## Effects of pH on the Formation of Volatile Products in Non-Enzymatic Browning of Maltose

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The effects of pH on the formation of maltol and furanmethanol as well as other minor reductones and furans on heating an aqueous solution of maltose under conditions in the absence (caramelization) and presence (Maillard reaction) of proline were examined. The experiments employing a pH-stat device clearly demonstrated the pH dependency on the formations of the products. The products of maltulose and proline-maltulose, which were postulated as the initial intermediates for the two pathways of maltose degradation in the presence of proline, were also examined. On the basis of the results, the reaction pathways of the formation of volatile products from maltose were discussed.

Keywords: maltose, non-enzymatic browning, volatile products, pH, reaction pathway

Volatile products generated from non-enzymatic browning of sugars during the processing and preservation of food and beverages greatly affect their flavor and taste. The composition of the products is normally very complex due to the divergent nature of the reaction pathways, which are affected by various factors (Ledl & Schleicher, 1990). Among the factors, the pH of the reaction system is particularly important, and substantial changes in the composition of the products depending on the pH condition have frequently been observed (Haleva-Toledo *et al.*, 1997).

In the past decade, the formation of typical products (such as maltol) and their intermediates in thermally generated products from disaccharides has been studied in detail (Pischetsrieder & Severin, 1998), since the products generated from 1,4-glycosidically linked disaccharides, i.e. maltose and lactose, differ from those of the corresponding monosaccharide, glucose. Whereas the reaction mechanisms have been extensively studied, there have been very few reports concerning the precise evaluation of the pH effects (Davidek *et al.*, 2002), probably due to the difficulties in monitoring various products generated under rapidly changing pH during the course of the reactions.

In addition, although some minor reductones and furans were reported as volatile products in disaccharide degradation (Berg & van Boekel, 1994), the mechanism of the formation had not been elucidated in detail. Because of their trace amounts and unstable properties, analysis of these compounds proved to be more difficult than that of the major products. In our preceding work (Mori & Ito, 2004), a method of quantitative analysis for such type of compounds in aqueous solution using solid-phase extraction (SPE) and gas chromatography-mass spectrometry (GC-MS) has been developed, and good recovery was achieved for most of the analytes.

The aim of this work was to elucidate the effect of pH on the formation of the volatile compounds in non-enzymatic browning of maltose in the absence (caramelization) or presence of proline (Maillard reaction). The Maillard reaction of the maltose/proline system has been investigated in detail by Yaylayan and Mandeville (1994). Employing some model systems, in which the pH was regulated by a pH-stat device, the formation of volatile products, including minor ones, could be traced quantitatively by the method developed in our preceding work. The products of maltulose and proline-maltulose (Amadori compound), which were postulated as the initial intermediates for the two pathways of maltose degradation (Berg & van Boekel, 1994; Pischetsrieder & Severin, 1998), were also examined. In this study, we dealt with the products generated in the early stage of the reaction proceeding at 100°C and below for several hours. On the basis of the results, the reaction pathways of the formation of volatile products from maltose were also discussed.

## **Materials and Methods**

Reagents and standards The following substances were obtained commercially: D-Maltose monohydrate, L-proline (Wako Pure Chemicals), D-maltulose, 3-hydroxy-2-methyl-4Hpyran-4-one (maltol), 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol), 3-methyl-1,2-cyclopentanedione (cyclotene), 2-furanmethanol, 2-furfural, 2-acetylfuran, and 5-hydroxymethyl-furfural (5-HMF) (Sigma-Aldrich). Pure samples of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) (Mills et al., 1970) and 2-hydroxyacetylfuran (2-HAF) (Miller & Cantor, 1952) were synthesized by the literature methods. Proline-maltulose (95% purity checked by HPLC) was prepared from L-proline and D-maltose monohydrate by the method of Moll and Gross (1981): ESI-MS, positive mode: 901.1 [2M+Na]<sup>+</sup>, 462.2  $[M+Na]^+$ ; negative mode: 877.1  $[2M-1]^-$ , 438.0  $[M-1]^-$ . The internal standards for GC-MS quantitation were hexanoic acidd<sub>11</sub> (98% D, Cambridge Isotope Laboratory Inc.) and p-bromophenethyl alcohol (98% purity, Sigma-Aldrich) in acetonitrile. Distilled deionized water was purified by Milli-Q<sup>®</sup> system (Millipore) and used in all procedures.

