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Correlation of methylglyoxal with acrylamide formation in fructose/asparagine Maillard reaction model system

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Abstract

α-Dicarbonyl compounds were highly reactive intermediates formed in Maillard reaction (MR), and *o*-phenylenediamine (OPD) was widely used as a trapping agent for α-dicarbonyl compounds. Both aqueous fructose/asparagine (Fru/Asn) and fructose/asparagine/*o*-phenylenediamine (Fru/Asn/OPD) model systems were heated at 150 °C for up to 30 min. Methylglyoxal (MG) was the main α-dicarbonyl compounds formed in MR, which was chosen as a representative of α-dicarbonyl compound to investigate the influence on acrylamide (AA) formation. The concentrations of AA, MG and Asn were detected during MR by HPLC method. The results indicated that the formation of AA increased with the heating time, and nearly 75% of AA was formed through the participation of α-dicarbonyl compounds. The amounts of formation and consumption of MG increased with heating time, and from 12 min of reaction, the consumed amounts of MG accounted for 62.1–90.3% on the basis of total amounts of MG formed in MR, suggesting that most of the MG took part in further reactions. Meanwhile, Asn concentration decreased with heating time in both models. The formation of AA and consumption of Asn were highly correlated with MG. Indeed, as MG concentration in MG/Asn model system decreased during heating at 150 °C, the concentration of AA significantly increased. The coefficient of correlation between consumed amounts of MG and the formed amounts of AA was 0.931, demonstrating that MG plays a role in AA formation.

Keywords: α-Dicarbonyl compounds; Acrylamide (AA); Methylglyoxal (MG); Maillard reaction (MR); Fructose (Fru); Asparagine (Asn)

1. Introduction

In April 2002, researchers in Swedish National Food Administration (SNFA) and Stockholm University announced that carbohydrate-rich foods which were heated or fried at high temperatures contained relatively high levels of AA, such as fried potato products (Swedish National Food Administration, 2002). After the announcement of AA in foods, confirmatory experiments were performed by other research groups and most foods were found to contain different amounts of AA (Ahn et al., 2002; Becalski, Lau, Lewis, & Seaman, 2003). These investigations cause a considerable concern as AA has been known to be a neurotoxic, genotoxic and carcinogenic compound in animals and is classified by IARC. (1994) as a probable human carcinogen.

Fundamental formation mechanism studies have revealed that Maillard reaction (MR) is a feasible important route to form AA (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Yaylayan, Wnorowski, & Locas, 2003; Zyzak et al., 2003), and a number of possible minor pathways have also been described, such as acrolein, acrylic acid and pyruvic acid (Stadler et al., 2003; Wnorowski & Yaylayan, 2003; Yasuhara, Tanaka, Hengel, & Shibamoto, 2003; Yaylayan & Stadler, 2005. MR is a complex chemical reaction, and the initial stages of MR involve the condensation of the amino compound with

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the carbonyl group of reducing sugar to form *N*-glucosylasparagine adduct, which is in equilibrium with the Schiff base (Mottram et al., 2002; Yaylayan et al., 2003). The *N*-glucosylasparagine can rearrange and dehydrate via deoxyosones to a multitude of low-molecular-weight compounds, including α-dicarbonyl compounds, such as 3-deoxyosuloses and 3,4-dideoxyosulos-3-enes (Hollnagel & Kroh, 2002; Martins, Jongen, & van Boekel, 2001). These α-dicarbonyl compounds are highly reactive intermediates, and play a key role in MR (Beck, Ledl, & Severin, 1988; Hollnagel & Kroh, 1998; Homoki-Farkas, Örsi, & Kroh, 1997). Mottram et al. (2002) proposed that α-dicarbonyl compounds could further react with Asn to form AA following the Strecker degradation pathway. In addition, decarboxylation of Schiff base is induced by heating (Yaylayan et al., 2003; Zyzak et al., 2003), and the decarboxylated Schiff base can be hydrolyzed to 3-aminopropionamide (3-APA) (Zyzak et al., 2003), an important transient intermediate, and then form AA (Granvogl, Jezussek, Koehler, & Schieberle, 2004; Granvogl & Schieberle, 2006; Stadler et al., 2004). In present paper, we are interested to find the evidence on the role of α-dicarbonyl compounds on AA formation during MR in Fru/Asn model system.

