Food Chemistry 119 (2010) 1–6

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Rapid Communication

Effects of melamine on the Maillard reaction between lactose and phenylalanine

Jinyu Ma [a], Xiaofang Peng [a], Ka-Wing Cheng [a], Ricky Kong [b], Ivan K. Chu [b], Feng Chen [a], Mingfu Wang [a,*]

[a] School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong, China
[b] Department of Chemistry, The University of Hong Kong, Pokfulam Road, Hong Kong, China

ARTICLE INFO

Article history:
Received 5 June 2009
Received in revised form 26 June 2009
Accepted 3 July 2009

Keywords:
Melamine
Maillard reaction
Interaction
Strecker aldehyde
Lactose

ABSTRACT

The effects of melamine on Maillard reaction were investigated in chemical model systems. The reaction products in the model systems with/without melamine were analysed by GC–MS and LC–MS/MS. Impact of melamine on Maillard browning in the above models was also examined by colourimetric methods. It was found that melamine can react directly with lactose and Strecker aldehydes formed in Maillard reactions to produce new adducts. In addition, the presence of melamine in chemical model systems also affects the formation of Maillard flavours and browning.

1. Introduction

Maillard reaction plays a major role in the generation of flavour and colour in thermally processed foods, and is of vital importance to the food industry. The reaction is initiated by the involvement of reactive carbonyl group of the reducing sugars (such as glucose, fructose and lactose) reacting with the nucleophilic amino groups of amino acids, peptides or proteins, and subsequently produce a large number of poorly characterised compounds, contributing to the colour and flavour of foods (Chuyen, 1998). Basically, the process of Maillard reaction can be divided into three stages. The first stage is initiated by the covalent attachment of reducing sugars to the N-terminal amino groups, resulting in the formation of reversible and unstable imines (or glycosylamine) which subsequently transform to Amadori rearrangement products. Subsequently, Amadori products undergo several reactions including dehydration, deamination, retroaldol and aldol reactions, enolizaiton and Strecker degradation to form Maillard intermediates containing many kinds of reactive carbonyl species (RCS), α-aminoketones, Strecker aldehydes and others (Hidalgo, Gallardo, & Zamora, 2005; Hofmann & Schieberle, 2000; Namiki, 1988; Schonberg, Moubasher, & Mostafa, 1948; Weenen, 1998). At the final stage of the reaction, the intermediates would involve in all kinds of dehydration, cyclisation and polymerisation reactions resulting in the formation of complicated Maillard reaction products composed of browning ingredients, volatile or flavour compounds (Schebor, Buera, Karel, & Chirife, 1999; van Boekel, 2006). For dairy products, it has been known for a long time that Maillard reaction is an important reaction happening in the milk drying process, and contributes to its unique flavour and browning.

Melamine, also known as cyanuramide or traminotriazine, is a synthetic nitrogenous compound with a 1,3,5-triazine skeleton. It is primarily used as a raw material for plastics stabilisers, countertops, house wares, flame retardants, fabrics and glues in industries. It is also a major component of some pigments. Recently, much attention has been paid to melamine since governmental bodies worldwide reported the detection of high levels of melamine in different foods, especially milk products (Astier, 2009; Xin & Stone, 2008). Due to its high nitrogen content (66% nitrogen by mass), melamine has been illegally added to foods to increase the tested (using Kjeldahl method), but not the real protein levels. Thousands of infants in China have developed melamine-related kidney diseases, and contaminated products have been re-called in more than 30 countries. It has been reported that melamine-contaminated milk products could cause urinary tract calculi (Zhang et al., 2009) and renal stone outbreak (Chiu, 2008; Yang & Batlle, 2008), although the detailed mechanism of melamine-induced nephrotoxicity has not yet been characterised. Until now, the formation of melamine-cyanuric acid cocrystals in kidneys has been identified as a major health consequence from the consumption of melamine-contaminated food products (Dobson et al., 2008). However, considering its structural characteristics, its occurrence at such high levels in adulterated food products (especially those that are subject to thermal processing) will likely have a major impact on many quality attributes, such as flavour, colour, contents

* Corresponding author. Tel.: +852 22990338; fax: +852 22990347.
E-mail address: mfwang@hkusua.hku.hk (M. Wang).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.07.007

of genotoxic compounds, and bioavailability of nutrients. Moreover, melamine itself might also react with other food components to form various new chemical entities, which if, consumed with the food products concerned, could also have significant health consequences. All these issues remain to be addressed.

