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Rapid Communication

Effects of *o*-phenylenediamine on methylglyoxal generation from monosaccharide: Comment on "correlation of methylglyoxal with acrylamide formation in fructose/asparagine Maillard reaction model system"

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Abstract

Methylglyoxal (MG), a reactive carbonyl compound, has recently garnered much attention because of its ability to modify proteins over time and yield advanced glycation end products (AGEs) that are thought to contribute to the development of diabetes mellitus and its complications. In a recent paper published in Food Chemistry by Yuan et al. [Yuan, Y., Zhao, G. H., Hu, X. S., Wu, J. H., Liu, J., & Chen, F. (2007a). Correlation of methylglyoxal with acrylamide formation in fructose/asparagines Maillard reaction model system. *Food Chemistry*, *108*(3), 885–890] authors showed a high correlation between methylglyoxal formation and acrylamide formation. However, in their study, model systems of aqueous fructose/asparagines (Fru/Asn) and fructose/asparagines/*o*-phenylenediamine (Fru/Asn/OPD) heating at 150 °C were used. The validity of these models relies on the assumption that OPD will only serve the role of a trapping agent for MG. In this short communication, we would like to call to attention that MG can also have a strong catalytic effect in the generation of MG from fructose. Therefore, it is concluded that the concentration of MG obtained in Fru/Asn/OPD model system cannot correspond to the total amount of MG formed by Maillard reaction of Fru and Asn as claimed by Yuan et al. [Yuan, Y., Zhao, G. H., Hu, X. S., Wu, J. H., Liu, J., & Chen, F. (2007a). Correlation of methylglyoxal with acrylamide formation in fructose/asparagines Maillard reaction model system. *Food Chemistry, 108*(3), 885–890, Yuan, Y., Zhao, G. H., Hu X. S., Wu, J. H., Liu, J., & Chen. F. (2007b). High correlation of methylglyoxal with acrylamide formation in glucose/asparagine Maillardreaction model. *European Food Research and Technology*. doi:10.1007/s00217-007-0658-0].

Keywords: *o*-Phenylenediamine; Methylglyoxal; Maillard reaction; Amine-assisted sugar degradation

1. Introduction

Since Louis Camille Maillard investigated the reaction between glucose and glycine, Maillard reaction, the chemical interaction involving carbohydrates and amino acids, peptides or proteins, has been studied for more than one hundred years. Besides the formation of colour and aroma, Maillard reaction has both nutritional and toxicological effects on processed food (De Revel, Pripis-nicolau, Barbe, & Bertrand, 2000; Hayashi & Shibamoto, 1985; Hodge, 1953). Recently, some intermediate compounds such as methylglyoxal (MG) and glyoxal (GO) have attracted much more attention since they are not only the precursors of colour and aroma but also serve as a significant clinical factor in diabetes and other disease complications (Baynes & Thorpe, 1999; Onorato, Thorpe, & Baynes, 1998; Thornalley et al., 2003). For example, MG, a major reactive

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carbonyl compound found in humans, is extremely reactive and readily modifies lysine, arginine, and cysteine residues on proteins to form advanced glycation endproducts (AGEs) which are linked to hyperglycemia and diabetes complications.

In 2002 when acrylamide, a probable genotoxic carcinogen, was reported in fried and baked foods, Mottram, Wedzicha, and Dodson (2002) were the first to show that the Maillard reaction between the amino acid asparagine and sugars provided the mechanistic route to acrylamide. They further proposed that MG could be an intermediate for the formation of acrylamide (Mottram et al., 2002). However, Stadler et al. (2004) later showed that Strecker degradation may plays a minor role in the generation of acrylamide. Two recent publications of Yuan et al. (2007a,b) have tried to demonstrate the correlation of MG with acrylamide formation in glucose/asparagine (Glc/Asn) and fructose/asparagine (Fru/Asn) Maillard reaction models. Their results showed that in both Glc/Asn and Fru/Asn model, the coefficients of correlation between the consumed amounts of MG and the formed amounts of AA was very high. Unfortunately, the methodology they used in both studies was questionable. They studied the total amounts of MG generated and consumed in Fru (or Glc)/Asn system by using two models of aqueous Fru (or Glc)/Asn and fructose (or glucose)/asparagine/*o*-phenylenediamine (Fru (or Glc)/Asn/OPD). The accuracy of these studies requires that all MG generated in Fru (or Glc)/Asn/OPD model be trapped by OPD, and OPD has to be inert to the MG formation and just play the role of a trapping agent. If OPD participates in the generation of MG from Fru or Glc under the experimental conditions (high temperature, basic pH), the models no longer work.

Due to the importance of methylglyoxal and acrylamide formations in model and food system, this short communication intends to clarify whether *o*-phenylenediamine could be used as a trapping agent of methylglyoxal during Maillard reaction at higher temperature.

2. Materials and methods

2.1. Materials

Phenylenediamine (OPD) and fructose were purchased from Sigma (St. Louis, MO, USA). 2-Methylquinoxaline (2-MQ; 97%), which was used an external standard was purchased from Aldrich (St. Louis, MO, USA). HPLC grade water, acetonitrile, methanol and ethanol were purchased from Fisher Scientific (Springfield, NJ).

