RESEARCH ARTICLE

Investigation of acrylamide formation on bakery products using a crust-like model

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Baking is a complex process where a temperature gradient occurs within the product as a result of simultaneous heat and mass transfers. This behaviour makes the physical parameters (baking temperature and product dimensions) as effective as the chemical parameters on the rate of acrylamide formation in bakery foods. In this study, the change of temperature in different locations of the sample was shown as influenced by the product thickness. The temperature values were close to each other in the sample having thickness of 1 mm (crust model). The product temperature rapidly increased to the oven temperature. A temperature gradient was recorded in the sample having a thickness of 10 mm. As a result, the product temperature did not exceed 100°C within a baking time of 30 min. The product thickness significantly influenced the rate of acrylamide formation during baking. Acrylamide concentration rapidly increased to 411±49 ng/g within 8 min in the crust model sample. However, no acrylamide was detected in the thicker sample within 15 min under the same conditions, because the moisture content was still above 10%. The crust model was considered useful to test the effectiveness of different mitigation strategies in bakery foods.

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1 Introduction

Detection of acrylamide levels in processed foods has been an intensive area of research shortly after the discovery of acrylamide in heated foods by the Swedish researchers in April 2002 [1]. Several researchers have established that the main pathway of acrylamide formation in foods is linked to the Maillard reaction and, in particular, the amino acid asparagine [2–5]. The presence of acrylamide in common heated foods has been considered an important food-related crisis by international authorities. Discovery of acrylamide in foods has highlighted that the agro-food industry is poorly prepared to face such problems.

Processed cereals are among the heat-treated foods in which acrylamide formation has been commonly encountered. These foods encompass a vast range of different products with many ingredients, processes, recipes and scales of operation. The resulting acrylamide concentrations in these foods change with great deviations, not only for different types of bakery products, but also for a single type of product from different suppliers (http://irmm.jrc.ec.europa.eu/html/activities/acrylamide/database.htm) [6]. Various strategies have been proposed so far for reducing acrylamide level in bakery products. The most common strategies have focused on reducing or diluting precursors [7]. The mitigation strategies that practically sound good include reducing asparagine level by asparaginase [8, 9], adding amino acids other than asparagine [10, 11], adding divalent cations [12, 13], removing ammonium salts [14, 15] and replacing reducing sugars [16, 17].

Apart from the modifications made on the recipe, baking conditions have a strong influence on acrylamide formation [17]. Since acrylamide formation is not homogenously distributed within the product, it is not easy to state the percentage contribution of recipe modification on the resulting acrylamide concentration. In bakery foods, only crust contains acrylamide while crumb is free of acrylamide.

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Abbreviation: APCI, atmospheric pressure chemical ionization

Hence, sampling becomes critical if both crust and crumb parts are present in the test sample. Some researchers used a crust-like dry cereal model system to study the effect of baking temperature and time on acrylamide formation during baking, but time-temperature history of the samples was not monitored [18].

The aim of the present study was to develop a crust model to study acrylamide formation in bakery products. The effect of product thickness on the rate of acrylamide formation and the formation of temperature gradient within the product was determined during baking process. The effects of some mitigation agents on acrylamide formation were also tested using the crust model.

2 Materials and methods

2.1 Chemicals and consumables

Acrylamide was purchased from Sigma (Deisenhofen, Germany). Formic acid, potassium hexacyanoferrate and zinc sulfate (all AnalaR grade) were purchased from Merck (Darmstadt, Germany). A total of 0.45 μm nylon membrane syringe filters were purchased from Agilent Technologies (Waldbronn, Germany). Oasis MCX (1 mL, 30 mg) solid phase extraction cartridges and Atlantis T_3 analytical column (4.6×150 mm, 3 μm) were supplied by Waters (Millford, MA, USA).

Carrez I solution was prepared by dissolving 15 g of potassium hexacyanoferrate in 100 mL of water, and Carrez II solution by dissolving 30 g of zinc sulfate in 100 mL of water.

2.2 Preparation of crust model samples

Bread dough was prepared by mixing 10 g of wheat flour with 6 mL of water. Cookie dough was prepared by mixing 80 g of wheat flour, 35 g of sucrose, 32 g of shortening, 0.8 g of non-fat dry milk, 1 g of salt, 0.8 g of sodium bicarbonate, 0.4 g of ammonium bicarbonate and 17.6 mL of deionized water according to the recipe described in AACC (American Association of Cereal Chemists) Method 10–54.