As melamine possesses three free amino groups, it is reasonable to presume that melamine added to foods might interact via Maillard reaction through competitively reacting with reducing sugars, or Maillard intermediates or products. To evaluate this possibility, we designed appropriate simple chemical models containing key food ingredients (sugars and amino acids), to which melamine is then added. In a previous study, glucose–phenylalanine chemical system was successfully employed to investigate the scavenging capability of naringenin on phenylacetaldehyde, a key intermediate reactive carbonyl species formed in the process of Maillard reaction (Cheng et al., 2008). Besides, lactose is the most abundant reducing sugar in milk which has been reported to be contaminated by melamine. Hence, we chose the reaction system composed of phenylalanine and lactose as a model reaction in the present work. Differences in the chemical profiles between Maillard model reactions with and without melamine were analysed with the application of GC–MS and LC–MS/MS. The structures of new adducts derived from the interactions between melamine and Maillard reaction-associated chemical species were tentatively assigned by interpretation of LC–MS/MS data. Finally, impact of melamine on colour development in the above models was assessed by the colourimetric method with a Chroma meter which measures changes of the L^*, a^* and b^* coordinates of the CIELAB colour space.

2. Materials and methods

2.1. Solvents and reagents

Phenylalanine, lactose, phenylacetaldehyde, melamine, di(ethylene) glycol, and sodium chloride were purchased from Sigma–Aldrich Company (St. Louis, MO, USA). All other solvents were of analytical grade and obtained from BDH laboratory Supplies (Poole, UK). The Reacti-Therm III heating module (model 18840) and the screw cap Tuf-Bond Teflon fitted glass reaction vials were purchased from Pierce (Rockford, IL, USA).

2.2. Model Maillard reactions

The reaction mixtures (shown in Table 1) were dissolved in 10 mL of di(ethylene) glycol (DEG, containing 14% water) in crew cap Tuf-Bond Teflon fitted glass reaction vials (40-mL volume) and heated in a Reacti-Therm III heating module at 125 °C for 60 min, meanwhile the time course (10, 20, 30, 40, 50 and 60 min) experiment was carried out with model D and E. At designated time points, reaction mixtures were immediately cooled in an ice–water bath, and the chilled samples were kept at −20 °C before determination of colour changes, GC–MS or LC-MS/MS analysis.

2.3. Sample preparation

The volatile compounds formed in the model systems were extracted based on the method described by Pini (Pini, de Brito, Garcia, Valente, & Augusto, 2004) with some modifications. The reaction mixture (1 mL) was added to a crew cap Tuf-Bond Teflon fitted glass reaction vial (40-mL capacity) with 4 mL of saturated sodium chloride containing *n*-dodecane (internal standard, 10 μL/L). The vials were sealed immediately after filling and samples stirred with a magnetic mixer at 600 rpm at ambient temperature (24 °C). The sample was equilibrated for 10 min, and then headspace-extracted with a Supelco SPME DVB-Carboxen-PDMS fibre for 30 min prior to GC–MS analysis. For identification of nonvolatile components, reaction mixtures from the phenylalanine–lactose (with or without melamine) models were subjected to LC-MS/MS analysis after 100-fold dilution with methanol without further sample processing.

For colour measurement, 1 mL out of 10 mL of each reaction mixture was diluted with 20 mL methanol and the sample was vortex-mixed for 1 min. Part of the diluted sample was directly measured with spectrophotometer at 420 nm (UV-1206, Shimadzu, Kyoto, Japan). Part of the diluted sample was transferred to a cuvette for colour measurement with a Chroma meter (Minolta CR-400, Konica Minolta, NJ, USA).

2.3. GC–MS analysis of volatile reaction products

GC–MS was carried out on an Agilent gas chromatograph (HP 6890 N, Santa Clara, CA, USA) with an Agilent GC–MS chemstation software coupled to an Agilent mass detector (HP 5973 N, EI mode, Santa Clara, CA, USA). Separation was performed on a DB-Wax capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). The following parameters were applied: sample desorption at 200 °C for 4 min in splitless mode; column flow, 1 mL/min (He); temperature programme, 40 °C for 4 min, ramp at 15 °C/min to 70 °C and hold for 6 min; ramp at 1 °C/min to 80 °C; ramp at 3 °C/min to 100 °C and then ramp 5 °C/min to 160 °C; post-run temperature, 230 °C for 5 min; MS temperature, 230 °C. The identification of volatile compounds was based on GC–MS analysis by comparing the mass spectra data with those of authentic compounds available in the mass spectra library. Peak area of volatile compounds was adjusted relative to the peak area of the internal standard (*n*-dodecane).

