mM) in 20 mM phosphate buffer pH 6.7 was used as the model sample of milk system. The butylamine concentration was adjusted so as to be almost the same amino group concentration as that of the primary amino group of casein in milk [5].

2.3. XTT assay procedure

The formation of AR in heated model solutions and milks was determined using the XTT assay, performed in a 96-well microtiter plate according to the method described by Shimamura et al. [4]. Each well contained 60 μL of 0.5 mM XTT prepared with 0.2 M potassium phosphate buffer (pH 7.0) saturated with menadione. Sample (40 μL) was added to the well and, after mixing in a microplate shaker at a speed of 500 rpm for 15 sec, the difference in the absorbance between 492 nm and 600 nm was read on a microplate reader (MPR A4i, Tosoh, Tokyo, Japan) as the absorbance at 0 min. After 20 min at room temperature, the difference in absorbance was again read and the increase in the absorbance was recorded as the ability of a sample to reduce XTT (XTT reducibility).

All assays were performed at least in duplicate and the results are shown as average values.

3. RESULTS AND DISCUSSION

3.1. Effect of heating temperature and time on the formation of AR

In an attempt to control the formation of AR in food processing and to optimize the producing conditions of AR, we investigate the effects of factors such as heating temperature, heating time, buffer concentration and the ratio of lactose and amino acid on the formation of AR in the Maillard reaction.

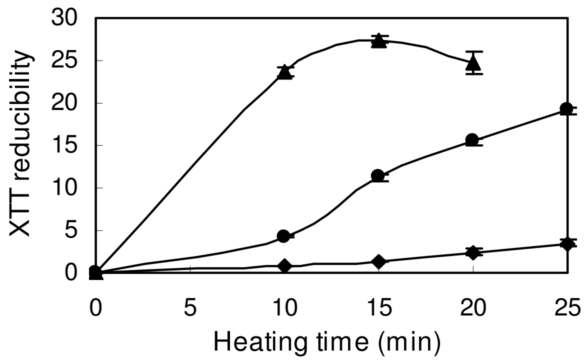

Figure 1. The effect of heating time and heating temperature on the formation of AR

The model solution of lactose (262 mM) and butylamine (1.16 M) in 1.28 M phosphate buffer (pH 7.0) were heated at 80°C (◆), 90°C (●), and 100°C (▲) for 0-25 min.

In our previous studies, it was already clarified that the reduction of XTT was mainly caused by AR formed in the Maillard reaction of lactose and butylamine [4]. Thus, the XTT reducibility of the Maillard reaction of model solution lactose and butylamin heated at 80 – 100 °C for 0 - 25 min was evaluated. As can be seen in Fig. 1, AR could be detected in the heated model solution and considerable increase in the XTT reducibility was dependent on the heating time and temperature. In addition, at higher temperatures, a more rapid increase of AR was noted. In the model solution of lactose (262 mM) and butylamine (1.16 M) in 1.28 M phosphate buffer (pH 7.0), AR formation increased during the prolongation of the heating time at heating temperature less than 100 ^{o}C. During the heating at 100 °C for 15 min, the XTT reducibility increased to 27 in value, while it was 17 and 3 in values at 90 °C and 80 °C for 25 min, respectively. Ukeda et al. [5] and Shimamura et al. [8] pointed out that AR formed by the Maillard reaction of lactose and amino compounds. In the heated model solution at 100 ^{o}C, the maximum amount of AR was obtained as soon after 10 min of heating time. After that, the decreases of the XTT reducibility was observed during the prolongation of the heating time. These might be referred to the progress of the advanced reactions of AR that commonly take place in the complicated sequences of the Maillard reaction during heating [3]. Our finding supported the presumption of previous reports that AR was formed in early stage of the Maillard reaction. Thus, AR can be obtained at maximum amount only in the initial stage of Maillard reaction. The study suggested the food technologies to find the suitable heating conditions for producing food abundant of AR.

