J Food Sci Nutr
Vol 13, p 134~137 (2008)
DOI: 10.3746/jfn.2008.13.2.134

J Food Science and Nutrition

The Effect of pH on the Formation of Acrylamide and Acrylate from Glucose and Fructose with Amino Acid Enantiomers in the Maillard Reaction

– Research Note –

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Abstract

This study was conducted to investigate the effect of pH on the formation of acrylamide and acrylate from glucose and fructose reacting with amino acid enantiomers by the Maillard reaction. The acrylamide content was increased with increasing pH, except for Fru/D-Asn system. Both acrylamide and acrylate contents were higher in the glucose-based system compared to the fructose-based system at pH 10.0. However, according to amino acid enantiomers, only the acrylamide content showed a difference in the fructose-based system. In addition, the acrylate content was increased with increasing pH except in the Glc/L-Asn system. Acrylate formation was observed specifically at pH 4.0 for both the Glc/D-Asn and Fru/D-Asn systems.

Key words: acrylamide, acrylate, amino acid enantiomers, Maillard reaction

INTRODUCTION

Detection of high concentrations of acrylamide in common heated foodstuffs in April 2002 caused considerable public concern, since acrylamide was found to be carcinogenic in rodents (1) and is classified as a probable human carcinogen (2). These findings caused the European Community (3), and the WHO (4) to initiate projects for the minimization of acrylamide content in commercial as well as in homemade foods.

A number of theoretical mechanisms have been proposed for the formation of acrylamide in heated food. Most probably, acrylamide in food results largely from the Maillard reaction between asparagine and a reactive carbonyl, proceeding through intermediates that include a Schiff's base (5-7). Several factors, such as the initial concentration of reactants and their ratio, temperature and time of processing, pH and water activity, have been shown to influence the formation levels of acrylamide in heat-processed foods (8). The influence of temperature on the formation of acrylamide has been repeatedly demonstrated (6,9-12).

Recent studies have indicated that polyvalent cations reduce acrylamide formation in thermally processed snack foods and bakery products (13,14). In this study, model Maillard and real food systems were employed to investigate the potential formation and degradation of acrylamide during heating in the presence of mono- and divalent cations.

In spite of acrylamide's toxicity in the monomer form, some microorganisms are able to utilize acrylamide as their sole carbon source for growth (15-19). All studies conducted to date show that there is an initial deamidation step that converts acrylamide to acrylic acid (acrylate) (16,17,19,20). The objective of this study, therefore, was to investigate the effect of pH on the formation of acrylamide and acrylate derived from glucose and fructose reacting with amino acid enantiomers in the Maillard reaction.

MATERIALS AND METHODS

Chemicals

D-glucose, D-fructose, L-asparagine and D-asparagine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetic acid, formic acid, sodium carbonate and sodium hydrogen phosphate were purchased from Merck Co. (Darmstadt, Germany). HPLC-grade water was purchased from J. T. Baker (Phillipsburg, NJ, USA). The analytical column, Zorbax SB-Aq (4.6×250 mm, 5 μm), was supplied by Agilent Technologies (Wilmington, DE, USA). Micro-spin centrifuge filters (0.45 μm) were obtained from Alltech Associates (Deerfield, IL, USA). Working standards of acrylamide were prepared at concentrations of 0.1, 0.2, 0.3, 0.5, 1.0 and 2.0 μg/mL in 0.01 mM acetic acid. Carrez I and Carrez II solutions were prepared by dissolving 15 g of potassium hexacyanoferrate and 30 g of zinc sulfate in 100 mL of water, respectively.

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Preparation of Maillard reaction products (MRPs)

Glucose, fructose and amino acids were dissolved in 100 mL of 0.5 M sodium acetate buffer, pH 4.0, 0.5 M phosphate buffer, pH 7.0 or 0.5 M sodium carbonate buffer, pH 10.0 to obtain a final concentration of 1 M. Four model systems were prepared, composed of glucose/L-asparagine (Glc/L-Asn), glucose/D-asparagine (Glc/D-Asn), fructose/L-asparagine (Fru/L-Asn) and fructose/D-asparagine (Fru/D-Asn). The reaction mixtures were then distributed among screw-capped glass, Schott tube (16×160 mm), each containing a minimum of 10 mL. Model solutions, prepared at least in duplicate, were heated without pH control at 100°C for 2 hr. The heating was carried out in a silicone oil bath and proper safety measures taken. After heating, model solutions were withdrawn and immediately cooled in ice water.

Extraction of acrylamide and acrylate from Maillard reaction products (MRPs)

The procedure described by Senyuva and Gokmen was used with minor modification (21). MRPs (1 mL) were put into a 10 mL glass tubes with caps. Carrez 1 (500 μL) and Carrez 2 (500 μL) solution were added to each tube and the volume was adjusted to 10 mL with 0.2 mM acetic acid. After mixing in a vortex mixer for 2 min, 1 mL of raw extract was transferred into a micro-spin centrifuge filter (0.45 μm) and centrifuged for 10 min at 10,000 rpm at 0°C. The clear extract (20 μL) was analysed by HPLC to quantify acrylamide.

HPLC analysis of acrylamide and acrylate

The quantifications of acrylamide and acrylate were performed by an Agilent 1100 HPLC system (Wilmington, DE, USA), consisting of an Agilent quaternary pump (Hewlett Packard, model G1311A, Wilmington, DE, USA), a variable wavelength detector (Hewlett Packard, model G1314A, Wilmington, DE, USA), an autosampler (Hewlett Packard, model G1313A, Wilmington, DE, USA) and a Chemstation software (Hewlett Packard, Wilmington, DE, USA). The chromatographic separations were performed on a Zorbax SB-Aq column (4.6×250 mm, 5 μm particle size, Agilent Technologies, Wilmington, DE, USA), using an isocratic mixture of 0.01 mM acetic acid in a 0.2% aqueous solution of formic acid, at a flow rate of 0.6 mL/min at 40°C. The data analysis was performed using Chemstation software (Hewlett Packard).