2.4. LC-MS/MS analysis of nonvolatile Maillard reaction products

The samples were analysed on a LC-MS/MS instrument equipped with an electrospray ionisation (ESI) source interfaced to a QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA). Liquid chromatography was run on a separation model (Agilent 1100; Agilent Technologies, Santa Clara, CA, USA) with a degasser, a quaternary pump and a thermostatted autosampler. Separation of Maillard reaction products was conducted on a Zorbax eclipse XDB-C_{18} column (5 μm, 2.1 × 150 mm; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was

Table 1
Aqueous and di(ethylene)glycol chemical model reactions.

Reactant	Reactant concentration (mM)							
	Model A	Model B	Model C	Model D	Model E	Model F	Model G	Model H
Phenylalanine	120			120	120	120		
Lactose		60		60	60		60	
Melamine			40		40	40	40	40
Phenylacetaldehyde								40

Model reactions were carried out in di(ethylene)glycol (14% of water) heated at 125 °C for 60 min.

composed of water (solvent A) and acetonitrile (B) of the following gradients: 0 min, 1% B; 5 min, 2% B; 30 min, 30% B; 35 min, 85% B; 40 min, 95%. Post-run time was 10 min. The flow rate was at 0.2 mL/min. The MS conditions were as follows: positive ion mode, spray voltage 5.3 kV, scan range 160–600 Da, DP 75 V. The scan model of enhanced product ion (EPI) was used to confirm the structure of three adducts, and collision energy was set at 10 V and 30 V for the peak at 1.7–1.9 min and 31.76 min, respectively.

3. Results and discussion

3.1. Effect of melamine on Maillard volatile product generation in phenylalanine–lactose model

A variety of volatile compounds can be formed through the Maillard reaction, and based on previous studies, pyrazines, aldehydes and furans are amongst the major classes of volatiles formed in a lot of Maillard model systems (van Boekel, 2006). In our study, 17 volatile compounds were identified and tentatively quantified by GC–MS from the lactose–phenylalanine model without the addition of melamine. These included eleven pyrazines (methylpyrazine, 2,5-dimethylpyrazine, 2-ethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, trimethylpyrazine, ethenylpyrazine, 2,6-diethylpyrazine, 2-methyl-5-propylpyrazine and 5H-5-methyl-6,7-dihydrocyclopentapyrazine), five aldehydes (benzaldehyde, alpha-phenylpropenal, alpha-methyl-benzeneacetaldehyde, benzeneacetaldehyde and alpha-ethylidene-benzeneacetaldehyde) and one furan (3-phenylfuran). We also found that the addition of melamine to the lactose–phenylalanine model did not lead to the generation of new volatile species. However, the presence of melamine in the model system did cause significant changes in the content of aldehydes and furans, but not pyrazines, which only account for a small part of the total volatile compounds formed. Fig. 1 displays the relative amounts of total pyrazines, aldehydes and furans in models with and without the addition of melamine at different time points (10, 30 and 60 min). It was found that melamine significantly lowered the contents of aldehydes in the Maillard models. Traditionally, melamine has been used as a raw material to synthesise thermoset polymers by reacting with formaldehyde in an aqueous solvent (Vo Thuy Diep et al., 2008). So it is highly possible that melamine under heating condition reacts directly with other aldehydes, thus lowering their concentrations in our model systems.

