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EFFECTS OF OXYGEN ON THE MAILLARD REACTION OF LACTOSE

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ABSTRACT

The effects of dissolved oxygen (DO) on the Maillard reaction of lactose were investigated. In the model solution of lactose and butylamine it was found that the concentration of aminoreductone (AR), Galactose (Gal), and Maillard reaction products,in deaerated sample were significantly higher than that in non-deaerated sample. The browning of deaerated sample measured by the absorbtion of the Maillard reaction product melanoidin at 420 nm was also higher than that in non-deaerated sample. The decrease in the oxygen concentration in the samples during heating at different conditions was recognized. It was found to correlate with the formation of Maillard reaction products (AR, Gal and browning compounds). Our results might give more insight in the Maillard reactions in food and suggest that food processing manufacturers should pay attention to the effects of dissolved oxygen in the process besides considering other factors such as reaction components and reaction conditions in order to receive the highest quality and nutritive value of the final products.

Keywords: oxygen, aminoreductone, galactose, Maillard reaction.

1. INTRODUCTION

Maillard reaction, a reaction between reducing sugars and compounds bearing free amino groups, is very important to food processing because it strongly influences the quality of final product [1]. Therefore, investigation of the Maillard reaction products is of paramount importance for the quality evaluation of foods. The Maillard reaction is extremely complicated; it can be classified by mechanisms involving oxidative and non-oxidative reactions [2]. Several investigations thoroughly studied the effects of oxygen on the advanced stage of Maillard reaction of proteins with glucose [2] and pentoses [3] under physiological conditions. However, most real food systems contain oligosaccharides [4]. In addition, they also occur as a consequence of thermal treatments in the manufacturing process at high temperature. Therefore it should be of a great value to study the effect of oxygen on the formation of the Maillard reaction products in food processing. De-aeration treatment is oftenly used in food processing to get rid of the effect of oxygen during food preservation. However, there are very few studies on the effect of deaeration on the Maillard reaction in food or food quality.

Lactose, a component of milk and dairy products, is one of the most important disaccharides in foods [5]. Up to now, the role of oxygen in the Maillard reaction of lactose has not been rigorously explored. In previous reports, we proposed an assay method for determining the ability of milk to reduce the tetrazolium salt XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2*H*-tetrezolium-5-carboxanilide) as a method of evaluating the extent of the Maillard reaction [6, 7]. It was also shown that aminoreductone (AR), which was formed during the Maillard reaction in a model solution of lactose, was mainly responsible for the reducibility of XTT [8]. In the pathway of the AR formation, galactose (Gal) was generated by the progress of the Maillard reaction [5].

In order to understand the effect of oxygen on the Maillard reaction in foods, we investigate in the present study the effects of oxygen on the formation of the Maillard reaction products of lactose such as AR and Gal under thermal treatments.

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. Incubation procedure

The solution containing lactose and butylamine was used as a model system for the Maillard reaction of lactose (model solution). Lactose monohydrate (127 mM) and butylamine (15 mM) were dissolved in 20 mM phosphate buffer (pH 6.7). The concentration of sugar was set to the value based on that of lactose in milk. Also, the butylamine concentration was adjusted to the same concentration of primary amino group of casein in milk (Ukeda et al., 1998).

The dissolved oxygen in the model solution was eliminated by purging with nitrogen for 20 min. The oxygen concentration in the solution was measured using the dissolved oxygen meter with an accuracy of $\pm$ 0.4 mg/l (DO-5509, Fuso Co., Ltd., Tokyo, Japan).

The model solution (1.2 mL) was heated under indicated conditions. Immediately after heating, the heated solution was cooled on ice to stop the reaction. And then, the formation of AR was determined using the XTT assay. The heated model solution was filtered through a Sep-Pak Plus C18 (Waters, MA; activated by 5 mL of ethanol and equilibrated by Milli-Q water) to remove brown components. The obtained clear solution was used for the determination of Gal.

2.3. XTT assay procedure

The formation of AR in heated model solution and milk was determined using the XTT assay, performed in a 96-well microtiter plate according to the method described by Shimamura et al. [8]. 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 s, 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 absorbance difference was again read and the increase in the absorbance was recorded as the ability of sample to reduce XTT (XTT reducibility).

All assays were performed at least twice and the results were shown as an average value.

2.4. Determination of Gal

The concentration of Gal was determined by F-kit (Lactose/ D-Galactose) according to the instruction. The principle of F-kit was as follows: Gal was oxidized by NAD to D-galactonic acid in the presence of galactose dehydrogenase at pH 8.6. At the same time, NAD was reduced to NADH. The concentration of NADH formed was stoichiometrically equal to that of Gal. The increase in NADH was measured at 340 nm (UV-VIS Spectrophotometer UV mini 1240, Shimadzu, Japan) before and after the addition of galactose dehydrogenase [7]. All tests were performed at least twice and the results were shown as average values.