The dough samples were rolled out to obtain the discs having a diameter of 6 cm with thickness of 1 or 10 mm. Since obtaining exactly the same thickness in crust model samples is difficult to achieve, the samples' weights were adjusted to a fixed value, in order to ensure about the same amount of dry matter in all samples. The discs were baked in the oven (Memmert UNE 400, Germany) at 180°C for different times up to 30 min, and 200°C for 15 min to obtain bread and cookies resembling model samples, respectively. In order to demonstrate the effect of some mitigation strategies on acrylamide formation using the crust model, wheat flour was mixed with calcium chloride (0.1 and 0.5 % w/w) or grape seed extract (0.1 and 0.5 % w/w).

2.3 Temperature measurement

Time-temperature data were acquired using a digital multimeter (Keithley Model 2700, Cleveland, OH, USA) during baking. Three thermocouples were inserted into the sample: the first thermocouple was placed into the centre of the disc, second into the edge of disc and the third in the oven.

2.4 Analysis of acrylamide by LC-APCI-MS

The samples were prepared for acrylamide analysis using a procedure described by us elsewhere [19]. Briefly, ground samples (1 g) were triple extracted with 10 mM formic acid (10, 5 and 5 mL). The combined extract was clarified by Carrez clarification. The extract was further cleaned up using Oasis MCX SPE cartridge. The final extract was filtered through 0.45 μm nylon filter and analysed by LC atmospheric pressure chemical ionization (APCI) MS.

An Agilent 1200 HPLC system (Waldbronn, Germany) consisting of a binary pump, an autosampler and a temperature controlled column oven, coupled to an Agilent 6130 MS detector equipped with multimode interface was used. The multimode interface was operated in positive APCI mode. The following interface parameters were applied: drying gas (N_2, 20 psig) flow of 5 L/min, nebulizer pressure of 20 psig, drying gas temperature of 350°C, capillary voltage of 2000 V and corona current of 5 μA. The analytical separation was performed on a Atlantis T_3 column (150×4.6 mm, 3 μm) using the isocratic mixture of 10 mM formic acid at a flow rate of 0.3 mL/min at 25°C. The LC eluent was directed to the MS system from 10 to 16 min using MSD software. Ions monitored were m/z 72 and 55 for the quantification of acrylamide in the samples. The quantitation was based on a calibration curve in a concentration range between 0.01 and 0.1 μg/mL. Each sample was analysed in duplicate and mean values with standard deviations were reported.

3 Results and discussion

Baking is a complex process where a simultaneous heat and mass transfers occur, which have certain consequences in the final product in terms of the chemical reactions involved. A temperature gradient occurs within the product during baking. Figure 1 shows the influence of thickness on the change of temperature in different locations of a disc during baking. The temperature values were close to each other in the disc having thickness of 1 mm, reaching the oven temperature in approximately 12 min at 180°C. On

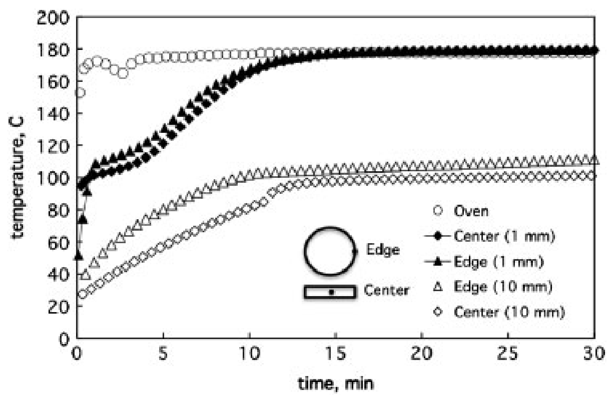

Figure 1. The effect of dough thickness on the time/temperature history of bread resembling model sample during baking at 180°C.

the other hand, the temperature did not exceed 100°C in the disc having thickness of 10 mm within 30 min due to evaporative cooling. Previous findings have successfully confirmed that acrylamide formation occurs at temperatures exceeding 100°C. This dictates the necessity of acquiring low moisture conditions in the product during baking for acrylamide formation. As shown in Fig. 2, the product thickness determines the rate of drying, hence the rate of acrylamide formation during baking. In the sample having thickness of 1 mm, acrylamide concentration increased rapidly at the onset of heating at 180°C, reaching an apparent maximum of 411 ± 49 ng/g within baking time of 8 min. Then acrylamide concentration decreased exponentially. However, no acrylamide was detected in the sample having thickness of 10 mm within 15 min of heating under the same baking conditions, because the moisture content was still above 10%. After 15 min, acrylamide concentration tended to increase linearly, reaching 217 ± 31 ng/g after 30 min at 180°C. This kind of kinetic behaviour has been previously shown during heating the binary mixtures of glucose and asparagine [20], roasting of coffee [21], frying of potato [22] and baking of wheat flour [23].