3.2. Analysis of the formation of melamine adducts in chemical model systems

To further examine the role of melamine in the phenylalanine–lactose model, LC–ESI-MS/MS analyses were performed to identify reaction products that may arise from reactions between melamine and lactose, lactose and phenylanine with/without melamine. With close examination and comparison of the LC-MS total ion chromatograms (TIC) between Maillard reaction models D (without melamine) and E (with melamine), some additional peaks were observed in the TIC of model E at 1.7–1.9 (predicted molecular weight, 450 and 468 Da) and 31.76 min (predicted molecular weight, 228 Da) (Fig. 2). The analyte with a molecular weight of 468 Da matched well with an adduct formed between lactose and melamine, and that of 450 Da could be generated by elimination of a water molecule from the lactose–melamine adduct. Further examination of spectra registered for samples from other model reactions showed that model G (melamine + lactose) also contained similar analytes at retention times of 1.7–1.9 min, which were absent from models B and C, suggesting these two compounds are formed by direct reaction between lactose and melamine, with one compound assigned as melamine-lactose and another one as dehydrated melamine–lactose. As the ion peak at 31.76 min was not identified from models B, C and G, and it was also absent from models A (containing only phenylalanine) and F (containing phenylalanine and melamine), it was reasonable to assume that this analyte is a product of reaction(s) between melamine and certain intermediate(s) formed from lactose and phenylalanine. It has been known that during heating, phenylalanine could undergo thermal degradation including Strecker degradation to form phenylacetaldehyde, which has been reported to form nucleophilic addition/condensation product with flavoniods such as naringenin (Cheng et al., 2008). Based on the predicted molecular weight (228 Da), the analyte could arise from nucleophilic addition of melamine to phenylacetaldehyde, followed by elimination of a water molecule. Consequently, direct reaction employing melamine and phenylacetaldehyde (model H) was carried out. As expected, an ion peak at 31.76 min was found which has a predicted molecular weight of 228 Da. Thus this phenomenon demonstrates that melamine could directly react with phenylacetaldehyde to form adduct(s).

For further confirmation, LC–MS/MS with EPI (enhanced product ion) scan mode was used to obtain more spectral information of the above three analytes. Their MS^2 spectra were presented in Fig. 3. Under the same experimental conditions (collision energy, 15 V), the precursor ion of *m/z* 469.2 generated ions of *m/z* 127.1 as the major fragment by the loss of lactose (Fig. 3A), whilst the precursor ion of *m/z* 451.2 produced ions of *m/z* 433.3, 289.3 and 127.2 by the loss of H_2O, galactose, lactose, respectively (Fig. 3B). In terms of stability, the analyte of *m/z* 451.2 was higher based on the observation that the relative abundance of its molecular ion was much higher than that of the *m/z* 469.2 analyte in their corresponding MS^2 spectrum. On the other hand, the dehydrated melamine–phenylacetaldehyde adduct is even more stable that a

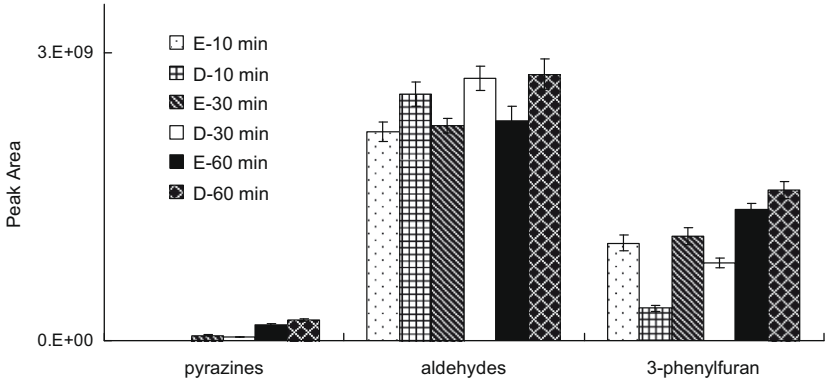

Fig. 1. The amount of Maillard volatile compounds formed in two chemical model systems (D: model D; E: model E). Results are expressed as means ± SD for $n = 3$.

