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Inhibition of acrylamide formation in asparagine/D-glucose model system by NaCl addition

Received: 4 January 2006 / Revised: 20 February 2006 / Accepted: 4 March 2006 / Published online: 29 March 2006
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Abstract Addition of 1, 5 and 10% of NaCl to equimolar mixture of asparagine/D-glucose was applied to study any affects of this food additive on acrylamide formation in the mixture. The samples were heated at 171.17°C for 10 min and the content of acrylamide was followingly determined by GC-MS technique using negative chemical ionization procedure. For comparison purposes, acrylamide formation was studied also in the mixture without NaCl addition at the same conditions. The results proved considerable inhibiting effects of NaCl on the acrylamide formation in all mixtures with addition NaCl, when the acrylamide content was lowered roughly by 32, 36 and 40% in comparison to mixture without NaCl addition. Very important is a fact, that effective lowering of acrylamide content was observed already at 1% of NaCl addition what could make possible to apply this knowledge also in real technological procedures of food processing.

Keywords Acrylamide · Asparagine · D-glucose · Inhibition · NaCl · GC-MS

Introduction

Roughly 3 years ago, it has been reported that acrylamide (AA) can be formed during thermal food processing [1]. Since AA is generally classified as “probably carcinogenic to humans”, these findings are considered alarming. The highest AA content was repeatedly found in French fries

and potato chips, and thus the attention was focused on heat-processed potato products [2–4]. Investigations of the reactions associated with the formation of AA revealed that the process is initiated with the reaction between reducing mono-saccharides and asparagine which indicates that AA might be a product of the Maillard reaction [5]. However, AA can be also formed from 3-aminopropionamide (β -alaninamide) which is generated from asparagine by decarboxylases in vegetable raw materials, e.g. potatoes [6]. The AA formation from asparagine and reducing mono-saccharides (D-fructose, D-glucose, D-galactose) can also take place in a dry equimolar mixture of the compounds during heating up to 190 °C [7]. The AA formation can be limited considerably by some additives such as amino acids and proteins [8, 9]. For example, croquettes prepared from fresh potatoes and coated with egg/breadcrumbs contained considerably reduced AA content after thermal processing [10]. The aim of this paper was to study inhibiting effects of NaCl as the common food additive, on the formation of acrylamide in the model system—dry asparagine/D-glucose mixture.

Materials and methods

Chemicals

AA of p.a. purity was purchased from Fisher Scientific Ltd. (Loughborough, UK) and 2,3,3-D3 AA (98%) was purchased from Cambridge Isotope Laboratories (Andover, MA). NaCl of p.a. grade was purchased from Lachema (Brno, Czech Republic). Acetone SupraSolv was obtained from Merck (Darmstadt, Germany), and asparagine monohydrate, D-glucose monohydrate, Acetonitrile R CHROMASOLV and Methanol CHROMASOLV from Sigma-Aldrich (Steinheim, Germany).

Techniques used

Agilent Technologies 6890N (Agilent Technologies, Palo Alto, USA) gas chromatograph equipped with an Agilent

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Table 1 Acrylamide content in the equimolar mixture of asparagine and D-glucose with additions of NaCl

	Addition of NaCl			
	0 wt.%, <i>n</i> = 27	1 wt.%, <i>n</i> = 27	5 wt.%, <i>n</i> = 27	10 wt.%, <i>n</i> = 27
Average AA content (μg AA/g mixture)	267	181	171	160
Standard deviation (μg AA/g mixture)	30	9	10	22
Relative standard deviation (%)	11	5	6	14
Relative content of AA (%)	100	68	64	60

n, number of analysis

Technologies 5973 inert mass selective spectrometer was used for the determination of the AA content in the samples.

Metal Block Thermostat was purchased from Liebsch, Germany, and EcoScan Temp JKT Temperature Meter equipped with Probe 3T520C was obtained from Eutech Instruments Europe B.V., Netherlands. A Nylon filter (0.45 μm) was purchased from Supelco (USA).

Experiment

Equimolar mixture of asparagine and D-glucose was prepared as follows: 14.4226 g asparagine monohydrate and 10.5774 g D-glucose monohydrate were mixed and homogenised thoroughly in a mortar dish. At this stage, the mixture was analysed for eventual AA presence without any heating treatment. Then, 0.2 g of the mixture (weighted nearest 0.0001 g) were placed into a glass tube and heated at the temperature 171.1 °C for 10 min. After that, the sample was cooled and analysed for AA content. The same conditions were maintained, when NaCl was added to the mixture at 1, 5 and 10%. Every heating experiment and determination was repeated for 27 times to obtain sufficient amount of data for calculation of standard deviation as well as relative standard deviation.

AA determination

After cooling, D3-AA as internal standard and 5 ml of acetonitrile/methanol 80:20 (v/v) mixture were added to a sample, the tube content was sonicated for 5 min, filtered and analysed by GC-MS. One microlitre of the extract was applied into a splitless injector (purge time 0.5 min at 250 °C) and separations were carried out using an Agilent 122-3232 30 m \times 0.25 mm \times 0.25 μm fused silica capillary column coated with a DB-FFAP phase. The column was held at 50 °C for 1 min, then heated to 250 °C at a rate of 10 °C/min. The carrier gas (helium) flow was maintained at 0.8 ml/min by an electronic control of pressure. Under these conditions AA and D3-AA eluted at 13.2 min. The data accumulation was not initiated until 12 min to avoid detection of the large peaks of acetonitrile and methanol. The content of AA was determined from the ratio of the peak area of AA to the peak area of the known amount of spiked D3-AA. The detection was carried out by the mass detector working in a selected ion monitoring mode; the ions were obtained by negative chemical ionization procedure using methane as the reagent gas. The mass of the most intense fragments was 70.15 and 73.15 *m/z*, respectively.

Results and discussions

Analysis of the mixture not treated by heat confirmed absence of AA at given analytical parameters which approved that neither components of the mixture were extracted from the mixture nor AA was formed in injection space during GG analysis. Results of samples treated by heat are summarised in Table 1. As follows from the Table, any addition of NaCl has considerable inhibiting effects on AA formation in the studied systems. But, the decrease is not linear with the addition of NaCl and the dependence is hyperbolic-like with the tendency to reach a limit value for NaCl additions over 10%. However, the high decrement in the AA content (by 32%) is already observed for the addition of 1% of NaCl, which is important for practical application in real technological procedures during thermal food processing, because just NaCl content round 1% represents its common content in many food products. On the other side, a difference between addition of 1 and 10% is far less effective, when AA content was lowered only by 8%. As show values of standard deviation and relative standard deviation, the analytical procedure itself is satisfactory reliable and suitable for monitoring of AA content in such systems, consisted of asparagine and reducing sugars.

Acknowledgement This work was supported by Science and Technology Assistance Agency of Slovak Republic under the contract no. APVT-27-030202.

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