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Food Chemistry 92 (2005) 693–700
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of temperature and time on the formation of acrylamide in starch-based and cereal model systems, flat breads and bread

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Received 5 April 2004; received in revised form 23 August 2004; accepted 23 August 2004

Abstract

Dry starch systems, containing varying amounts of asparagine and glucose, freeze-dried rye-based flat bread doughs, flat bread and bread, were baked at varying temperatures and times according to central composite designs. In the starch-based model system the amount of acrylamide went through a maximum when the level of asparagine increased. No such maximum was found for glucose. In the starch system, freeze-dried flat bread doughs and flat breads, the amount of acrylamide formation went through a maximum at approximately 200 °C, depending on the system and the baking time. The amount of acrylamide was reduced at long baking times. However, in bread crust, the amount of acrylamide increased with both baking time and temperature in the interval tested.

Keywords: Acrylamide; Cereal products; Asparagine; Glucose; Heating time; Temperature

1. Introduction

Because acrylamide is classified as a probable carcinogen by the International Agency for Research on Cancer (IRAC, 1994), the discovery by Swedish scientists of acrylamide in starch-rich foods (Tareke, Rydberg, Karlsson, Eriksson, & Törnqvist, 2002) has created much concern among regulating authorities, the food industry and the public. However, it has been claimed that dietary acrylamide does not constitute any risk to human health (Mucci, Dicman, Steineck, Adami, & Augustsson, 2003), but this study has been criticised because it was not designed to detect small increases in cancer incidents (Friedman, 2003).

It has been convincingly shown that acrylamide in food is mainly formed by a reaction between asparagine and reducing sugars (Becalski, Lau, Lewis, & Seaman, 2003; Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Weisshaar & Gutsche, 2002). Two mechanisms, varying in details, have been proposed (Becalski et al., 2003; Zyzak et al., 2003). It has also been suggested that acrylamide can be formed from lipid-rich foods by a reaction between ammonia and acrolein (Yasuhara, Tanaka, Hengel, & Shibamoto, 2003), and some data indicate that the type of oil used for deep frying can influence the formation of acrylamide (Becalski et al., 2003). However, evidence clearly points to the reaction between asparagine and reducing sugars as the main culprit.

Several strategies for reducing acrylamide have been proposed and are under investigation. Some results indicate that different additives, such as rosemary, amino acids or proteins, reduce the level of acrylamide (Becalski et al., 2003; Rydberg et al., 2003). Jung, Choi, and Ju (2003) found that reduction of the pH dramatically reduced the formation of acrylamide during preparation of corn chips, both by frying and baking. However, the main focus has been on heating conditions and

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doi:10.1016/j.foodchem.2004.08.030

composition of the system, but none of these studies have been done on cereal-based foods. Neither has the possible interaction between time and temperature been systematically investigated.

Initial results clearly indicated that the amount of acrylamide increased with frying and baking temperature (Tareke et al., 2002). These results were corroborated by Grob et al. (2003) and Rydberg et al. (2003) studying french fries. Similar results have also been found in model systems. Thus, Becalski et al. (2003) found acrylamide when heating amino acids and glucose at 175 °C, but not at 120 or 140 °C. Studies in aqueous model systems have indicated that the maximum amounts of acrylamide are formed approximately at the temperature used in deep frying of potato chips and french fries (Mottram et al., 2002). Similar results were also found by Becalski et al. (2003) using a dry system comprising asparagine and glucose in a 1:1 ratio. However, no similar studies in dry model systems or dry cereal-based food products have been reported.

In the first report on acrylamide in food, Tareke et al. (2002), showed that increased heating time for mashed potatoes in a microwave oven increased the amount of acrylamide. Also, in french fries, a similar increase has been reported (Haase, Matthaus, & Vosmann, 2003; Rydberg et al., 2003), and Becalski et al. (2003) reported that the level of acrylamide in potato chips increased with increasing frying time. However, in model systems heated for 10–30 min, they found a reduction in acrylamide with time except for the lowest temperature where the amount of acrylamide went through a maximum after 20 min. Similarly, Stadler et al. (2002) found a decrease in acrylamide with time when pyrolysing a mixture of asparagine and glucose, and Rydberg et al. (2003) found a maximum, after approximately 20 min, when heating potato strips at 200 °C. In their study on french fries, Grob et al. (2003) concluded that the most important measure for minimising the formation of acrylamide was to determine the right end-point, that is not to overcook the fries.