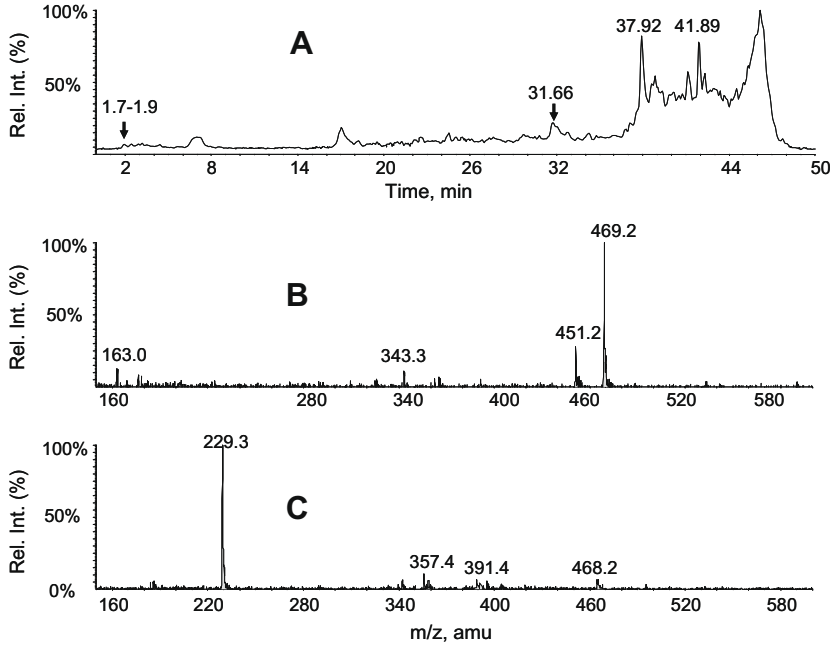

Fig. 2. The typical HPLC-MS chromatogram and MS spectra from model E. A: the total ion chromatogram; B and C are the mass spectra at 1.80 and 31.76 min, respectively.

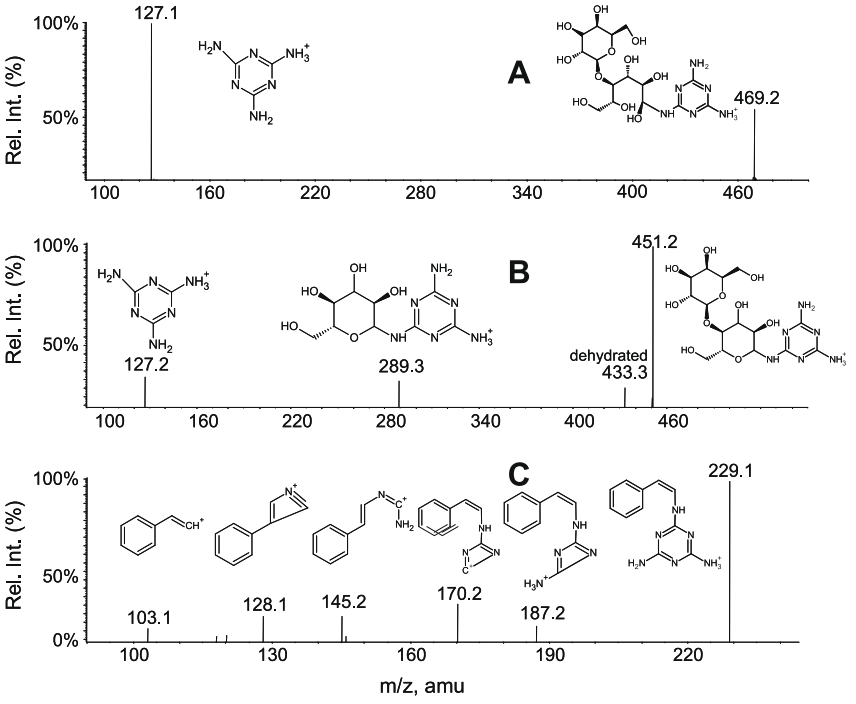

Fig. 3. The MS/MS spectra and the proposal fragments for the three adducts. A: melamine–lactose; B: dehydrated melamine–lactose; C: dehydrated melamine–phenylacetaldehyde.

much higher level of collision energy (30 V) was required to fragment the molecular ions to derive structural information. As shown in Fig. 3C, the precursor ion (*m/z* 229.1) generated ions of *m/z* of 187.2, 170.2, 145.2, 128.1, and 103.1 by the loss of NH_2CN, CH_5N_3, $C_2H_4N_4$, $C_2H_7N_5$, $C_3H_6N_6$, respectively. This fragmentation behaviour is consistent with that of protonated melamine (Yang et al., 2009) and is also consistent with the assigned structure. All these MS/MS data further confirmed that the three compounds are adducts of melamine–lactose, dehydrated melamine-lactose and dehydrated melamine–phenylacetaldehyde.