3. RESULTS AND DISCUSSION

To investigate the effects of oxygen on the Maillard reaction of lactose, the model solutions of lactose and butylamine were incubated at 100 ^{o}C and 120 ^{o}C for the indicated time under aerobic and non-aerobic conditions with 8.8 mg L^{-1} and 0.8 mg L^{-1} dissolved oxygen, respectively.

3.1. Effects of oxygen on the generation of AR in the Maillard reaction of lactose

The formation of AR in the Maillard reaction was recognized by the increase of XTT reducibility [7, 8]. As can be seen in Fig. 1, the formation of AR was accelerated in the absence of oxygen during heating. After 20 min of incubation, the XTT reducibility under non-aerobic condition considerably increased by 28.05 % and 15.12 % compared to that under aerobic condition at 100 ^{o}C and 120 ^{o}C, respectively. There are several possible explanations for the increase of AR formation during the Maillard reaction under the non-aerobic condition. First, it was investigated that AR is a strong reducing substance that can act as an antioxidant or prooxidant, mainly due to the reductone structure [5]. In the presence of oxygen, this highly reactive substance can easily form a complex with oxygen leading to the reduction of AR after formation. Thus, at the same heating condition, the XTT reducibility of the model solution containing the dissolved oxygen at high concentration should show lower value than that of the model solution containing negligible amount of oxygen. The second possibility is that oxygen might directly alter the pathway of the AR formation. Although the participation of oxygen during the Maillard reaction has previously been suggested [2, 3], most of studies spoke for the effect of oxygen on the generation of advanced glycation end products during the Maillard reaction.

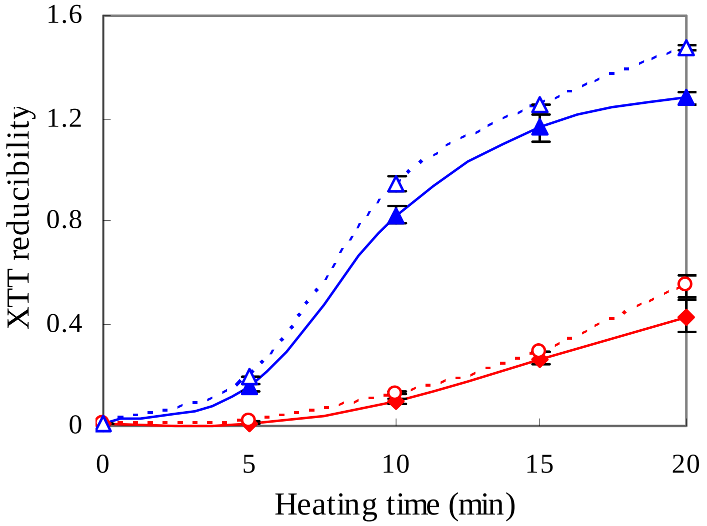

Figure 1. Effects of oxygen on the generation of AR in the Maillard reaction of lactose.

The model solutions of lactose (127 mM) and butylamine (15 mM) in a 20 mM phosphate buffer (pH 6.7) containing 8.8 mgL-1 (solid line) and 0.8 mgL-1 oxygen (dotted line) were heated at 100 ^{o}C (circle) and 120 °C (triangle) for 0 - 20 min. Data points represent means ± SD of results obtained from two independent experiments for each reaction condition.

3.2. Effects of oxygen on the generation of Gal in the Maillard reaction of lactose

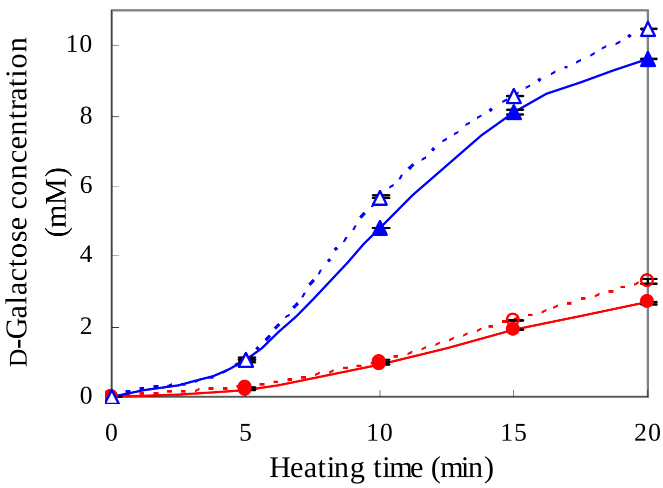

Figure 2. Effects of oxygen on the generation of D-galactose in the Maillard reaction of lactose.