3.2. Effect of buffer concentration on the formation of AR

Buffer agents were added in many diverse food to control the pH of the system [7]. Limited data exist on the effects these buffers have on the Maillard reaction and the formation of AR. To find out the suitable condition and to establish the good model solution for the producing of AR in the future study, the effect of phosphate buffer concentration on the formation of AR was also investigated. The formation of AR was evaluated in phosphate buffer solutions of various concentration at pH 7 and 90 ^oC. As showed in Fig. 2, the rates of AR formation increased with the increasing phosphate buffer concentration. The concentration of AR in heated model solution at 2 M of buffer concentration was 2 times higher than at 0.04 M of buffer concentration. Similar observations about the increase in the Maillard reaction rate with the increasing of buffer concentration was also presented. In line with our results, Bell reported that the rates of glycine loss and browning increased with increasing phosphate buffer concentration [7]. The results obtained in our study indicated that the phosphate anion should be a catalytic compound for the production of AR.

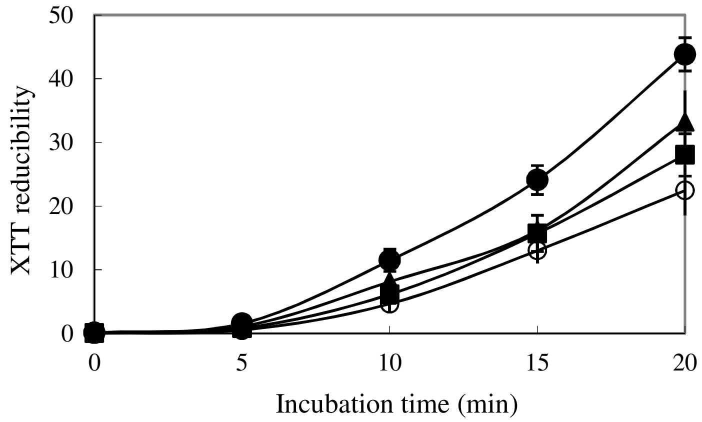

Figure 2. The effect of buffer concentration on the formation of AR

The model solution of lactose (127 mM) and butylamine (15 mM) in 0.04 M (●); 0.5M (▲); 1 M (■) and 2 M (○) phosphate buffer (pH 7) was heated at 90 ^oC for 0 - 20 min.

3.3. Effect of reducing sugar/amino ratio on the formation of AR

In the system containing reducing sugar and amino compounds, the Maillard reaction always accompanies with the isomerization/degradation reaction of sugar. Thus, we proposed that the ratio of sugar/amino concentration might affect on the formation of AR during heating. It was tried to determine changes in AR formations when the ratios of lactose/amino group concentration in the model solution before heating were altered. As showed in Fig. 3A, at the same amount of lactose and the same heating condition, the increasing in AR formation incorporated in the increase of the butylamine/lactose ratio from 0.1 to 5. In the model solution containing abundant amount of butylamine, the maximum amounts in the XTT reducibility values were obtained as soon after 10 min of the heating time (Fig. 3B). These might be referred to the progress of the advanced reactions of AR that commonly take place in the complicated sequences of the Maillard reaction during heating [3] after all the reducing sugar was taken part in the initial stage of the reaction. Thus, based on our results, it can be postulated that the formation of AR closely depends on the ratio of lactose and amino compounds concentration in the solution before heating.