o-Phenylenediamine (OPD) is used as a trapping agent that converts the α-dicarbonyl compounds to their stable quinoxaline derivatives (Bednarski, Jedrychowski, Hammond, & Nikolov, 1989; De Revel, Pripis-nicolau, Barbe, & Bertrand, 2000; Geoffrey, Christine, & Laurence, 2006; Glomb & Tschirnich, 2001; Hollnagel & Kroh, 1998, 2002), which can be detected by HPLC. In a previous study, we identified the main compounds with α-dicarbonyl structure formed in MR by HPLC–MS, and MG was the predominant α-dicarbonyl compounds formed in MR. Meanwhile, the changes of amounts of MG, AA and Asn were also investigated in GlcAsn model system (Yuan et al., 2007). In the present study, we focus on the changes of amounts of MG, AA and Asn in Fru/Asn model system to find the relationship between amounts of MG and AA and relationship between amounts of MG and Asn. Consequently, to explain how MG contributes to the formation of AA in Fru/Asn model system.

2. Materials and methods

2.1. Reagents

All solvents used were of HPLC and other chemicals were of analytical grade. AA (>99.5%) and Fru were obtained from Sigma–Aldrich (Beijing Superior Chemical & Instruments Co., Ltd., Beijing, China). MG (40% in water), Asn, OPD, *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (2-ME) were obtained from Fluka Biochemika Company (Beijing, China). Other chemicals were obtained from Beijing Chemicals Co. (Beijing, China). HPLC-grade methanol was purchased from Fisher Scientific International Inc (Beijing, China).

2.2. Preparation of the MR model systems

Based on the method described by Becalski et al. (2003), Fru and Asn were selected as reactants for the formation of AA. Fru and Asn were dissolved in phosphate buffer solution (0.2 M, pH 7.5), respectively, and at a concentration of 0.5 mmol/L each; and 0.54 g OPD were dissolved in 100 mL methanol (0.5 mmol/L).

MR model systems were prepared as followed: (1) Fru/Asn/OPD model system. An aliquot (1 mL) of Fru, Asn and OPD solution was mixed thoroughly with MS1 Mini-shaker (IKA Co., Ltd., Works, Malaysia) and heated at 150 °C in sealed glass tubes (180 × 18 mm) in an oil bath; (2) Fru/Asn model system. An aliquot (1 mL) of Fru, Asn, and phosphate buffer solution (0.2 M, pH 7.5) was mixed thoroughly, and treated as Fru/Asn/OPD model system; (3) MG/Asn model system. MG and Asn were dissolved in phosphate buffer solution (0.2 M, pH 7.5), respectively, and at a concentration of 0.5 mmol/L each. An aliquot (1 mL) of MG, Asn, and phosphate buffer solution (0.2 M, pH 7.5) was mixed thoroughly and treated as above.

The changes of pH values of solution were not significant during heating in both model systems, thus the pH values were not adjusted in the experiment. At predetermined heating times, samples were taken and immediately cooled in an ice-water bath, and 1 mL of mixture was taken and diluted to 10 mL by adding 9.0 mL of ultra-pure water, prior to analysis. Experiments were carried out in triplicates; where necessary, additional experiments were conducted to obtain reliable results.

2.3. Analysis of AA by HPLC

The analysis method of AA was carried out as described by Rośen and Hellenas (2002) and Knol et al. (2005) with slight modification. This method has been confirmed by HPLC–MS/MS in our study. The diluted sample solution was centrifuged (4500*g*, 10 min) and filtrated through a 0.45 μm PVDF syringe filter. The determination of AA concentration was performed using a HPLC system (Knauer Co., Berlin, Germany) equipped with a K-501pump and K-2501 UV detector, and a reversed ODS-C_{18} (250 × 4.6 mm, 5 μm, Hypersil, Thermo Electron Co., Waltham, MA, USA). Twenty microlitres of solution were injected onto the column and eluted isocratically at 30 C with 2% methanol in water at a flow rate of 700 μL/min. The detection wavelength was 205 nm. AA was quantified by external calibration, in which the concentrations of AA standard solutions were ranged from 0.05 to 1.00 μg/mL.