The model solutions of lactose (127 mM) and butylamine (15 mM) in a 20 mM phosphate buffer (pH 6.7) containing 8.8 mgL^{-1} (solid line) and 0.8 mgL^{-1} oxygen (dotted line) were heated at 100 ^{o}C (circle) and 120 °C (triangle) for 0 - 20 min. Data points represents means ± SD of results obtained from two independent experiments for each reaction condition.

In previous study, we observed that Gal which was formed during the formation of AR in the Maillard reaction of lactose was a product of the cleavage of Amadori compound. If the first possibility was a major reason for the XTT reducibility in the presence of oxygen, the formation of Gal should not be significantly different in both aerobic and non-aerobic conditions. In contrast with this hypothesis, as shown in Fig. 2, similar results to the formation of AR were obtained from measurement the Gal formation: the absence of oxygen enhanced the generation of Gal from the Maillard reaction at 100 °C and 120 °C by 23.48 % and 8.94 %. These evidences indicated that the change in the XTT reducibility and the Gal concentration was mainly the result of a corresponding change during the process of the Maillard reaction by the presence of oxygen.

3.3. Effects of oxygen on the generation of the final product in the Maillard reaction of lactose

In the final stage of Maillard reaction, the browning of the reaction solution is generated due to low molecular weight colored compounds and due to high molecular weight conjugated chromophores, commonly referred as melanoidins [10]. Final products of Maillard reaction (browning products) have been measured spectrophotometrically as the absorbance at 420 nm [11]. To investigate the effect of oxygen on the Maillard reaction, we also monitored the formation of melanoidins during heating. Figure 3 shows the browning generated by the Maillard reaction of lactose and butylamine at 100 °C and 120 °C under aerobic and non-aerobic conditions. At 100 °C, although the formation of browning products was negligible, there was slightly increased browning under non-aerobic condition during heating, compared to that under aerobic condition. The significant increase of the browning products under non-aerobic compared to aerobic conditions was recognized at 120 °C. This result is in agreement with literature data describing the acceleration of the Maillard reaction of glucose in non-aerobic condition [2].

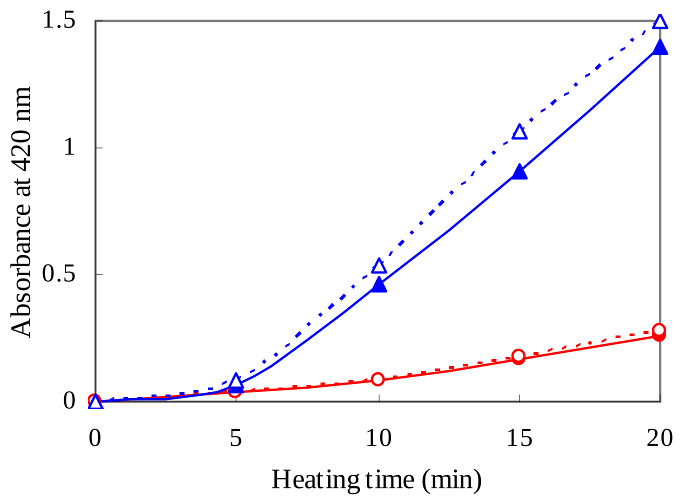

Figure 3. Effects of oxygen on browning in the Maillard reaction of lactose.

The model solutions of lactose (127 mM) and butylamine (15 mM) in a 20 mM phosphate buffer (pH 6.7) containing 8.8 mgL^{-1} (solid line) and 0.8 mgL^{-1} oxygen (dotted line) were heated at 100 °C (circle) and 120 °C (triangle) for 0 - 20 min.

3.4. Reduction of oxygen in the model solution during heating

The concentration of oxygen in the model solution before heating was 8.8 mgL^{-1}. As shown in Fig. 4, the dissolved oxygen was drastically reduced to 2 and 1.4 mgL^{-1} after 10 min heating at 100 ^{o}C and 120 ^{o}C, respectively. The reduction of oxygen content during the Maillard reaction suggested that oxygen might participate in the Maillard reaction of lactose.

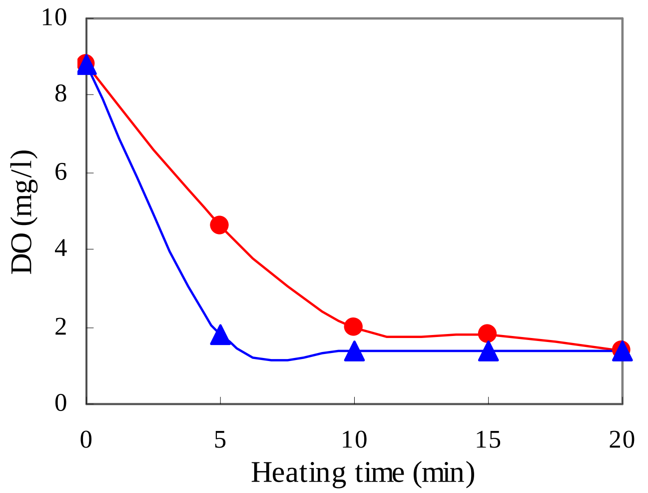

Figure 4. Reduction of oxygen concentration in the model solution during heating at 100 ^{o}C (●) and 120 °C (▲) for 0 - 20 min.