Moreover, the three melamine adducts were quantified in a kinetic study. As shown in Fig. 4, the contents of the dehydrated adduct of lactose-melamine and melamine–phenylacetaldehyde both increased, whilst that of lactose–melamine adduct decreased with time. This phenomenon suggests that in Maillard models containing lactose and melamine, a nucleophilic addition product might be formed in early stage of reaction, followed by the removal of a water molecule to form the stabilised dehydrated adduct, thus in the model system, reasonable amount of dehydrated lactose–melamine was observed. For dehydrated melamine–

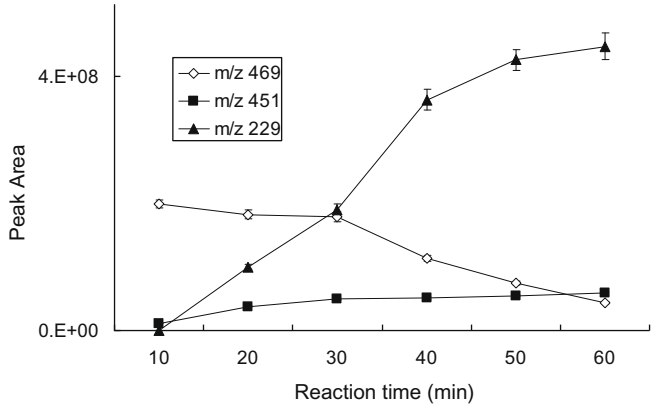

Fig. 4. The peak area of three adducts in model E at different time points. Results are expressed as means ± SD for $n = 3$.

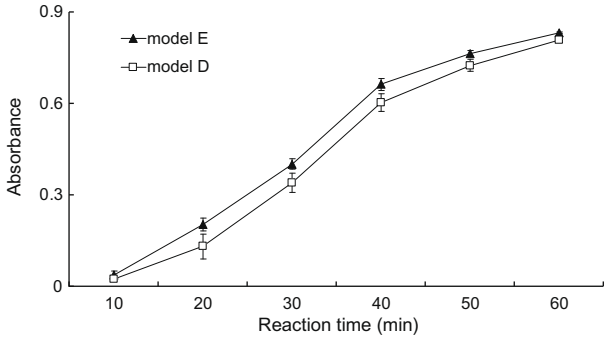

Fig. 5. UV-absorbance at 420 nm of Maillard reaction products at different time points. Results are expressed as means ± SD for $n = 3$.

phenylacetaldehyde, it can be viewed as a stable final product. With the progress of Strecker degradation of phenylalamine, more Strecker aldehyde (in the case of phenylalanine, the Strecker aldehyde is phenylacetaldehyde) is formed, which correspondingly reacts with melamine to form dehydrated melamine-phenylacetaldehyde.

With the observation of new adducts and these structures assigned by LC/MS/MS, it is confirmed that melamine could take part in a Maillard type of reactions by directly reacting with reducing sugars and/or Maillard intermediates such as those arise from the reaction between lactose and phenylalanine, especially Strecker aldehydes.

3.3. Effect of Melamine on Maillard Browning in phenylalanine–lactose model

Maillard browning involves the formation of a complex mixture of coloured compounds from an array of reactions. Generally, the browning intensity of the overall intermediates and final browning products in the Maillard reaction is described by UV-absorbance or colour dilution factors (CD_{total}) (Yeo & Shibamoto, 1991). In the present work, changes in UV-absorbance ($A_{420\ nm}$) were monitored to investigate the effects of melamine on Maillard browning in lactose–phenylalanine models (Fig. 5). It is apparent that samples from model E exhibited higher UV-absorbance at each time point than those from model D. This suggests that melamine may affect Maillard browning to some extent. Similar result was also observed by measuring a^* value of the same reaction solutions using a Chroma meter (Data not shown).

Overall, the addition of melamine into the phenylalanine–lactose chemical model can affect the formation of browning products, which might be attributed to the capability of melamine to competitively react with lactose at the early stage of Maillard reaction, thus accelerating the formation of N-substituted glycosylamine and others, which subsequently undergoes fragmentation to generate reactive carbonyl species (RCS). The enhanced generation of RCS, which are important precursors for browning reactions, such as those involved in the formation of melanoidins (Adams, Kitryte, Venskutonis, & De Kimpe, 2009), might therefore lead to enhanced colour development in Maillard models where melamine was present. However all these explanation experimental supports.

4. Conclusions

Findings of this study suggest that melamine can directly react with reducing sugars to consequently interfere with Maillard reactions in chemical model systems. These reactions can give rise to new chemical entities, which could have the potential to exhibit various biological activities including toxicity. Moreover, melamine was found to influence the levels of different kinds of volatile products and colour development in Maillard models. The presence of melamine in foods could therefore have a significant impact on various organoleptic properties. However, the real effects caused by the involvement of melamine in Maillard reaction on both food industry and life science need to be further investigated.