*Model reactions* A solution of D-maltose monohydrate with or without L-proline in water was placed in a glass vessel and heated at 90°C with stirring in an oil bath. The pH of the

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solution was initially adjusted to 4, 5, 6, 7, or 8 with HCl or NaOH solution. Experiments were performed at the constant pH maintained by using a pH-stat device (Mettler-Toledo DL55 titrator v.2.4) filled with NaOH solution (1 or 0.1 mol/l) and equipped with an electrode (Mettler HA405-DPA-SC-S8/120) permitting measurements at above 90°C. Aliquots of the reaction mixture were taken at regular time intervals and analyzed.

Analyses of volatile products Analysis of the target compounds was carried out by the procedure described in our preceding report (Mori & Ito, 2004) with minor modification. Two aliquots of the reaction mixture (2 ml) were diluted to 20 ml with water, and the pH of each solution was adjusted to 2 (for the analysis of furoic acid) or 4.5 (for the other reductones and furans) with aq. HCl or NaOH solutions. The internal standard (hexanoic acid-d<sub>11</sub> for the acid, or *p*-bromophenethyl alcohol for the other reductones and furans) was added, and treated further according to the described SPE techniques. Samples were made in duplicate. GC-MS quantitation was carried out on FFAP capillary column and in SIM mode.

For the recovery study, the reaction mixture obtained by heating of maltose/proline (each 1 M conc.) at 90°C for 2 h at constant pH 8 maintained by using a pH-stat device was employed as the model matrix. To 2 ml of the solution, 50  $\mu$ l of a standard solution of the analytes in acetonitrile and internal standard solution (50  $\mu$ l) was spiked. The mixture was worked-up and analyzed in the usual manner. The recoveries were calculated by comparison with the values in the direct injection of the standard solution.

## **Results and Discussion**

In our preceding paper (Mori & Ito, 2004), we reported a preliminary study on the volatile products generated upon heating a mixture of maltose and proline (each 1 M conc.) at 100°C for 8 h in 0.2 M phosphate buffer (pH 6.5). Using the SPE pretreatment and GC-MS technique, the polar and unstable products could be detected and quantified. In that case, maltol and 2-furanmethanol were the major products, and relatively small amounts or trace levels of other reductones (DDMP, furaneol, and cyclotene) and furan derivatives (2-furfural, 2-acetylfuran, furoic acid, 5-HMF, and 2-HAF) were also detected. The contents of the standard solution of the analytes and SIM parameters for GC-MS quantitation used in this study are summarized in Table 1. The recovery of most analytes was essentially good except that of the highly unstable and polar DDMP and furoic acid (Table 2).

*Formation of volatile products from aqueous maltose/proline system* An aqueous solution of maltose and proline (each 1 M conc., initial pH 8, adjusted with aq. NaOH solution) was heated at 90°C for 24 h. Aliquots of the reaction mixture were taken at regular time intervals, and the pH value and volatile products were determined (Fig. 2). In this case, maltol and furanmethanol were the major products and a small amount of 5-HMF was gradually formed after 8 h. The pH dropped, most likely due to

 Table 1. Contents of standard solutions, and summary of SIM parameters used for the analysis of the volatile products from maltose degradation.

		-	-	
	(mg/10 ml)	Retention time (min)	Target ion (amu)	Qualifier ion (amu)
2-Furfural	6.3	8.3	96	95
2-Acetylfuran	5.8	8.9	95	110
Furanmethanol	9.8	11.4	98	81
2-HAF	8.3	19.6	95	126
Furoic acid	106.3	30.8	112	95
5-HMF	8.8	31.5	98	126
Cyclotene	5.9	14.9	112	69
Maltol	49.8	18.4	71	126
Furaneol	51.7	20.2	128	57
DDMP	8.4	26.7	144	101



Fig. 1. GC chromatogram (TIC) of the volatile products generated from aqueous maltose/proline (each 1 M conc.) system heated at 90°C for 2 h under constant pH 8 condition.

the increase of acid concentration (Davidek *et al.*, 2002) during the course of the reaction, and reached 4.3 after 24 h. Be-cause of the rapid drop of pH, it was hard to evaluate the effect of pH on the formation of the products, particularly under basic to neutral conditions. Even if buffer solution was used to regulate the pH of the reaction system, an eventual drop of pH could not be avoided.

