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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Comparison of Mitigation Strategies to Reduce Acrylamide in Prepared Maillard Model Systems

Mahwash Aziz[1], Imran Pasha[1], Faqir Muhammad Anjum[1] and Mahr-un-Nisa[2]
[1]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan
[2]Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad, Pakistan

Abstract: Maillard model systems were prepared by using asparagine and glutamine as amino acids and glucose and fructose as reducing sugars. Acrylamide is the toxic compound that is produced during Maillard reaction among amino acids and sugars. So, in the current study, various mitigation strategies were utilized in these model systems to reduce the toxic effects of acrylamide. These mitigation strategies include vacuum treatment, application of calcium chloride as a cation and utilization of pectin. Afterward, acrylamide concentration was determined in these model systems by Gas Chromatography-mass Spectrometry (GC/MS) technique and progressive decrease was observed in acrylamide content by using different treatments. Maximum acrylamide concentration was observed in control i.e., 22.37±1.9 µg/g while minimum concentration (3.51±0.5 µg/g) was recorded in pectin-treated model system. Data pertaining to utilization of model system for acrylamide analysis by GC/MS indicated that model systems affected the acrylamide concentration momentously. Maximum acrylamide concentration (11.33±8.3 µg/g) was observed in Glucose-Asparagine model system, whilst minimum concentration (9.63±8.1 µg/g) was examined in Fructose-Glutamine model system. Consequently, pectin as a hydrocolloid reduced the higher content of acrylamide in comparison with vacuum treatment and calcium chloride.

Key words: Maillard reaction, pectin, acrylamide, calcium chloride

INTRODUCTION

Various intermediate products are formed as a result of reaction among amino acids and sugars during Maillard reaction. These intermediate compounds include numerous low molecular weight compounds, high molecular weight polymers and some other compounds. The preliminary steps of Maillard reaction include the formation of low molecular weight intermediate compounds that include furanones derivatives, furfural and dicarbonyl compounds. Subsequently, these compounds react with some other reactive compounds like amino acids, amines, hydrogen sulphide, aldehydes and ammonia. As a result, many aroma producing molecules are developed for instance, pyrazines, furans, oxazoles, pyrroles, thiazole and some other heterocyclic compounds (Kitts *et al*., 2012; Konishi and Kobayashi, 2012). Finally, the high molecular weight melanoidins are formed by the condensation of pyrroles or pyrrole derivatives (Fay and Brevard, 2005; Hellwig and Henle, 2012).

In addition to the above aromatic compounds production, the toxic compound; acrylamide is also produced during Maillard reaction (Mulla *et al*., 2010). Haase *et al*. (2012) corroborated the production of acrylamide during Maillard reaction by the association between reducing sugars and asparagine. Both the asparagine and reducing sugars (Claus *et al*., 2008) play an important role in the acrylamide formation. The deamination and decarboxylation of asparagine result in the production of acrylamide (Hidalgo *et al*., 2010; Meyer *et al*., 2010).

On the basis of discovery of toxic effects of acrylamide, various organizations gave awareness about its genotoxic and carcinogenic impacts. For the purpose, a connoisseur discussion on the insinuation of acrylamide in food products was hosted by the World Health Organization (WHO) and the United Nations Food and Agriculture Organization (FAO) in June 2002. These organizations harassed the requirement to set up a research network for acrylamide analysis to attain a better perceptive of human revelation and its probable health impacts. After this consultation, many experts analyzed the occurrence of acrylamide in various cooked food items and also learnt the means of acrylamide formation in foods (Sanny *et al*., 2012). The detection of acrylamide in the Maillard model systems and foods (Garabagiu and Mihailescu, 2011; Zhuang *et al*., 2012) directed to analyses to explore the level of this toxic compound (IARC (International Agency for Research on Cancer), 1994) and stimulated some appropriate analytical measures for acrylamide analysis.

Two factors are involved to decrease the prospective for acrylamide formation that includes optimization of thermal process to mitigate acrylamide formation and the reduction of its precursors in raw material. Both these factors must be believed delicately, as on the

Corresponding Author: Mahwash Aziz, National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan

whole the nutritional and organoleptic features of the foods should be sustained. The optimal thermal treatment can be examined through the process imitation and molecule formation, in conjunction with validating experimental measurements (Carrieri *et al*., 2009).