2.4. Analysis of MG by HPLC

The analysis of MG was based on the method described by Bednarski et al. (1989) and de Revel et al. (2000). Five millilitres of the sample solution obtained

from Fru/Asn mixture were taken, and 100 μL of 0.1 mmol/L OPD (1.08 g OPD was dissolved in 10 mL methanol) were added into the solution and mixed thoroughly. The pH value of sample solution was adjusted to 8.0 with 1.0 mol/L sodium hydroxide and the solution was heated at 60 ℃ for 3 h for the derivatization of MG to quinoxaline derivative. After the derivatization, the pH of sample was adjusted to 3.0 with 1.0 mol/L hydrochloric acid. Two millilitres of chloroform were added and mixed vigorously to extract the quinoxaline derivative of MG. The sample was centrifuged at 4500*g* for 10 min, and the bottom chloroform layer was taken. The extraction was repeated three times, all chloroform layers were combined and evaporated under N_2 at 40 °C, and the resulting residue was dissolved in 1.0 mL of HPLC-grade methanol. The solution was filtered through a 0.45 μm filter (Millipore Corporation, Bedford, MA, USA) and then used for HPLC analysis. For Fru/Asn/OPD model system, 5 mL of the sample solution were directly extracted three times with chloroform to extract the quinoxaline derivative of MG as mentioned above, and without any further derivatization.

The derivatives were analyzed using an HPLC system (Knauer Co., Berlin, Germany) equipped with a K-501pump and a K-2501 UV detector as that for AA analysis. Twenty microlitres of sample were injected onto the column, and eluted isocratically at 35 °C with 68% methanol in water at a flow rate of 1.0 mL/min. The detection wavelength was 313 nm. MG was quantified by external calibration, in which the concentrations of MG standard solution ranged from 10 to 500 μg/mL.

2.5. Analysis of Asn by HPLC

Asparagine was quantified by an HPLC system (Knauer Co., Berlin, Germany) equipped with a K-501pump and a K-2501 UV detector that employed precolumn derivatization using OPA/2-ME reagent at pH 9.0 according to the method described by Tcherkas, Kartsova, and Krasnova (2001) and Paramás, Bárez, and Marcos (2006). Twenty microlitres of filtrate were injected onto the reversed Kromasil–C_{18} (250 × 4.6 mm, 5.0 μm, Eka Chemicals Company, Bohus, Sweden) and the derivative of Asn was eluted in a gradient with 10 mmol/L sodium phosphate buffer (pH 6.85) (Eluent A) and methanol (Eluent B). The elution program was as follows: 0–2.60 min 100% → 80% A; 2.61–3.20 min 80% → 60% A; 3.21–14.00 min 60% → 55% A; 14.00–15.00 min 55% → 80% A. The flow rate of eluent was 1.0 mL/min; and the detection wavelength was 340 nm. Asn was quantified by external calibration, in which the concentrations of Asn standard solutions ranged from 200 to 2000 μg/mL.

2.6. Statistical analysis

Statistical analysis was performed with SPSS 12.0 software, and *t* test was used for comparison of means from multiple samples in different treatments. Graphs were drawn with OriginPro 7.5 software.

3. Results and discussion

3.1. Changes of AA concentration in Fru/Asn/OPD and Fru/Asn reaction model systems

Both Fru/Asn/OPD and Fru/Asn reaction model systems were heated at 150 °C for up to 30 min to investigate the change of AA formation in the presence or absence of α-carbonyl compounds. As shown in Fig. 1, AA concentration was increased in both model systems with heating time. To compare the difference between the two model systems, the change process was generally divided into two phases. In phase 1, the slow growth of AA concentration in both systems was observed, implying that precursors of AA were accumulated during this phase. In phase 2, from about 15 min upon heating, the rapid increase of AA concentration was observed in Fru/Asn, and the increase rate of AA was much faster than that in Fru/Asn/OPD model. When the mixture was heated for 30 min, 0.0020 ± 0.0001 mmol/L of AA was detected in Fru/Asn system, but only 0.0005 mmol/L of AA was detected in Fru/Asn/OPD system, which only took up 25.0% compared with that in Fru/Asn system. In Fru/Asn/OPD system, the added OPD trapped all α-dicarbonyl compounds formed in MR to their stable quinoxaline derivatives, and restricted the further reaction of α-dicarbonyl compounds in MR (De Revel et al., 2000; Hollnagel & Kroh, 1998, 2002). The difference in the formed amounts of AA between the two systems indicated that nearly 75% of AA was formed through the participation of α-dicarbonyl compounds while 25% of AA was produced through other reaction pathway(s) where α-dicarbonyl compounds were not required. This conclusion is consistent with previous observations (Bednarski et al., 1989; De Revel et al.,