2.2. Preparation of Fru and Fru/OPD models

Most of the parameters used here were the same as those described by Yuan et al. (2007a,b). Fru and OPD were dissolved in phosphate buffer solution (0.2 M, pH 7.5) and methanol, respectively at a concentration of 0.5 mmole/L. An aliquot (1 mL) of Fru and phosphate buffer solution was mixed and shaken vigorously by vortexing for 5 s, and so was an aliquot (1 mL) of Fru and OPD. These two models were heated at 150 °C in sealed glass tubes in an oil bath for 30 min. All reacted samples were cooled in an ice bath. The Fru model was used for the next derivation process. Fru/OPD was centrifuged at 14 × 1000 rpm (16,000*g*) for 5 min before HPLC analysis.

2.3. Analysis of MG by HPLC

The derivatization procedure was based on the methods described by de Revel et al. (2000) and Lo et al. (2008). For methylglyoxal analysis, the derivatization method could be optimized to a shorter time (de Revel et al., 2000). Under our analysis condition, the recoveries of methylglyoxal were the same for derivatization time of 30 min, 1 h or 2 h (data not shown here). Fru model was adjusted pH to 8.0 by using sodium hydroxide. The solution was added with OPD (0.5 mmole/L) in glass vials, glass vials were capped and shaken vigorously by vortexing for 5 s. The reaction was performed in a 60 °C water bath and 50 rpm for 30 min. After centrifuging at 14 × 1000 rpm (16,000*g*) for 5 min, samples were ready for HPLC analysis.

A Dionex UltiMate 3000 LC Modules equipped with a pump (model: LPG-3400 pump, Sunnyvale, CA), UV–Vis detector (model: VWD-3400 detector), and an autosampler (model: WPS-3000 SL) were used. A Luna C18 (Phenomenex, Torrance, CA) column (150 × 4.6 mm i.d. 3 μm particle size) was used for 2-methylquinoxaline derivative analysis. The column temperature was maintained at 25 °C in column oven (Dionex model: STH 585). The mobile phase for the HPLC system consisted of HPLC grade water with 0.15% acetic acid (v/v; solvent A) and acetonitrile (solvent B) with a constant flow rate set at 0.8 mL/min. HPLC gradient programs were performed for MG analysis as followings: 8% solvent B, and it increased to 40% over 10 min, to 48% over additional 2 min, to 60% over additional 1 min. After 13 min, solvent B increased to 80% for additional 2.5 min and 5 min for equilibrium. 2-Methylquinoxaline was detected with a UV wavelength at 313 nm and the injection volumes were 15 μL. The external standard quantification method was applied in this study. Every single peak area for the quantification was laid in the linear range of each standard curve.

2.4. Statistical analysis

Data were expressed as means ± standard deviation (SD) and represent two independent analyses. Statistical significance was examined using Student's *t*-test comparison between the means. A p value of <0.05 was considered statistically significant.

3. Results and discussion

Two major pathways of MG formation are Namiki pathway and monosaccharide autoxidation in both of

Table 1
Methylglyoxal (MG) generated from heating fructose (Fru) and fructose/phenylenediamine (Fru/OPD) systems

Reactant	pH	Heating temperature (°C)	Heating time (min)	MG generated (mmole/L)
Fru	7.5	150	30	0.0199 ± 0.0012
Fru/OPD	7.5	150	30	0.1210 ± 0.0129

which MG is generated from 3-deoxyosone (Booth, Khalifah, Todd, & Hudson, 1997; Nagai & Horiuchi, 2003). For monosaccharide autoxidation, 3-deoxyosone is formed from 2,3-enol after dehydration, whereas in the Namiki pathway, 3-deoxyosone is formed through deamination of Amadori products. Indeed, the amine group in amino acids catalyzes the formation of 3-deoxyosone and increases the level of MG, therefore, MG generated in monosaccharide-amino acid system should be higher than that from the system with monosaccharide only. As shown in Table 1, in our model system of thermal degradation of fructose only, the level of MG formed was 0.0199 ± 0.0012 mmole/L which was slightly lower than that in the Fru/Asn system from Fig. 2 in Yuan's paper (Yuan et al., 2007a). However, *o*-phenylenediamine (OPD) contains two amine groups which could catalyze the transformation of fructose and other reducing sugars into reactive carbonyl compounds such as MG. Therefore, in the system of Fru/Asn/OPD, OPD could play a dual role of a catalyst for MG formation as well as a trapping agent for MG derivatization. In the present study, the model of Fru/OPD was used to verify the catalytic function of OPD in MG formation. After being heated at 150 °C for 30 min, the model contained MG of 0.1210 ± 0.0129 mmole/L which was 6-fold higher than that in model system containing only fructose. The Fru and Fru/OPD models clearly showed OPD could dramatically increase MG generation in the Maillard Reaction. Therefore, it is concluded that the concentration of MG obtained in Fru/Asn/OPD or Glc/Asn/OPD model system cannot correspond to the total amount of MG formed by Maillard reaction of Fru and Asn as claimed by Yuan et al. (2007a,b).

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