Then mechanism of acrylamide removal is different while using the mitigation strategies. Acrylamide molecule is physically eradicated from the whole system during the process of heating. It is thought that the removal of acrylamide can be done on the basis of its physical and chemical properties, as it has low molecular weight (71 Da) (Budavari *et al*., 1996). The appropriate environmental conditions including pressure and temperature are fisible to remove the acrylamide content during food processing. It has been studied previously that the reduction of the acrylamide content can be done on the basis of its physical properties (Nicoli and Anese, 2006; Anese *et al*., 2010). In addition to use physical treatments to reduce acrylamide, many chemicals can also be utilized to mitigate the acrylamide level during food processing. These food additives include cysteine, citric acid, hydrocolloids and divalent cations for the inhibition of acrylamide in model systems as well as in food products (Gokmen and Senyuva, 2007; Ou *et al*., 2008; Pedreschi *et al*., 2008).

The present study was designed to assess and reduce the acrylamide content produced during Maillard reaction in model systems. The mandate of the study was to compare different mitigation strategies applied for the reduction of acrylamide content in prepared model systems.

MATERIALS AND METHODS

Proteins, asparagine and glutamine and the sugars, fructose and glucose were purchased from Merck (Germany). Acrylamide and methacrylamide were obtained from Sigma (USA). All reagents were of analytical grade or of the highest grade available.

Preparation of different model systems: Different Maillard model systems were prepared by using amino acids and reducing sugars. These model systems include glucose/glutamine, fructose/glutamine, glucose/asparagine and fructose/asparagine model systems. For the purpose, 0.5g sugar and 0.5g amino acid were weighed accurately in 100ml volumetric flask and mixed in distilled water. pH was adjusted to 4.5, 7 and 10 with the 0.1N HCl and 0.1N NaOH and the volume was made up to mark. Then all these systems were transferred to Reflux apparatus and heated at boiling temperature for one hour. These model systems for sugars and amino acids were prepared following the method described by Chen and Chi-Tang (1999). All the model systems were prepared by using this method. After cooling, the prepared model systems were transferred to glass bottles. The bottle's covers were sealed with aluminum foil properly and then bottles of model systems were kept in refrigerator at 4°C.

Extraction of model systems: For the extraction of volatile model systems, Clevenger apparatus was used. For the extraction of volatiles individual model systems were subjected to dichloromethane solvent. Solvent trapped all flavourous compounds from liquid system and was collected in a receiving flask and this procedure continued for at least 4-6 h until the solvent in receiving flask become colored and liquid model system become clear. The flasks containing flavourous volatiles and solvent were removed and the contents were transferred to plastic bottles. These bottles were then stored in freezer. Finally, this extract was subjected to rotary evaporator for the separation of solvent. The volatiles were then collected in sealed bottles and stored at freezing temperature.

Strategies to reduce acrylamide formation: The following treatments were applied in Maillard model systems according to the treatment plan mentioned in Table 1.

Vacuum treatment: Vacuum treatment was carried out to reduce acrylamide formation according to the procedure described by Anese *et al*. (2010). Experiments of acrylamide removal were made by using an apparatus consisting of an oven connected to a rotary vacuum pump. The samples, previously measured in petri dishes, were introduced into the oven once the desired temperature was reached. Afterwards, the rotary

Table 1: Treatment plan for reduction of acrylamide in model systems

Treatments	Control	VT	H	DC
T_1	MS_1	-	-	-
T_2	MS_2	-	-	-
T_3	MS_3	-	-	-
T_4	MS_4	-	-	-
T_5	-	MS_1	-	-
T_6	-	MS_2	-	-
T_7	-	MS_3	-	-
T_8	-	MS_4	-	-
T_9	-	-	MS_1	-
T_{10}	-	-	MS_2	-
T_{11}	-	-	MS_3	-
T_{12}	-	-	MS_4	-
T_{13}	-	-	-	MS_1
T_{14}	-	-	-	MS_2
T_{15}	-	-	-	MS_3
T_{16}	-	-	-	MS_4

T= Treatment, VT: Vacuum treatment, H: Hydrocolloids, DC: Divalent cations.
MS_1 = Glucose+Glutamine model system,
MS_2 = Fructose+Glutamine model system,
MS_3 = Glucose+Asparagine model system,
MS_4 = Fructose+Asparagine model system

pump was immediately switched on. The time needed to achieve the desired vacuum ranged from 2-15 min depending on the set pressure value and the water content of the samples. The pressure used for this treatment was 6.67Pa, applied at 60°C for 5 min. After the treatments, samples were immediately removed from the oven, wrapped in aluminum foil and stored in desiccators until analyses were performed.

Use of hydrocolloids: The mitigation of acrylamide was also done by using hydrocolloids following the method of Zeng *et al.* (2010). The quantity of prepared samples of model systems was measured. Afterwards, 2% pectin was added in prepared model systems accordingly.