Several investigations have shown that the Maillard reaction of disaccharides leads to the formation of substances that are not known to arise from monosaccharides [9]. However, in the present study, the effect of oxygen on the Maillard reaction of lactose is in line with literature describing the increase of the Maillard reaction products of glucose and pentose in the absence of oxygen. Hayase et al. [2] suggested that oxygen did not affect the formation pathway to Amadori compounds, but it might participate in the cleavage of the Amadori compound in the presence of metals and alter the formation pathway of the final products. In phosphate buffer, a trace of metal ion was present [12]. Kawakishi et al. [12] also showed that the oxidative degradation of the Amadori compound went through complexes with copper ion in the presence of oxygen as an ion transportation [12]. Because the presence of oxygen inhibited the direct modification of the Amadori compound, as a consequence, the formation of the Maillard reaction products was delayed in following stages. More recently, Litchfield et al. [3] also presented that the browning of proteins by pentoses occurs efficiently in the absence of oxygen and in the presence of transition metal chelators. In the present study, the formation of AR and Gal in the model solution containing oxygen at high concentration was also inhibited comparing with that in the de-oxygen model solution. From these results, we can suggest that oxygen not only affected the formation of the advanced glycation end products, but it also affected the initial stage of the Maillard reaction of lactose. The inhibition by oxygen in the formation of the Maillard reaction products of lactose had not been sufficiently investigated. However, it could be concluded that the participation of oxygen and a trace of metal in phosphate buffer could alter the reaction pathway of the Maillard reaction. In the absence of oxygen, AR and Gal were directly generated from the cleavage of the Amadori compound.

4. CONCLUSION

The effect of oxygen on the Maillard reaction has so far received little attention, even though there has been sporadic report on the increase of the Maillard reaction products under the absence of oxygen. However, most of the investigations addressed the effect of oxygen on the Maillard reaction in vivo, investigating the formation of the advanced glycation end products of monosaccharides. The present study clearly indicated that the absence of oxygen indeed accelerated the Maillard reaction of disaccharides in foods from the early stage of the reaction by using the model solution of lactose and butylamine. Although the mechanism of the participation of oxygen in the reaction is still unclear, this finding could contribute a valuable knowledge to food science and food manufacturing. It must be emphasized that the elimination of oxygen reduces the oxidation of the food components during manufacturing and storage; on the other hand, it has an opposite effect on the acceleration of the Maillard reaction. During processing, the manufacturer should should pay attention to the participation of oxygen to receive the highest quality and nutritive value of the final products.

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TÓM TẮT

ẢNH HƯỞNG CỦA OXY TRONG PHẢN ỨNG MAILLARD CỦA LACTOZA

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Nghiên cứu này khảo sát ảnh hưởng của hàm lượng oxy hòa tan (DO) lên phản ứng Maillard của lactose. Trong mô hình mẫu lactose và butylamine, các sản phẩm của phản ứng Maillard như aminoreductone (AR), galactoza (Gal) tạo thành trong mẫu đã loại bỏ oxy là cao hơn đáng kể so với mẫu không loại oxy. Màu nâu của mẫu đã loại bỏ oxy xác định bằng độ hấp thụ tại bước sóng 420 nm, thể hiện sự tạo thành của melanoidin, một sản phẩm của phản ứng Maillard, cũng cao hơn so với trong mẫu không loại oxy. Kết quả nghiên cứu cho thấy nồng độ oxy trong các mẫu trong quá trình gia nhiệt ở điều kiện nhiệt khác nhau giảm tương ứng với sự hình thành các sản phẩm của phản ứng Maillard (AR, Gal và các hợp chất màu nâu). Kết quả nghiên cứu cho thấy khi nghiên cứu về phản ứng Maillard trong thực phẩm, không chỉ cần kiểm soát thành phần ban đầu và điều kiện phản ứng mà còn cần quan tâm tới hàm lượng oxy có mặt trong thực phẩm. Trong khi chế biến, các nhà sản xuất cũng cần quan tâm đến sự có mặt của oxy để nhận được sản phẩm có giá trị dinh dưỡng và chất lượng tốt nhất.

Từ khóa: ôxy, aminoreductone, galactoza, phản ứng Maillard.