Influence of Water Activity on the Development of Antioxidative Effect during the Maillard Reaction between Histidine and Glucose

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Influence of water activity on the development of antioxidative effect by the Maillard non-enzymatic browning reaction between histidine and glucose in the presence of microcrystalline cellulose was examined. The formation of brown pigment during the Maillard reaction increased as water activity of the model system decreased and initial rates of color formation reaction at 120°C were about twice those at 100°C regardless of water activity. Furthermore the initial rate of antioxidative effect development was found to increase with decreasing water activity. Maillard reaction products thus prepared was applied to the production of kamaboko-type sardine product as an antioxidant and the oxidation of sardine lipids was effectively inhibited during storage at 4°C.

The intricate chemical reaction between amino and carbonyl compounds, known as the Maillard browning reaction, has gained increased attention in recent years. The reaction is very common during cooking, processing, and storage of many foods. In most cases, browning is undesirable because of the development of off-flavor and poor color as well as the loss of nutritional value. However, in coffee, baked goods, potato chips, and some other processed foods, controlled browning is indispensable for their desirable characteristics. A considerable amount of researches pertaining to the Maillard reaction have been carried out and reviewed by many workers.¹⁻⁴⁾

Polyunsaturated fatty acids in foods are particularly prone to autoxidation. There are a number of examples which indicate that autoxidation plays a crucial role in the formation of off-flavor and toxic substances. The oxidation of fatty acids at first leads to the formation of fatty acid hydroperoxides by a free radical chain reaction. The hydroperoxides subsequently undergo several reactions which bring about the formation of such products as aldehydes, ketones, and other volatile compounds. As a result, rancid flavor is developed and limits the shelf-life of many food products.

The progress of lipid oxidation can be prevented either by adding antioxidants or packaging in vacuum or inert gas. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated

hydroxytoluene (BHT) and propyl gallate have frequently been used for the processing of foods. However, natural antioxidants are preferred from a food hygienic point of view. In recent years a large number of Maillard reaction products (MRP) were demonstrated to have antioxidative effect and applied as antioxidants to model and food systems.⁵⁻⁸⁾ Despite there being much published work on antioxidative effect on these compounds, no antioxidants formed by the reaction have so far been identified, nor has the mechanism of their antioxidative effect been completely elucidated.

The aims of this work were to study the influence of water activity on the development of antioxidative effect during the Maillard reaction between histidine and glucose and to apply histidine-glucose MRP to kamaboko-type sardine products as antioxidants.

Materials and Methods

Preparation of the Model System

L-Histidine monohydrochloride monohydrate (1.25 g, Nihon Rikagaku Yakuhin Co.) and anhydrous D-glucose (1.08 g, Kokusan Kagaku Co.) with molar ratio of 1:1 were dissolved in 30 ml of 0.1 M phosphate buffer (pH 7.0). After pH value of the solution was adjusted to 9.0 with 20% KOH, microcrystalline cellulose powder (Avicel, Asahi Kasei Co., 10 g) was slurried with

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this solution. The slurry was frozen in a blast freezer (-30°C) and then freeze-dried. Microcrystalline cellulose is inert to the browning reaction and served as a solid support for the model system. After freeze-drying, samples were crushed with a mortar and pestle to a fine powder and then equilibrated to the desired water activity (A_w) *in vacuo* over an appropriate saturated salt solutions. For the adjustment of water activity, saturated solutions of MgCl_2 (A_w 0.33), $\text{Mg}(\text{NO}_3)_2$ (A_w 0.51), and KCl (A_w 0.84) were employed. The samples were allowed to equilibrate for 3–5 days at room temperature until the proper water activity was obtained. The freeze-dried sample *per se* had a water activity of 0.15. Water activity of the sample was checked with a Shibaura A_w meter WA-351 (Shibaura Denshi Co.).

Heat Processing

Samples equilibrated to given water activities were heated at 100 and 120°C for up to 70 min. For heating purposes, 1 g of the sample was placed in a glass vial (1.0 cm i.d. $\times$ 4.2 cm) and sealed with a screw cap to minimize heat transfer limitations in come-up time. At 100°C samples were heated in a water bath, and at 120°C in a fluidized silica sand bath. At given time intervals, samples were withdrawn and cooled in an ice-water bath.

Determinations of Color and Antioxidative Effect

The Maillard browning reaction was monitored by measuring brown pigment formation. Brown pigment was extracted by incubating 100 mg of heated sample with 5 ml of distilled water at 40°C for 2 h, followed by centrifugation at 1,000 rpm for 15 min. The development of brown color was determined as absorbance of the supernatant at 420 nm on a Shimadzu spectrophotometer Model UV-160 after an appropriate dilution. The amount of brown pigment formed was expressed as absorbance at 420 nm per g solid.