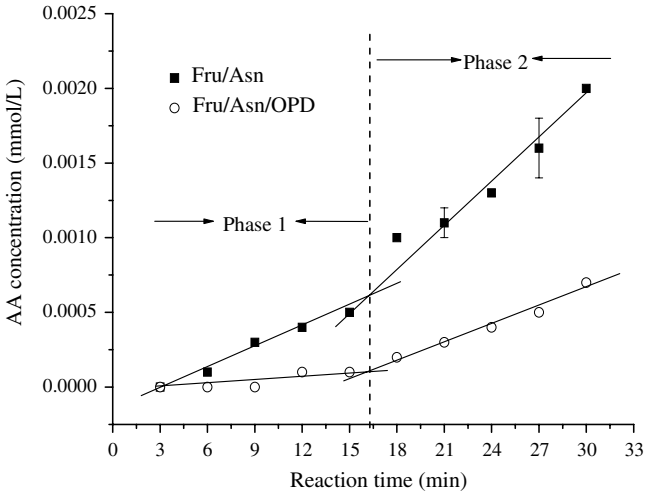

Fig. 1. Changes of AA concentration in Fru/Asn/OPD and Fru/Asn model systems heating at 150 °C up to 30 min.

2000; Geoffrey et al., 2006; Glomb & Tschirnich, 2001; Hollnagel & Kroh, 1998, 2002).

3.2. Changes of MG concentration in Fru/Asn/OPD and Fru/Asn model systems

In a previous study, MG was identified by HPLC/MS as the most predominant α-dicarbonyl compounds formed in our particular model system (data not given). The amounts of MG in Fru/Asn/OPD and Fru/Asn model systems were detected as shown in Fig. 2. The concentration of MG obtained in Fru/Asn/OPD model system corresponded to the total amount of MG formed by MR, and the concentration of MG in Fru/Asn system was that of the unreacted MG after MR. Therefore, the amount of MG consumed in MR was calculated by the concentration of MG formed in Fru/Asn/OPD minus that in Fru/Asn.

In Fru/Asn/OPD, the MG formation rate increased linearly with heating time during the first 18 min, and the rate increase was 0.014 mmol/L/min. After 18 min of heating, the rate was increased slowly, just 0.005 mmol/L/min. In contrast, steady state concentration of MG was very low in Fru/Asn model system. After the reaction for 30 min much less amount of MG was detected, suggesting that most MG probably participated in the MR and were consumed during heating. From 12 min of heating, the consumed amount of MG increased from 62.1% to 90.3% on the basis of total amount of MG in Fru/Asn system (Fig. 2, inset). Simultaneously, the amount of AA formed in Fru/Asn was ~4-fold larger than that in Fru/Asn/OPD after 18 min of heating (Fig. 1). The concurrence between consumption of MG and increase of AA suggested that MG was involved in the formation of AA as one kind of α-dicarbonyl intermediate. However, other reactions involved in the formation of some small molecules, such as formic and acetic acid could not be excluded (Hofmann, Bors, & Stettmaier, 1999; Hollnagel & Kroh, 2000; Martins et al., 2001).

3.3. Changes of Asn concentration in Fru/Asn/OPD and Fru/Asn model systems

The changes of Asn concentration in Fru/Asn/OPD and Fru/Asn reaction model systems are shown in Fig. 3. The Asn concentration decreased with heating time in both models. After 30 min of heating treatment, about 0.234 ± 0.020 mmol/L of Asn could be detected in Fru/Asn/OPD system and the consumption was about 53.2%. In Fru/Asn system, 0.226 ± 0.006 mmol/L of Asn could be measured, and the consumption was about 54.8%. Statistical analysis indicated that the difference between both models was not significant ($p > 0.05$). This might be determined by the reaction characteristics of the MR, namely, Asn was a major reactant and a large amount of Asn was consumed during MR, but only a small amount of Asn took part in the formation of MG, which eventually reacted with the remaining Asn to generate AA (Mottram et al., 2002). Therefore, it was difficult to detect the difference in the change of Asn concentration between the two model systems.