Becalski et al. (2003) found the highest amount of acrylamide in a mixture of asparagine and glucose in the molar ratio 0.5–1.0, indicating that the composition of the system influences how much acrylamide is formed. Amrein et al. (2003) studied the potential for acrylamide formation in 74 potato samples from 17 cultivars grown at various locations during 2002. The variability in acrylamide potential correlated with the product of the concentration of asparagine and reducing sugars. The differences between samples and cultivars were primarily related to glucose and fructose. Fructose was found to be twice as effective as glucose in promoting acrylamide formation. Similar results were obtained by Biedermann-Brem et al. (2003) and Chuda et al. (2003). Grob et al. (2003) found the amount of reducing sugars in the raw potato to be the determining variable for acrylamide formation, underlining the importance of choosing the right cultivar and storing it at appropriate temperatures.

The aim of the present study was to determine the effects of time and temperature on the formation of acrylamide in starch gels and cereal food products, and whether or not the effect of different levels of asparagine and glucose influenced the dependence on time and temperature.

2. Materials and methods

2.1. Experimental design and data analysis

All experiments were done according to central composite designs (Cochran & Cox, 1971) with three repetitions of the centre point in order to estimate the experimental error. This design makes it possible to calculate response surface equations of the type shown in Eq. (1).

$$\text{Response} = c_1A + c_2B + c_3C + \cdots + c_nA^2 + c_{n+1}B^2 + \cdots + c_mAB + c_{m+1}AC + \cdots + c_zBC + \cdots, \quad (1)$$

where $A, B, C, \ldots$ are the variables and $c_1, c_2, \ldots$ are the coefficients.

The independent variables were asparagine, glucose (only for the starch model system), baking time and baking temperature. The designs and data analyses were done by the Minitab statistical software, version 14.1 (Minitab Inc. State College, PA 16801, USA).

2.2. Starch model system

Wheat starch (10.00 g) was gelatinised in water (100 ml) by heating with stirring at 100 °C for 3.5 min. Asparagine and glucose (100–3000 μg/g starch) were added as solutions, and the mixture stirred for an additional 30 s. The slurry was poured into aluminium forms, $25 \times 8 \times 2$ cm, allowed to set at room temperature and freeze-dried. The samples was baked in an electrically heated stone baking oven (Sveba, AB Svenska Bakungsfabriken, S-51300 Fristad, Sweden) for 5–30 min at 120–260 °C (Table 1), and kept frozen until they were analysed for acrylamide. The set point of the oven thermostat was continuously checked by a thermometer.

2.3. Dry cereal model system and flat breads

Doughs contained wholemeal rye flour, (53%) water (46%) and salt (0.9%). The doughs were rolled to an even thickness of 0.6 mm using a commercial rolling machine. The doughs were cut in half, one half-baked immediately and the other freeze-dried prior to baking

Table 1
Processing conditions, additions and measured acrylamide contents (mg/kg) in the starchy model system

Sample no.	Temperature (°C)	Time (min)	Added Glc (μg/g starch)	Added Asn (μg/g starch)	Acrylamide
S1	260	17.5	1550	1550	150
S2	225	23.8	2275	825	960
S3	225	23.8	2275	2275	1570
S4	225	23.8	825	825	450
S5	225	23.8	825	2275	1150
S6	225	11.2	2275	2275	3620
S7	225	11.2	2275	825	4260
S8	225	11.2	825	2275	5230
S9	225	11.2	825	825	2070
S10	190	30.0	1550	1550	6780
S11	190	17.5	3000	1550	15550
S12	190	17.5	1550	1550	7390
S13	190	17.5	1550	1550	8670
S14	190	17.5	1550	1550	10900
S15	190	17.5	1550	100	450
S16	190	17.5	1550	3000	12140
S17	190	17.5	100	1550	6050
S18	190	5.0	1550	1550	12020
S19	155	23.8	2275	2275	6260
S20	155	23.8	2275	825	2600
S21	155	23.8	825	2275	3570
S22	155	23.8	825	825	1740
S23	155	11.2	2275	2275	5250
S24	155	11.2	2275	825	1500
S25	155	11.2	825	2275	4030
S26	155	11.2	825	825	1110
S27	120	17.5	1550	1550	310

Table 2
Processing conditions and measured acrylamide contents (mg/kg) in flat bread products with (FF) and without (F), a freeze drying step of the dough before baking

Sample no.	Temperature (°C)	Time (min)	Acrylamide (FF)	Acrylamide (F)
FF1 and F1	120	17.5	50	30
FF2 and F2	140	8.7	130	30
FF3 and F3	140	26.3	1160	560
FF4 and F4	190	5.0	3220	<20
FF5 and F5	190	17.5	3800	1790
FF6 and F6	190	17.5	3870	1480
FF7 and F7	190	17.5	3370	2060
FF8 and F8	190	30.0	3690	1780
FF9 and F9	239	8.7	3090	2620
FF10 and F10	239	26.3	40	50
FF11 and F11	260	17.5	<20	<20

using the same temperature and time ranges as for the starch model system (Table 2). After baking, the samples were kept frozen until they were analysed for acrylamide.