The apparatus used in this study for the determination of antioxidative effect was similar to that reported by Farr *et al.*⁹⁾ Hemin (chlorohemin, Tohyo Kasei Kogyo Co.) was employed as a catalyst for lipid autoxidation. Linoleic acid (200 mg, Tokyo Kasei Kogyo Co.) dissolved in ethanol (10 ml) and air-saturated 0.2 M phosphate buffer (pH 7.0, 14 ml) was placed in a thermostatically controlled reaction vessel (volume: 25 ml, temperature: 25°C) and MRP solution (1 ml) was added with stirring. Thus, the completely homogeneous solution was prepared without the use of an emulsifier such as Tween-80. The reaction

vessel was fitted with an oxygen electrode (Clark-type, Able Co.). To start the autoxidation of linoleic acid, 0.1 ml of 1 mM hemin in a mixture (1:1, w/w) of 0.02 M phosphate buffer (pH 7.0) and 95% ethanol was injected. The response of the oxygen electrode was recorded with the aid of an electrometer (Model HE-101A, Hokuto Denko Co.) and a one mV recorder (Rikadenki Co.). The addition of hemin resulted in rapid decrease in dissolved oxygen content. When antioxidative samples were added to the reaction solution, a longer time was required for the decrease of dissolved oxygen. Therefore, antioxidative effect was calculated as $T(a)/T(c)$, where $T(a)$ is time elapsed for 50% reduction of dissolved oxygen in the reaction solution with antioxidative sample, and $T(c)$ is corresponding time interval in the control.

Application of MRP to Kamaboko-type Sardine Product

Minced sardine *Sardinops melanostictus* meat (lipid content: 11.5%, moisture content: 68.0%) was washed twice with 10 volumes of ice-cold NaHCO_3 solution (0.2% w/v) for 10 min. The washed meat was centrifuged and adjusted to a moisture content of 84–85%. Sardine meat thus treated was ground with 2% (w/w) NaCl and 0, 0.5, 1.0, 2.0, 3.0% (w/w) MRP powder (A_w 0.15, 40 min heating at 120°C). As references, BHA (Wako Junyaku Kogyo Co.) and L-ascorbic acid (Kokusen Kagaku Co.) were used in the level of 0.095% (w/w), respectively. The amount of BHA or ascorbic acid was equivalent to the solid content of MRP added in the level of 0.5%. The meat paste thus prepared was wrapped in polyvinylidene chloride film and heated at 80°C for 20 min. After heating, gels formed were cooled in ice-water for 30 min. The kamaboko-type product (called "Tsumire" in Japanese) thus obtained were stored at 4°C for up to 13 days. Peroxide value (POV) of lipid extracted with chloroform and methanol mixture (2:1) and thiobarbituric acid (TBA) number of meat were measured at intervals during storage. POV was determined by the iodometric method.¹⁰⁾ The TBA number was measured according to the procedure reported by Sinnhuber and Yu¹¹⁾ and expressed as mg of malonaldehyde per kg of sample meat. Tristimulus color values of kamaboko-type sardine product were determined by a Digital Color and Color Difference Meter (Model 1010, Nippon Denshoku Kogyo Co.) standardized with a white color tile ($L=90.9$, $a=0$, $b=3.1$).

Results and Discussion

Development of Brown Color

Fig. 1 shows the development of brown color during the Maillard reaction between histidine and glucose as a function of heating time, temperature and water activity. The rate of color development was constant at the initial stage of the reaction and approached a plateau at the later stage, regardless of heating temperature and water activity. The induction period prior to visual detection of an increase in brown color was not observed or at least it was not detected within the accuracy of the determinations used in this study. Data not included here showed more than half of glucose disappeared within 20 min at 100°C and 5 min at 120°C even at A_w 0.84, indicating that the conditions for the Maillard reaction were very favorable.

Initial rates of the brown pigment formation

Fig. 1. Effect of water activity, heating temperature and time on the development of color (Abs. at 420 nm) during the Maillard reaction between histidine and glucose (molar ratio 1:1) at pH 9 (20-fold dilution).

Δ: 100°C heating, ●: 120°C heating.

Fig. 2. Relationship between water activity and the initial rate of color development as measured at 420 nm.

Δ: 100°C heating, ●: 120°C heating.

(Δ Abs./g solid·min) calculated from slopes of the linear portion of Fig. 1 are plotted against water activity in Fig. 2. The browning rate increased as water activity decreased. This agrees with data reported by Tanaka *et al.*¹²⁾ on the Maillard reaction between histamine and glucose, while was not in accord with the results of Warmbier *et al.*¹³⁾ which showed a maximum rate around A_w 0.5. It is noteworthy that initial rates at 120°C were approximately twice those at 100°C regardless of water activity tested.

Development of Antioxidative Effect

The development of antioxidative effect during the Maillard reaction between histidine and glucose is presented in Fig. 3 as a function of heating time, temperature and water activity. The overall tendency towards development of antioxidative effect was similar to that of color development (Fig. 1). This might suggest that the formation of polymerized brown pigments is at least responsible for the development of antioxidative effect. Moreover, the reducing power of MRP increased rapidly for the period of initial 15 min at 100°C and 5 min at 120°C at any water activities tested, and plateaued out thereafter (data not shown). The development of reducing power during the initial stage of the Maillard reaction

Fig. 3. Development of antioxidative effect during the Maillard reaction between histidine and glucose (molar ratio 1:1) at pH 9.
 Δ : 100°C heating, $\bullet$: 120°C heating.

might contribute to the development of antioxidative effect under the conditions used in this study.