Statistical analysis

All experimental data were analyzed by analysis of variance (ANOVA) and significant differences among means from triplicate analysis at ($p<0.05$) were determined by Duncan's multiple range tests using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL)

RESULTS AND DISCUSSION

Asparagine is the only amino acid capable of directly generating acrylamide. Consequently it is considered the main source of acrylamide in food. The studies related to the detailed mechanism of this transformation have indicated that sugars and other carbonyl compounds play a specific role in the decarboxylation process of asparagine-a necessary step in the generation of acrylamide. Mechanistic studies have proposed that N-glycosides and related compounds formed in the early phase of the Maillard reaction are key intermediates leading to acrylamide (6,7,22). The first step in acrylamide production is the formation of a Schiff base between the carbonyl and α-amino group of asparagines by means of the dehydration of the N-glycosyl compound. Table 1 showed the acrylamide and acrylate content by amino acids enantiomers from the Maillard reaction with increasing pH. At pH 4.0, acrylamide was not found in all systems. However, acrylate was found in Glc/D-Asn (2.93 mM) and Fru/D-Asn systems (6.79 mM). The content of acrylate was higher in the fructose-based system than glucose-based system. At pH 7.0, acrylamide and acrylate were found in all systems, expect in the Fru/L-Asn system, in which acrylate was not found. The content of acrylamide was the highest in the Fru/D-Asn system (22.38 mM). There was no significant effect of amino acid enantiomers on the content of acrylamide in the glucose-based system. On the other hand, the content of acrylamide was significantly different in the fructose-based system according to amino acids enantiomer

Table 1. Contents of acrylamide and acrylate by amino acid enantiomers in the Maillard reaction[1)]

		Acrylamide	Acrylate
pH 4.0	Glc/L-Asn	−	−
	Glc/D-Asn	−	2.93 ± 0.09^{b}
	Fru/L-Asn	−	−
	Fru/D-Asn	−	6.79 ± 0.20^{a}
pH 7.0	Glc/L-Asn	$12.78\pm0.38^{2)b3)}$	14.54 ± 0.44^{a}
	Glc/D-Asn	12.68 ± 0.36^{b}	7.22 ± 0.20^{c}
	Fru/L-Asn	10.25 ± 0.31^{c}	−
	Fru/D-Asn	22.38 ± 0.67^{a}	8.93 ± 0.27^{b}
pH 10.0	Glc/L-Asn	31.23 ± 0.94^{a}	12.07 ± 0.36^{a}
	Glc/D-Asn	32.68 ± 0.98^{a}	12.44 ± 0.37^{a}
	Fru/L-Asn	17.35 ± 0.52^{b}	8.77 ± 0.26^{b}
	Fru/D-Asn	15.74 ± 0.47^{c}	9.31 ± 0.28^{b}

[1)]Data expressed as mM/L of acrylamide and acrylic acid.
[2)]Values are mean±standard deviation of three experiments.
[3)]Means in a column followed by different superscripts are significantly different at the $p<0.05$ level.

($p<0.05$); the content of acrylamide exhibited the following order: Fru/D-Asn>Glc/L-Asn≥Glc/D-Asn>Fru/L-Asn. In addition, the content of acrylate was the highest in the Glc/L-Asn system (14.54 mM). The content of acrylate exhibited the following order: Glc/L-Asn>Fru/D-Asn>Glc/D-Asn. At pH 10.0, acrylamide and acrylate were found in all systems. The content of acrylamide was highest in the Glc/D-Asn system (32.68 mM). The content of acrylamide and acrylate was higher in the glucose-based system than fructose-based system. The content of acrylamide exhibited the following order: Glc/D-Asn≥Glc/L-Asn>Fru/L-Asn>Glc/D-Asn. Differences in the acrylamide contents were present only in the fructose-based system according to amino acids enantiomers ($p<0.05$). In addition, the content of acrylate was the highest in the Glc/D-Asn system (12.44 mM). The content of acrylate exhibited the following order: Glc/D-Asn ≥Glc/L-Asn>Fru/D-Asn>Glc/L-Asn. Stadler et al. (6) investigated the role of different carbohydrates in the generation of acrylamide and observed that D-fructose, D-galactose, lactose and sucrose released acrylamide with comparable yields in model reactions with asparagine monohydrate heated at 180°C. However, Biedermann et al. (23) demonstrated fructose appeared to be twice as effective in promoting acrylamide formation as glucose when added to dry potato (5%) and heated at 150°C for 30 min in the model experiments. Conversely, Claeys et al. (24) found glucose to be more efficient in generating acrylamide than fructose when the heating temperature was higher than 140°C.

In conclusion, this study observed differences in the acrylamide and acrylate production by the Maillard reaction by pH, sugar type, and amino acid enantiomers. The content of acrylamide increased with increasing pH, except in the Fru/D-Asn system. The acrylamide and acrylate contents were higher in the glucose-based system than in the fructose-based system at pH 10.0. However, according to amino acid enantiomers, only the acrylamide content showed a difference in the fructose-based system. In addition, the acrylate content increased with increasing pH, except in the Glc/L-Asn system. Acrylate formation was observed specifically at pH 4.0 for the Glc/D-Asn and Fru/D-Asn systems. Therefore, these results can be used for understanding of the formation of acrylamide and acrylate in the Maillard reaction, and could be extended to the reaction of amino acids with reactive carbonyl compounds in general.

ACKNOWLEDGEMENTS

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2006-511-C00133).

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(Received April 17, 2008; Accepted May 21, 2008)