Divalent cation method: Divalent cation was also used to decrease the acrylamide configuration as described by Gokmen and Senyuva (2007). For the purpose, prepared model systems were measured and 2% $CaCl_2$ was used according to the treatment plan.

Determination of acrylamide concentration by GC/MS: Analysis of acrylamide was carried out through GC/MS (Agilent, USA) according to the method of Nemoto *et al.* (2002) and Lee *et al.* (2011). An injection volume of 2 µL was made using a 50% split injector. The GC column was DB-5 MS (30 m length x 0.25 mm i.d. x 0.25 µm film thickness, J and W Scientific, Folsom, CA, USA). The oven temperature program was as follows: 50°C (5 min), then 20°C min^{-1}-120°C and hold for 15 min. The injection temperature was 120°C. The mass spectrometer was operated in Electron Impact (EI) mode with ionization energy of 70eV, a scanning range of m/z = 45-550 and a scan rate of 20 scans s^{-1}.

Statistical analysis: Finally, the data was statistically analyzed following the method of Steel *et al.* (1997). One-factor factorial CRD was applied on model systems and safety study.

RESULTS AND DISCUSSION

Analysis of acrylamide concentration by GC/MS: Mean squares revealed significant effect of treatments, model systems and their interaction on acrylamide by GC/MS. Acrylamide concentration was determined by GC/MS and progressive decrease in acrylamide analysis was observed by using different treatments (Table 2). Maximum acrylamide concentration was observed in control i.e., 22.37±1.9 µg/g and this concentration was decreased by using the mitigation strategies. Calcium chloride reduced the acrylamide content in comparison with control. Moreover, the application of vacuum treatment reduced higher acrylamide content as compared to control and calcium chloride. Conversely, minimum concentration (3.51±0.5 µg/g) was recorded in pectin-treated model system. The Maillard reaction requires water and oxygen for the whole process

Table 2: Analysis of acrylamide content by GC/MS in treatments

Treatments	Acrylamide by GC/MS (µg/g)
Control	22.37^{a}±1.9
Vacuum oven	8.62^{c}±1.2
$CaCl_2$	10.62^{b}±0.6
Pectin	3.51^{d}±0.5

Means carrying the same letters are not significantly different

Table 3: Analysis of acrylamide content by GC/MS in model systems

Model systems	Acrylamide by GC/MS (µg/g)
Fructose-asparagine	11.09^{a}±0.10
Fructose-glutamine	9.63^{b}±0.01
Glucose-asparagine	11.33^{a}±0.30
Glucose-glutamine	10.06^{b}±0.30

Means carrying the same letters are not significantly different

completion. However, the above mitigation treatments bound the availability of water, so the Maillard reaction cease up to the Schiff base formation and further reaction do not proceed. Subsequently, acrylamide formation does not occur during Maillard reaction.

Data pertaining to utilization of model systems for acrylamide analysis by GC/MS given in Table 3 indicated that model systems affected the acrylamide concentration momentously. Highest acrylamide content was observed in asparagine containing model systems as asparagine results in maximum acrylamide formation. So, the maximum acrylamide concentration was observed in Glucose-Asparagine and Fructose-Asparagine model systems i.e., 11.33±8.3 and 11.09±0.10 µg/g, respectively. Conversely, minimum concentration (9.63±8.1 µg/g) was examined in Fructose-Glutamine model system.

The Fig. (1a) revealed the chromatogram formation by acrylamide standard after 7.50 min. Likewise, in Fig. 1b, the chromatogram represents that higher concentration of acrylamide is formed in control model systems after 7.55 min. However, the chromatogram formed by GC/MS in pectin-treated model systems revealed the minimum acrylamide concentration as mentioned in Fig. 1c.

Acrylamide was determined by Paleologos and Kontominas (2005) and it ranged from 77.5-942.9 µg/kg in different food samples. The findings are also in agreement with Viklund *et al.* (2010) who observed that blanching reduced the acrylamide up to 71-73%. Findings of Gokmen and Senyuva (2007) reported that the absence of cations in asparagine and fructose model system results in a maximum of 0.045 and 0.085 µmol of acrylamide at 150 and 180°C, respectively. However, the use of Ca^{2+} in the model system reduced acrylamide formation by 59%. So, the divalent cation like Ca^{2+} and Maillard model system in equimolar concentration results in entire inhibition of the acrylamide formation.