It was clear from the results that decreasing the thickness is a good approach to obtain a model system simulating the crust. The crust layer is the part of product where acrylamide formation takes place during baking. There is no doubt that the physical conditions dictate the type and extent of chemical reactions.

The temperature gradient that occurred in the cookie sample during heating resulted in a gradient of acrylamide concentration. Figure 3 illustrates the distribution of acrylamide along the vertical cross-section of the cookie sample baked at 200°C for 15 min. Acrylamide concentration was 377 ± 28 ng/g in the surface of the cookie sample (outer layer with 1 mm thickness) while the inside was free of acrylamide. Acrylamide mostly concentrated on the surface and nearby regions as a thin layer, because the conditions in terms of temperature and moisture became

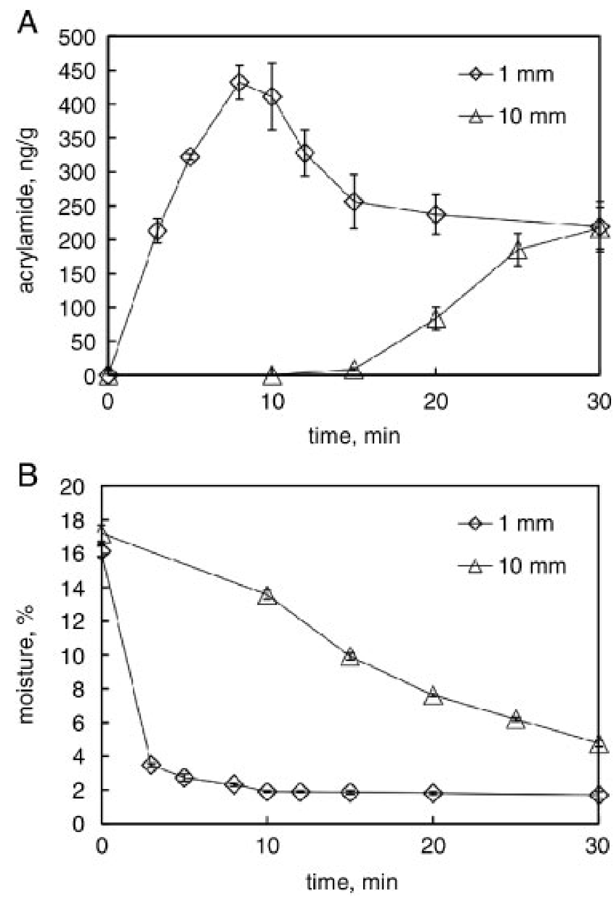

Figure 2. The effect of dough thickness on (A) acrylamide formation and (B) drying rate in bread resembling model sample during baking at 180°C

favourable only in these regions from the viewpoint of acrylamide formation. Crust temperature in combination with water content had a significant effect on acrylamide formation, higher temperatures resulting in higher acrylamide concentrations.

It has been previously reported that more than 99% of acrylamide in bread was detected in the crust [24] while no acrylamide was detected in the crumb [25]. However, some studies indicated that acrylamide is present in both shell and centre zones of breakfast cereal biscuits. The researchers measured the temperatures of centre and shell zones of the biscuit immediately on removal from the oven using a thermal camera. It was reported that centre zone temperature of the biscuit never exceeded 80°C, but acrylamide concentration in the cooler zone was 128 ng/g [26]. Some researchers studied the effect of crust temperature and water content on acrylamide formation during the baking of white bread. They measured the water content and acrylamide concentration of outer and inner crust fractions immediately after baking. The results indicated that the outer crust had a significantly lower water