References

Adams, A., Kitryte, V., Venskutonis, R., & De Kimpe, N. (2009). Formation and characterisation of melanoidin-like polycondensation products from amino acids and lipid oxidation products. *Food Chemistry, 115*, 904–911.

Astier, A. (2009). Milk adulterated with melamine: Another scandal in public health. *Annales Pharmaceutiques Francaises, 67*, 1–2.

Cheng, K. W., Wong, C. C., Cho, C. K., Chu, I. K., Sze, K. H., Lo, C., et al. (2008). Trapping of phenylacetaldehyde as a key mechanism responsible for naringenin's inhibitory activity in mutagenic 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine formation. *Chemical Research in Toxicology, 21*, 2026–2034.

Chiu, M. C. (2008). Melamine-tainted milk product (MTMP) renal stone outbreak in humans. *Hong Kong Medical Journal, 14*, 424–426.

Chuyen, N. V. (1998). Maillard reaction and food processing. Application aspects. *Advances in Experimental Medicine and Biology, 434*, 213–235.

Dobson, R. L. M., Motlagh, S., Quijano, M., Cambron, R. T., Baker, T. R., Pullen, A. M., et al. (2008). Identification and characterization of toxicity of contaminants in pet food leading to an outbreak of renal toxicity in cats and dogs. *Toxicological Sciences, 106*, 251–262.

Hidalgo, F. J., Gallardo, E., & Zamora, R. (2005). Strecker type degradation of phenylalanine by 4-hydroxy-2-nonenal in model systems. *Journal of Agricultural and Food Chemistry, 53*, 10254–10259.

Hofmann, T., & Schieberle, P. (2000). Formation of aroma-active strecker-aldehydes by a direct oxidative degradation of Amadori compounds. *Journal of Agricultural and Food Chemistry, 48*, 4301–4305.

Namiki, M. (1988). Chemistry of Maillard reactions: Recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. *Advances in Food Research, 32*, 115–184.

Pini, G. F., de Brito, E. S., Garcia, N. H. P., Valente, A. L. P., & Augusto, F. (2004). A headspace solid phase microextraction (HS-SPME) method for the chromatographic determination of alkylpyrazines in cocoa samples. *Journal of the Brazilian Chemical Society, 15*, 267–271.

Schebor, C., Buera, M. d. P., Karel, M., & Chirife, J. (1999). Colour formation due to non-enzymatic browning in amorphous, glassy, anhydrous, model systems. *Food Chemistry, 65*, 427–432.

Schonberg, A., Moubasher, R., & Mostafa, A. (1948). Degradation of alpha-amino-acids to aldehydes and ketones by interaction with carbonyl compounds. *Journal of the Chemical Society, 2*, 176–182.

van Boekel, M. A. J. S. (2006). Formation of flavour compounds in the Maillard reaction. *Biotechnology Advances, 24*, 230–233.

Vo Thuy Diep, T., Himmelsbach, M., Haunschmidt, M., Buchberger, W., Schwarzinger, C., & Klampfl, C. W. (2008). Improved analysis of melamine–formaldehyde resins by capillary zone electrophoresis–mass spectrometry using ion-trap and quadrupole-time-of-flight mass spectrometers. *Journal of Chromatography A, 1213*, 83–87.

Weenen, H. (1998). Reactive intermediates and carbohydrate fragmentation in Maillard chemistry. *Food Chemistry, 62*, 393–401.

Xin, H., & Stone, R. (2008). Tainted milk scandal. Chinese probe unmasks high-tech adulteration with melamine. *Science, 322*, 1310–1311.

Yang, V. L., & Batlle, D. (2008). Acute renal failure from adulteration of milk with melamine. *Scientific World Journal, 8*, 974–975.

Yang, S., Ding, J., Zheng, J., Hu, B., Li, J., Chen, H., et al. (2009). Detection of melamine in milk products by surface desorption atmospheric pressure chemical ionization mass spectrometry. *Analytical Chemistry, 81*, 2426–2436.

Yeo, H. C. H., & Shibamoto, T. (1991). Flavour and browning enhancement by electrolytes during microwave irradiation of the Maillard model system. *Journal of Agricultural and Food Chemistry, 39*, 948–951.

Zhang, L., Wu, L. L., Wang, Y. P., Liu, A. M., Zou, C. C., & Zhao, Z. Y. (2009). Melamine-contaminated milk products induced urinary tract calculi in children. *World Journal of Pediatrics, 5*, 31–35.