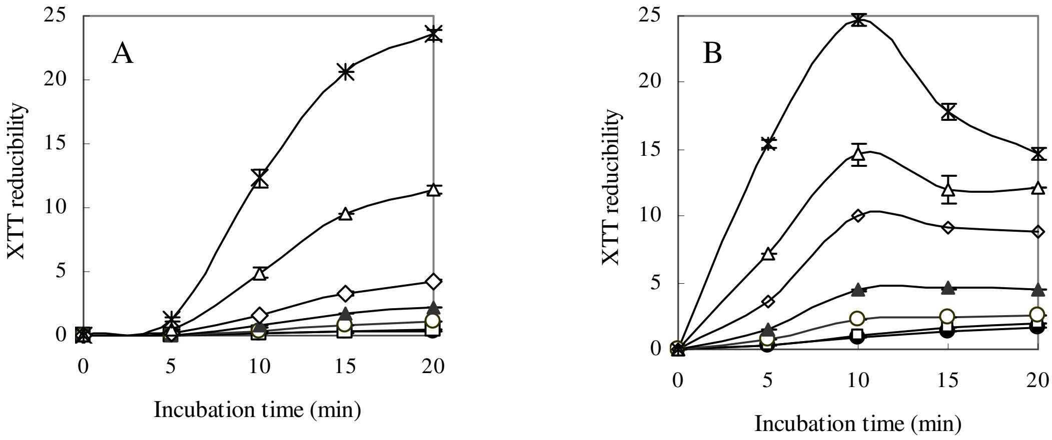

Figure 3. The effect of reducing sugar/amino ratio on the formation of AR at 100°C (A) and 120 °C (B). Lactose (127 mM) and butylamine (●: 12.7 mM; □: 15 mM; ○: 31.75 mM; ▲: 63.5 mM; ◇: 127 mM; △: 317.5 mM; ✳: 635 mM; -: 0 mM) in 1.28 M phosphate buffer (pH 7.0).

4. CONCLUSION

On the basis of our results, the formation of AR was closely depends on heating time, heating temperature, buffer concentration and the ratio of reducing sugar/amino compound concentration. Furthermore, the obtained results might give more insight in the discovery of Maillard reactions in food because it suggested tendency of the AR formation and to control the formation of AR during food processing and model system base on the control of initial constituents and reaction conditions. The study creates a basis for the production of AR followed by the use of AR as a functional ingredient in food supplements. In the following studies, the optimization producing conditions of AR by using the model solution of reducing sugars and amino compounds will be performed.

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TÓM TẮT

ẢNH HƯỞNG CỦA CÁC YẾU TỐ ĐẾN SỰ TẠO THÀNH AMINOREDUCTONE TRONG PHẢN ỨNG MAILLARD

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Để tạo cơ sở cho quá trình điều chế aminoreductone (AR) và sử dụng AR như thành phần chức năng bổ sung vào thực phẩm, chúng tôi nghiên cứu ảnh hưởng của các yếu tố như là nhiệt độ, thời gian gia nhiệt, nồng độ dung dịch đệm và tỉ lệ đường khử/amino axit trong dung dịch ban đầu tới sự tạo thành AR trong phản ứng Maillard. Trong mô hình phản ứng của lactoza (262 mM) và butylamin (1,16 M) trong 1,28 M dung dịch đệm phốt phát (pH 7,0), ở nhiệt độ gia nhiệt thấp hơn 100 ^{o}C, hàm lượng AR tạo thành tăng lên trong suốt quá trình gia nhiệt. Tại 100 ^{o}C, lượng AR đạt cực đại ngay sau 10 phút gia nhiệt. Tiếp đó, lượng AR trong dung dịch giảm dần do các quá trình phản ứng tiếp theo của chuỗi phản ứng Maillard. Tốc độ tạo thành AR tăng khi nồng độ dung dịch đệm tăng. Thêm vào đó, hàm lượng AR tạo thành tăng lên cùng với sự tăng lên của tỉ lệ về nồng độ butylamin và lactoza từ 0,1 đến 5. Kết quả nghiên cứu của chúng tôi có thể cung cấp cái nhìn sâu sắc hơn trong nghiên cứu về phản ứng Maillard trong thực phẩm vì nó cho thấy xu hướng của sự hình thành AR. Kết quả nghiên cứu cũng giúp kiểm soát sự tạo thành AR trong quá trình chế biến thực phẩm và xây dựng mô hình phản ứng dựa trên việc kiểm soát nồng độ các thành phần ban đầu và điều kiện phản ứng.

*Từ khóa***:** phản ứng Maillard, aminoreductone, điều kiện gia nhiệt.