2.4. Breads

Breads were made from white wheat flour (31.6%) wholemeal rye flour (31.6%) water (34.2%) salt (0.7%) dry yeast (0.3%) and fat (1.5%) using commercial baking equipment. Hearth breads were baked in a fan oven (Revent, Revent International AB, S-19427 Upplands Vås-

Table 3
Processing conditions and measured acrylamide contents (mg/kg) in bread crusts

Sample no.	Temperature (°C)	Time (min)	Acrylamide
B1	180	30.0	100
B2	195	19.4	<20
B3	195	40.6	<20
B4	230	15.0	<20
B5	230	30.0	200
B6	230	30.0	160
B7	230	30.0	140
B8	230	45.0	330
B9	265	19.4	230
B10	265	40.6	340
B11	280	30.0	340

by, Sweden) at 180–280 °C for 15–45 min (Table 3). Slices of approximately 150 g were cut from the centres of three breads, the crumb and crust separated manually; the crusts were frozen and later analysed for acrylamide.

2.5. Analysis of acrylamide

The analysis of acrylamide was done by the Norwegian Air Research Institute (NILU), by a method similar to that of Rosen and Hellenas (2002), using high resolution time of flight mass spectrometry instead of tandem mass spectrometry.

The samples (3 g) were homogenised in water (30 ml) containing d3-acrylamide (100 μg/l) as an internal standard. Acrylamide was extracted by sonication for 30 min. The extract was purified by adding 500 μl of Carrez I and Carrez II reagents, respectively. The precipitates were removed by centrifugation at 4000 rpm for 10 min and the supernatant (3 ml) was filtered through SPE columns, Isolute Multimode, 300 mg (IST, Hengoed, UK), pre-treated with acetonitrile (1 ml) and water (2×2 ml). The first portion (1 ml) was discarded and the remaining portion was collected and passed through a 0.22-μm syringe filter Millex-GS (Millipore, Bedford, USA). 500 μl of the filtrate were passed through a Microcon YM-3 centrifuge spin filter (Millipore, Bedford, USA) (13,000 rpm, 10–20 min) until a sufficient volume for injection into the chromatographic system had been obtained. Acrylamide was separated from the sample matrix by a high performance liquid chromatography system (Agilent HP-1100) system equipped with a Waters Atlantis pre-column in front of the analytical column (3.9 mm × 20 mm, 3 μm, No. 186001313, and 3.9×150 mm, 3 μm, No. 186001317, respectively). The injection volume was 100 μl and the mobile phase was 100% water at a flow rate of 0.8 ml/min up to 6 min with subsequent column flushing (100% acetonitrile). The detector was a Micromass LCT orthogonal Time-Of-Flight (TOF) mass spectrometer equipped with a Z-spray ion source operated in the atmospheric pressure chemical ionisation positive mode APCI (+). The cone voltage was 15 V and the monitoring ions were *m*/*z* 72 and *m*/*z* 75 for acrylamide and the internal standard, respectively, with a signal peak width typically 30 mDA. Limit of detection (signal-to-noise ratio: 3) depends on instrument tuning and ion source contamination and corresponds, typically, to 10–30 μg/kg acrylamide in the sample.