3.4. Analysis of relationship between MG and AA with that between MG and Asn

To confirm the result of the present study, the relationship between the consumed amount of MG and Asn by MR and the formed amount of AA by α-dicarbonyl compounds in the MR was investigated as shown in Fig. 4. The change process assumably consisted of three phases. In phase 1, the consumed rate of Asn, MG and the formed rate of AA changed relatively slowly. The MR was in an initial lag phase, in which Asn could react with Fru to form intermediates, such as *N*-glucosylasparagine adduct or

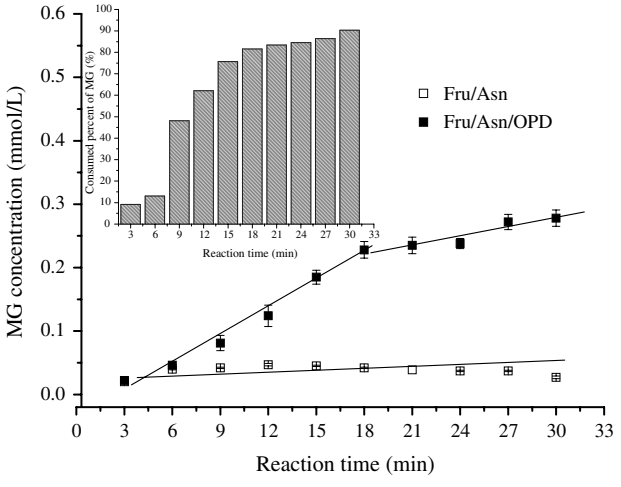

Fig. 2. Changes of MG concentration in Fru/Asn/OPD and Fru/Asn model systems heating at 150 °C up to 30 min. (Inset) The percent of consumed amounts of MG in total formed amounts of MG.

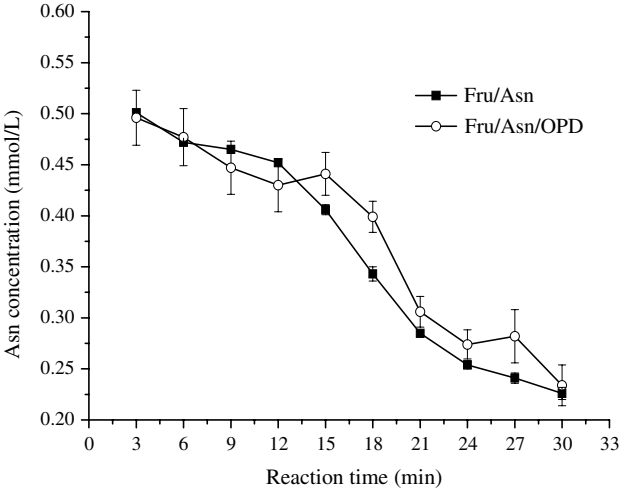

Fig. 3. Changes of Asn concentration in Fru/Asn/OPD and Fru/Asn model systems heating at 150 °C up to 30 min.

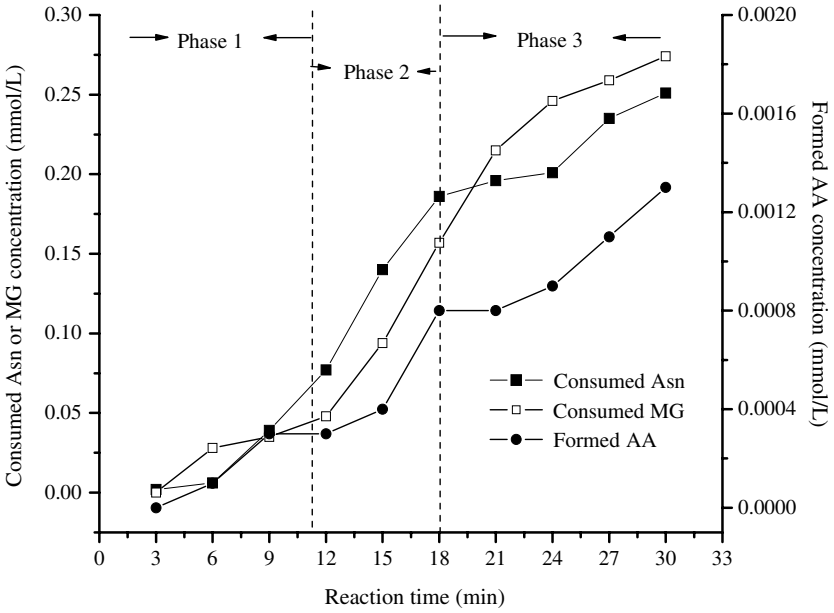

Fig. 4. The relationship between MG concentration and AA concentration, and MG concentration and Asn concentration in Fru/Asn model system heating at 150 °C up to 30 min.