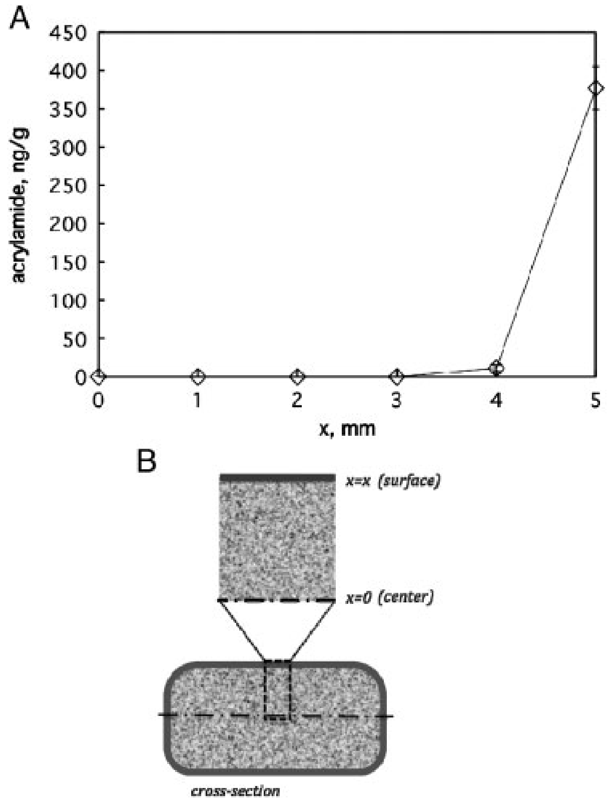

Figure 3. (A) The concentration profile of acrylamide along the vertical cross-section of a cookie sample (10 mm thickness) baked at 200°C for 15 min; (B) cross-section.

content and higher acrylamide concentration than the inner crust [27].

Testing of any mitigation strategy under the real food conditions is problematic, because both chemical and physical parameters have strong effects on the resulting acrylamide level. In order to focus only on the chemical parameters, one should limit the physical effects by controlling the dimensions of food being heated. The crust model to study parameters affecting acrylamide formation in bakery foods offered advantage with this respect, because reduced thickness in the crust model prevented the formation of temperature gradient within the product. In addition, the crust model limited the lag time required for the product temperature to reach the oven temperature.

In order to show the convenience of crust model on testing the effectiveness of mitigation strategies for bakery foods, cookie and bread crust resembling models were prepared with calcium chloride and grape seed extract, respectively. Figure 4 shows the effect of different amounts of calcium chloride on acrylamide formation in cookie resembling model system with different thicknesses. Acrylamide concentrations without calcium chloride were 128 ± 19 and 552 ± 16 ng/g for cookies having thickness of 10 and 1 mm, respectively. It was clear from the results that acrylamide concentrations of cookies were attenuated for thicker sample. This caused difficulty for the detection of acrylamide during acrylamide analysis by LC-MS. The crust model gave comparable results in terms of acrylamide mitigation by means of calcium chloride.

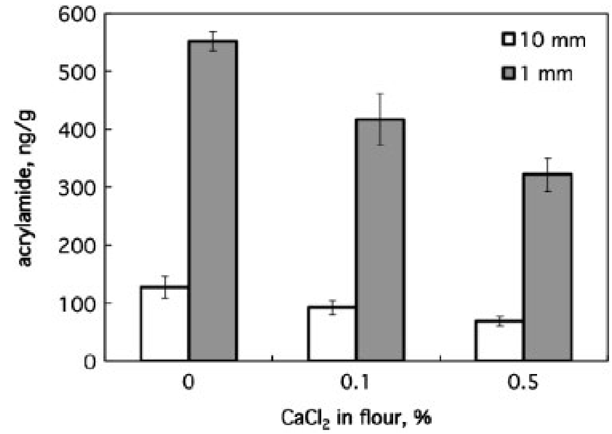

Figure 4. Effect of different amounts of calcium chloride on the formation of acrylamide in cookie resembling model systems during baking at 200°C for 15 min.

The effect of antioxidants on the formation of acrylamide was examined in bread resembling model system by the addition of different amounts of grape seed extract into wheat flour. Decreasing the thickness significantly increased the resulting acrylamide concentration in bread during baking at 180°C for 30 min. The results indicated that the addition of any concentration of grape seed extract had no effect on acrylamide formation.

4 Concluding remarks

Apart from the chemical parameters, baking temperature and product dimensions have strong influence on the rate of acrylamide formation in bakery foods. The product thickness is an important parameter that determines the rate of drying and consequently the rate of acrylamide formation during baking. Decreasing the thickness is a good approach to obtain a model system simulating the crust where the acrylamide formation takes place during heating. With this respect, the crust model was considered to be useful to test the effectiveness of any mitigation strategy. Avoiding crumb formation increases analytical accuracy and sensitivity, because the whole portion is brought to analysis.

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