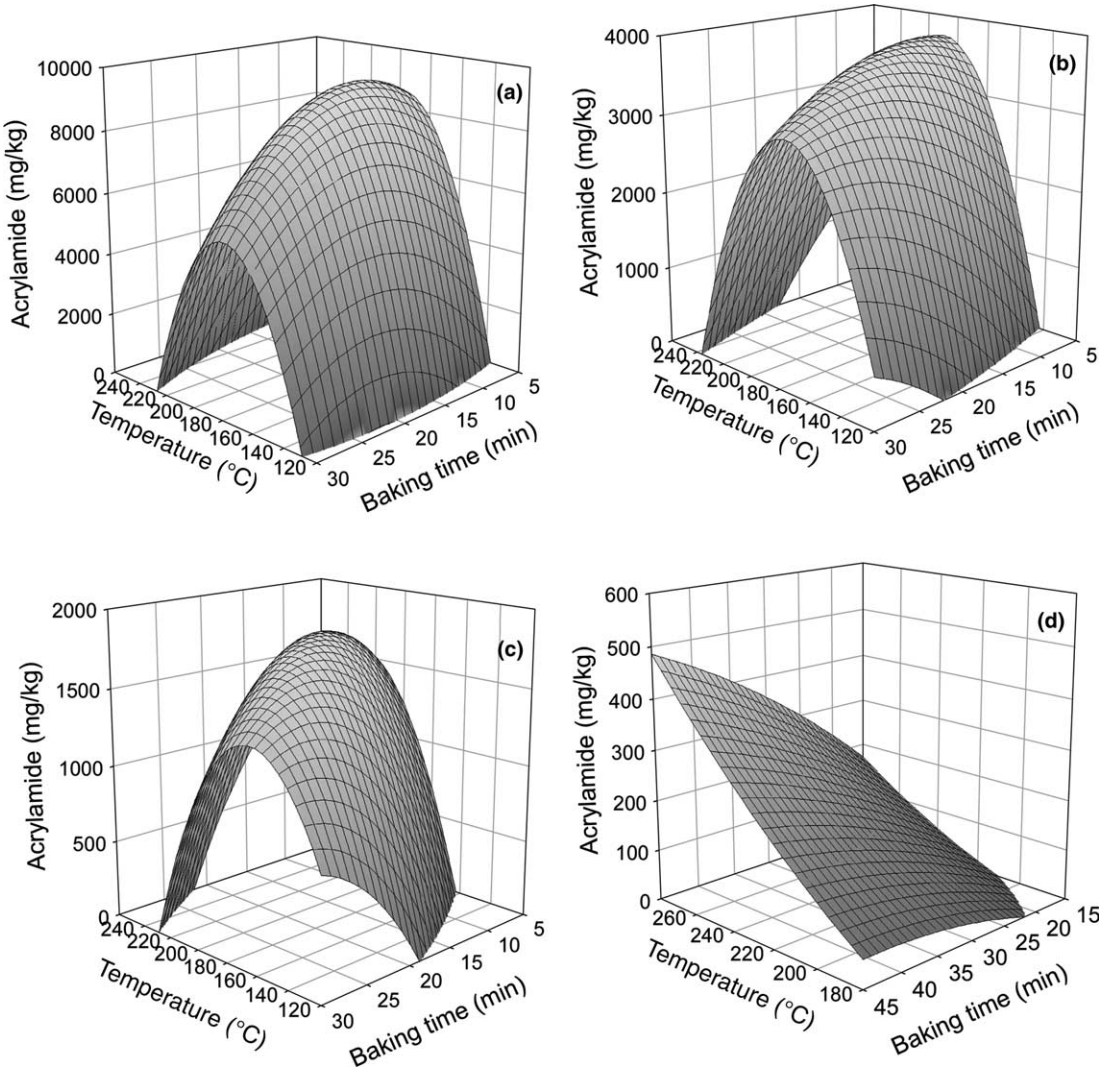

Fig. 1. Response surface plots of acrylamide versus time and temperature for the centre point of the starchy model (a), freeze-dried flat breads (b), flat breads (c), and bread crusts (d).

3. Results

The actual amounts of acrylamide found in the samples are shown in Tables 1–3. The highest content was found in the starch system and the least in bread crusts. More acrylamide was formed in the freeze-dried flat breads than in the flat breads (Tables 1–3 and Fig. 1).

The colour of the baked starch gels varied from nearly white at a baking temperature of 120 °C to almost black at high temperatures and/or long baking times. In the freeze-dried flat breads and flat breads the colour varied from light brown at low temperatures to almost black at high temperature and long baking time. The bread crusts, varying between 16.9% and 22.4%, averaging 19.7% of the slices analysed, ranged from light and thin to dark and thick, corresponding to the amount of acrylamide.

The variability between the centre points for the starch model system was large (Table 1, samples S12–S14). However, the variability between the centre points in the three other systems was considerably smaller (Table 2, samples FF5–FF7, F5–F7 and Table 3, samples B5–B7).