Schiff's base as well as α-dicarbonyl compounds (Mottram et al., 2002; Yaylayan et al., 2003), therefore, the accumulated rate of MG also showed a slow increase in Fig. 4. In phase 2, the consumed amounts of Asn and MG and the formed amount of AA were rapidly increased, and the rate of consumed Asn and consumed MG was nearly the same, which showed that Asn and MG simultaneously took part in the reaction. However, increase of AA showed a slower rate in the early period and then increased to the same rate with MG and Asn, proving that MG was the main intermediate to form AA. In phase 3, the consumed rate of MG was still higher, but the formed rate of AA was declined. This implied that MG could not only took part in the formation of AA, but also form some intermediate or other small molecular during MR. This could be explained in some references that MG is an active intermediate with α-dicarbonyl compound structure, which has two oxo-groups and very flexible H of the methyl group (Homoki-Farkas et al., 1997).

3.5. Changes of AA and MG concentrations in MG/Asn model system

To provide the direct proof that MG takes part in AA formation, MG/Asn model system was also heated at 150 °C for up to 30 min. The changes of AA and MG concentrations are shown in Fig. 5, and there was a similar profile to the changes in Fru/Asn system. The AA concentration was increased with heating time, after 30 min the AA concentration in MG/Asn reached 0.00065 ± 0.00002 mmol/L. However, compared with the changes of AA concentration in Fru/Asn, phase 1 lasted for a shorter time, about 9 min which was about 1/2 of that in Fru/Asn. This showed that MG may shorten the lag time for the reaction process to form AA, since there is a necessary process to accumulate to form reactive intermediate such as α-dicarbonyl compounds in Fru/Asn system (Brands & Van Boekel, 2001). Meanwhile, the MG concentration was decreased with heating time (Fig. 5, inset), and the consumed amounts of MG were well correlated to the formed amounts of AA ($r = 0.931$). This indicated that MG was consumed as the reaction precursor of AA during heating. MG could react with Asn to form some intermediate, which would further form AA. The formation mechanism of AA by the participation of MG is currently under investigation.

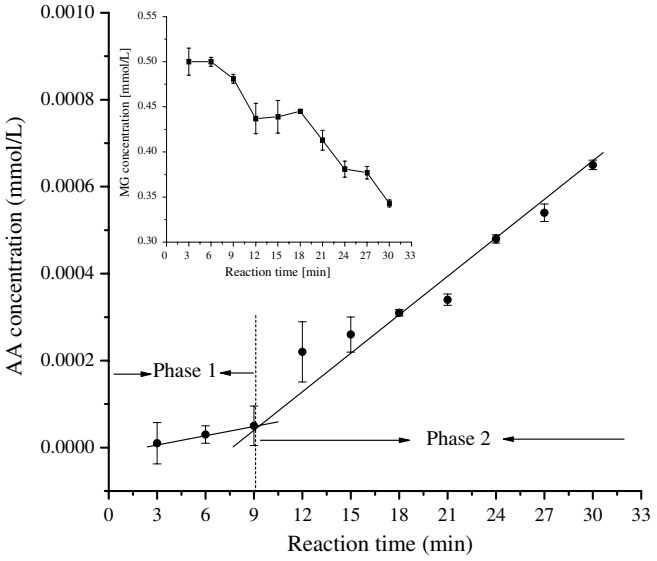

Fig. 5. Changes of AA concentration in MG/Asn model system heating at 150 °C up to 30 min. (Inset) Changes of concentration of MG in MG/Asn model system heating at 150 °C up to 30 min.

4. Conclusions

We give priority to MR routes of AA formation in present study although there are numerous and plausible reaction routes by which amino acids and sugars may form AA. MG is one of the most predominant α-dicarbonyl compounds formed during MR, and the role of MG on the AA formation is evident from the correlations between MG and AA, as well as MG and Asn in Fru/Asn reaction mixtures. In addition, the consumed amounts of MG were well correlated with the amounts of AA formed in MG/Asn.

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