The effects of pH on the formation of volatile products The formation of maltol, furanmethanol and the other minor products was studied at 90°C for 2 h using the same initial concentration (1 M) of maltose with an equimolar amount of proline. The experiments were carried out at constant pH 4, 5, 6, 7 and 8 using a

**Table 2.** Recoveries of the analytes in the model matrix $^{a}$ .

	5				
	Spiking conc. (ng)	Mean recovery (%)	Coefficient of variation (%)		
2-Furfural	6.3	88.8	0.6		
2-Acetylfuran	5.8	90.3	0.6		
Furanmethanol	9.8	89.5	1.8		
2-HAF	8.3	96.8	3.4		
Furoic acid	106.3	76.2	11.5		
5-HMF	8.8	101	3		
Cyclotene	5.9	95.8	0.8		
Maltol	49.8	103.4	2.3		
Furaneol	51.7	100.3	0.4		
DDMP	8.4	33.7	17.4		

<sup>*a*)</sup> Model matrix: the reaction mixture of maltose and proline (each 1 M conc.) upon heating at 90°C for 2 h at pH 8. Details are described in Materials and Methods.



**Fig. 2.** The change of pH (upper) and the formation of volatile products upon heating an aq. solution of maltose and proline (each 1 M conc., initial pH 8 adjusted with aq. NaOH) at 90°C for 24 h.  $\Box$ : 2-furanmethanol;  $\blacklozenge$ : 2-furfural;  $\triangle$ : 2-acetylfuran;  $\blacktriangle$ : 5-HMF;  $\diamond$ : 2-furoic acid; \*: 2-HAF;  $\bigcirc$ : DDMP;  $\blacklozenge$ : maltol;  $\times$ : cyclotene;  $\blacksquare$ : furaneol.

pH-stat device. As can be seen in Fig. 3, the formation of the volatile products heavily depends on pH. Under pH 4-5 conditions, almost no volatile products were detected except a small level of 5-HMF at pH 4. At pH 5, furanmethanol began to form in a trace amount, and its level increased with increasing pH. Whereas maltol formation was very low below pH 6, its level increased at a higher rate than that of furanmethanol with increasing pH until it was the primary product at pH 8. DDMP and 2-acetylfuran were formed in rather large amounts at pH above 7. Under basic condition we observed the formation of small amounts of furaneol and cyclotene, which were considered to be the secondary products in the reaction systems (Haleva-Toledo et al., 1997; Wnorowski & Yaylayan, 2000), but these results are not discussed further in this study. The formation of 5-HMF was limited to a very small amount in these pH-regulated (pH 4-8) model systems.

When an aqueous solution of maltose was heated in the absence of proline at 90°C for 2 h, furanmethanol was the predominant volatile product, which was formed at pH above 7 along with a small amount of DDMP (Fig. 3). Trace levels of the maltol, and other reductones and furan derivatives were detected at pH 8. The yield of furanmethanol at pH 8 was similar to that obtained at pH 7 in the presence of proline.

Since the thermal degradation of maltose in an aqueous solution at pH above 7 affords a considerable amount of furanmethanol, it was suggested that maltose is involved in at least two competing reactions: formation of furanmethanol by the degradation of the sugar itself, and the reaction to give maltol, upon



**Fig. 3.** The effects of pH on the formation of volatile products generated upon heating a 1 M aq. solution of maltose at 90°C for 2 h in the presence (upper) and absence (lower) of an equimolar amount of proline.  $\Box$ : 2-furanmethanol;  $\blacklozenge$ : 2-furfural;  $\triangle$ : 2-acetylfuran;  $\blacktriangle$ : 5-HMF;  $\diamondsuit$ : 2-furoic acid; \*: 2-HAF;  $\bigcirc$ : DDMP;  $\blacklozenge$ : maltol;  $\times$ : cyclotene;  $\blacksquare$ : furaneol.

heating the aqueous solution in the presence of proline. Berg and van Boekel (1994) studied the degradation of lactose in the presence of lysine in a heated milk system and concluded that the



Fig. 4. The amounts of maltol, DDMP, and four furan derivatives formed upon heating (at 90°C for 2 h) the 0.2 m aqueous solutions of maltose and maltulose with or without equimolar conc. of proline at constant pH 8. The data from proline-maltulose (initial concentration: 10 mm) is also shown. The amounts of 2-furoic acid and 5-HMF were omitted because of their trace-level yields.

reaction comprises two main pathways: (1) lactose isomerization into lactulose via 1,2-enolization, and subsequent degradation to galactose, formic acid and C5/C6 compounds (caramelization); and (2) a series of reactions initiated by the formation of the Amadori compound from lactose and an amino-compound (Maillard reaction). The present results on maltose degradation were in agreement with their conclusions.