Fig. 4 depicts the influence of water activity of the model system on the initial rate of antioxidative effect development. It is clear that this also increases with decrease in water activity, particularly for $A_w < 0.5$ at 120°C. Considering this together with results for the rate of color development (Fig. 2), it appears that the activation energy for the development of antioxidative effect is larger than that for the color development particularly at the lower water activity region.

Addition of MRP to Kamaboko-type Sardine Product

From the results obtained above, the optimum condition for the development of antioxidative

Fig. 4. Relationship between water activity and the initial rate of antioxidative effect development as measured by the oxygen electrode method.
 Δ : 100°C heating, $\bullet$: 120°C heating.

effect in this study was as follow; A_w 0.15, 40 min heating at 120°C. In the next experiments, antioxidative effect towards fish lipids was examined by incorporating this optimum system to kamaboko-type sardine product. Despite some applications of antioxidative MRP for cookies,⁷⁾ cereals,¹⁴⁾ and sausage,⁸⁾ little work has been carried out on the use of MRP for fish meat products which are rich in polyunsaturated fatty acids.^{15,16)}

Fig. 5 illustrates the development of POV in sardine lipids extracted from kamaboko-type product during the storage at 4°C. It is obvious that lipid oxidation was effectively retarded with the addition of MRP, especially at levels of 2 and 3%. BHA (0.095%) also inhibited the oxidation of sardine lipids and its effectiveness was close to that of MRP at the level of 2.0%. Effect of ascorbic acid (0.095%) was poorer than that of BHA, but was superior to that of MRP (0.5%). Rancid odor of kamaboko-type sardine product became apparent when POV of the product was larger than 30 meq/kg. POV reached 30 meq/kg within 3–4 days for the control, 9 days at 0.5% MRP, and 11 days at 1% MRP, indicating that the shelf-life of kamaboko-type product increased significantly with the addition of MRP even below

Fig. 5. Changes in POV in kamaboko-type sardine product containing 0-3% Maillard reaction products during the storage at 4°C.

○: Control, ×: 0.5%, △: 1.0%, ●: 2.0%, □: 3.0%, ◇: BHA (0.095%), ▽: L-ascorbic acid (0.095%).

Fig. 6. Changes in TBA number in kamaboko-type sardine product containing 0-3% Maillard reaction products during the storage at 4°C.

○: Control, ×: 0.5%, △: 1.0%, ●: 2.0%, □: 3.0%, ◇: BHA (0.095%), ▽: L-ascorbic acid (0.095%).

1%. Moreover, the addition of 2-3% MRP almost completely inhibited the oxidation of sardine lipids over 13 days.

The effect of storage time on TBA number of kamaboko-type sardine product is shown in Fig. 6. The overall pattern of TBA number increase is similar to that of POV. In particular, the use of MRP at levels of 2-3% considerably retarded the development of rancidity over 13 days. From Figs. 5 and 6, it was concluded that MRP prepared in this study from histidine and glucose was very effective for the prevention of lipid oxidation in kamaboko-type sardine product during the storage. The addition of MRP at levels of 1-3% seems to be too great as a food additive. However, the actual weight of MRP is approximately one sixth of the weight of preparation used here,

Table 1. Tristimulus color values of kamaboko-type sardine product containing 0-3% MRP*

	Percentage of MRP Added				
	0	0.5	1.0	2.0	3.0
L	52.5	50.0	48.5	45.3	43.3
a	0.3	1.1	2.5	3.0	4.3
b	9.3	11.2	13.4	14.1	15.1

* MRP: Maillard reaction products prepared by heating histidine and glucose (molar ratio 1:1) in the presence of microcrystalline cellulose powder at 120°C for 40 min and A_w 0.15.

since the remainder was crystalline cellulose powder, which is inert to the Maillard reaction, as adsorbant.

Since antioxidant effect of MRP from histidine and glucose was evaluated from the degree of autoxidation of linoleic acid in this study (Fig. 3), it is not clear whether antioxidative MRP prepared are also effective towards other fatty acids such as EPA and DHA, which are dominant in sardine lipids and more prone to autoxidation than linoleic acid. However, it can be concluded that MRP prepared in this study effectively prevent the oxidation of such fatty acids, because the development of both POV and TBA of sardine lipids was retarded significantly by the addition of MRP.

Although the texture, taste and flavor of kamaboko-type sardine product were not altered by the addition of MRP, the color of the product varied with the level of MRP added. Table 1 shows the changes in color of the product prior to the storage. Kamaboko-type sardine product became less white (L value increased), more red (a value increased) and more yellow (b value increased) with the increasing amount of MRP. However, the color of products did not change during the storage at 4°C. From this point, the preparation or separation of colorless antioxidative MRP has been under way in our laboratory.

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