The current results are in harmony with Granda *et al.* (2004) as they observed the reduction in acrylamide content by using vacuum frying, as low temperatures can be used without changing the sensory attributes of the

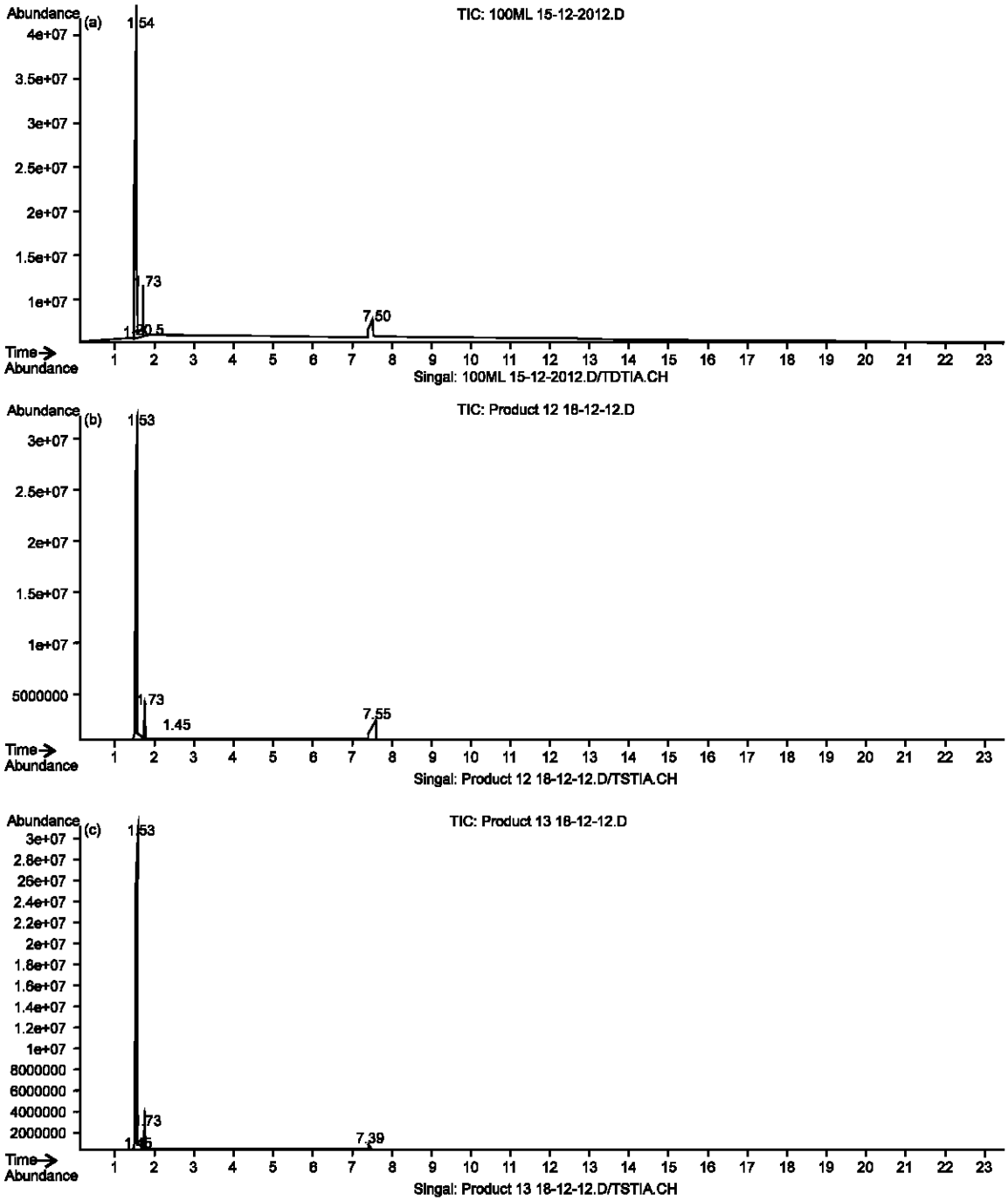

Fig. 1(a-c): (a) Acrylamide standard chromatogram, (b) Chromatogram of acrylamide in control model systems and (c) chromatogram of acrylamide in pectin-treated model systems

crisps. Similarly, Anese *et al.* (2010) found that the application of vacuum treatment from 5-15 min with the pressure of 6.67 Pa and temperature of 60°C results in the maximum acrylamide removal. These conditions were applied on biscuits and fried potato chips and inhibited 43 and 18% acrylamide, respectively. Earlier, Zeng *et al.* (2010) observed that the use of hydrocolloid (pectin) inhibited the formation of acrylamide in potato products.

So it is deduced from the above results that pectin reduced higher value of acrylamide as compared to other mitigation strategies. Moreover, acrylamide concentration varies in Maillard model systems prepared from various amino acids and sugars.

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