*Products from maltulose and proline-maltulose (Amadori compound)* As can be seen in Fig. 3, in the experiment at pH above 7, the maltose/proline system afforded the other reductones and furans in substantial amount. To evaluate the participation of the two routes for the formation of these volatile products, quantitation of the products from maltulose and proline-maltulose (Amadori compound), which are postulated as the initial intermediates of the two pathways, was carried out at constant pH 8 in the presence or absence of proline.

Each 0.2 M conc. aq. solution of maltose or maltulose with or without equimolar conc. of proline was heated at 90°C for 2 h at constant pH 8. The amounts of maltol, DDMP, and four furan derivatives (2-furoic acid and 5-HMF were omitted because of their trace-level yields) contained in each reaction mixture are depicted in Fig. 4. The spectrum of the products from maltulose itself was essentially the same as that of maltose, whereas the amount of furanmethanol was somewhat higher. In the presence of proline, however, the product spectrum from maltulose was quite different from that of maltose. When maltulose was treated with an addition of proline, the main products were furanmethanol and DDMP, while maltose/proline gave maltol as the main product. DDMP, which was a major product in the reaction of glucose and proline (Mills et al., 1970), must be formed from the hexose-unit released in the course of the formation of furanmethanol. The higher yield of DDMP from maltulose than that of maltose at pH 8 indicates that β-elimination via maltulose mainly participates in the release of the hexose-unit.

On the other hand, 10 mM conc. of proline-maltulose (Amadori compound) in aqueous solution was heated at pH 8 to give maltol as the predominant product along with a significant



Fig. 5. Proposed mechanism of the formation of volatile products from maltose and proline at pH 8.

amount of 2-furfural. However, the other products including furanmethanol were formed in trace or negligible amounts.

These results demonstrated that furanmethanol is mainly formed via maltulose in the caramelization pathway even in the presence of proline. In contrast, proline-maltulose (Amadori compound) was mainly concerned in the formation of maltol. The formation of a small amount of maltol detected in the maltulose/proline system can be explained by the equilibrium between maltulose and maltose under the reaction conditions.

Formation of volatile products from maltose in the presence of proline at pH 8 As outlined in Fig. 5, maltose in the presence of proline can undergo two main transformations into maltulose leading to formic acid and furanmethanol or into the Amadori compound, which further degrades to maltol and other products. The former pathway had been investigated by several researchers mainly in the lactose degradation in heated milk systems. Patton (1950) proposed the mechanism of the conversion of lactose into furanmethanol and formic acid in heated milk for the first time. He had pointed out that the pH necessary for the hydrolytic cleavage of the galactosyl residue ranged between 4-6. However, as the overall reactions to furanmethanol occurred at pH 6 and above, we considered that  $\beta$ -elimination of the sugar residue is a more plausible mechanism. Later, one of the possible intermediates, 3-deoxypentulose, a ketose derivative, was isolated by Troyano et al. (1992) in heated milk and in alkaline model mixtures containing maltose and cellobiose. Berg and van Boekel (1994) postulated this compound to be the precursor of furanmethanol. However, they did not consider that the pathway leading to furanmethanol is the main one, because it was formed in much smaller concentration than formic acid. The present results showed that the pathway involving 3-deoxypentulose might be one of the main routes for maltose degradation at pH 7-8.

Hollnagel and Kroh (2002) reported that 3-deoxypentosulose is the predominant  $\alpha$ -dicarbonyl compound specifically formed from 1,4-linked oligosaccharides, i.e. maltose and maltotriose, under both caramelization and Maillard reaction conditions. They proposed the mechanism of the formation from the Amadori compound of maltose, and suggested that 3-deoxypentulose and furanmethanol are other possible products derived from similar reaction pathways. However, the present results on the degradation of proline-maltulose showed that their proposed mechanism was not viable at least under the conditions examined in this study. Rather than that, we considered that 2-furfural produced from proline-maltulose under constant pH 8 condition could be generated through the cyclization-dehydration processes of 3-deoxypentosulose. This coincided with the fact that maltose and maltulose in the absence of proline also produced 2furfural in rather high yields.

In addition, under the basic conditions, the possibility that furfural is converted into furanmethanol and furoic acid via Cannizzaro-type reaction could not be ruled out. When aqueous solutions of furfural (0.1 M) were treated alone and with 1 M proline at 90°C for 2 h at constant pH 8, the concentrations were markedly decreased to 55% and 1.3%, respectively. However, neither these products (furanmethanol and furoic acid) nor the other volatile components discussed in this study could be detected in substantial amounts. These data indicated that reaction via the Cannizzaro-type route from furfural does not participate in the reaction, but that the low yields of furfural in the reactions of maltose and maltulose in the presence of proline must be due to the degradation under the reaction conditions.

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