The response surface coefficients and *P*-values are given in Table 4. Only in the case of bread crusts were the linear terms in time and temperature significant ($P < 0.05$). Thus, the amount of acrylamide in bread crusts increased with time and temperature (Fig. 1(d)).

For the two dry systems (starch gels, freeze-dried flat breads and conventional baked flat breads), the quadratic term in temperature was significant or nearly so, indicating that the amount of acrylamide goes through a maximum at around 190–210 °C as the temperature increases (Fig. 1(a)–(c)). In the case of freeze-dried flat breads the term temperature × time was significant, showing that the temperature which gives the most acrylamide depends on the baking time, as can also be seen from Fig. 1(b).

In the starch system, the linear term in asparagine was significant ($P < 0.05$) and the quadratic term nearly so ($P < 0.1$), indicating that the amount of acrylamide goes through a maximum as the amount of asparagine increases. No such effects were found for glucose.

The percent dry matter in the flat breads varied between 88.0 (190 °C, 5 min) and 99.6 (190 °C, 17.5 min). A general positive relationship between acrylamide and dry matter was found (Fig. 2). The two samples with high dry matter and little or no acrylamide were both baked at high temperature to a very dark colour.

4. Discussion

In our experiments, commercial baking ovens were used, since this is close to the real life situation. The ovens had less precise temperature control than ovens used in other investigations, i.e., gas chromatography ovens (Rydberg et al., 2003), and some variation in the temperature was to be expected. Consequently, some variation in the amount of acrylamide formed in the centre points was also expected, making it more difficult to find significant effects. On the other hand, all significant effects found are probably relevant in a real-life production situation.

In the starch model, the freeze-dried flat breads and the flat breads, the amount of acrylamide went through a maximum as the temperature increased but, in the bread crusts, the amount increased with increasing temperature (Fig. 1). In the starch system, the effect of time and temperature was the same, irrespective of the amount of asparagine and glucose present (Fig. 3).

Table 4
Coefficients for the response surface equations and *P*-values (Eq. (1)) for the four experimental systems

	Starch system		Freeze-dried flat breads		Flat breads		Bread crusts	
	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*	Coefficient	*P*
Constant	−109183	0.001	−34915.6	0.000	−18235.2	0.015	222.134	0.013
Temp	981.88	0.673	353.34	0.393	158.709	0.424	−4.19808	0.009
Time	1079.13	0.262	545.324	0.512	501.335	0.858	−2.95919	0.045
Glc	3.96379	0.143	n.a.		n.a.		n.a.	
Asn	14.7133	0.033	n.a.		n.a.		n.a.	
Temp × Temp	−2.31029	0.002	−0.810714	0.001	−0.322534	0.076	0.0113333	0.679
Time × Time	−13.7627	0.472	−3.472	0.384	−4.51467	0.367	−0.118519	0.697
Glc × Glc	−0.00035692	0.800	n.a.		n.a.		n.a.	
Asn × Asn	−0.0024996	0.094	n.a.		n.a.		n.a.	
Temp × Time	−3.80857	0.338	−2.33143	0.030	−1.77143	0.124	0.0733333	0.505
Temp × Glc	−0.00899015	0.789	n.a.		n.a.		n.a.	
Temp × Asn	−0.0205172	0.545	n.a.		n.a.		n.a.	
Time × Glc	0.0315862	0.867	n.a.		n.a.		n.a.	
Time × Asn	−0.0329655	0.861	n.a.		n.a.		n.a.	
Glc × Glc	−0.00014625	0.928	n.a.		n.a.		n.a.	

n.a., not applicable.

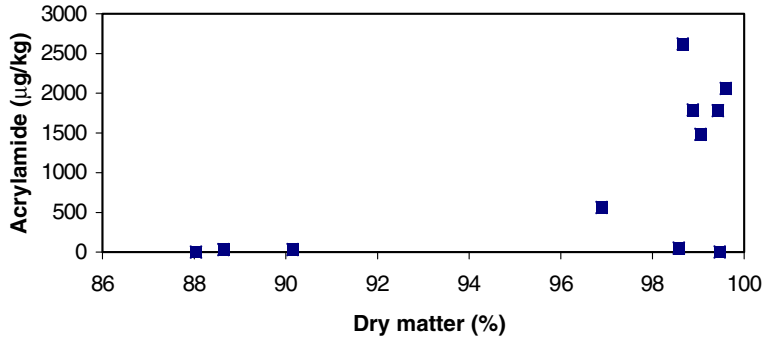

Fig. 2. Correlation between acrylamide and dry matter in flat breads.

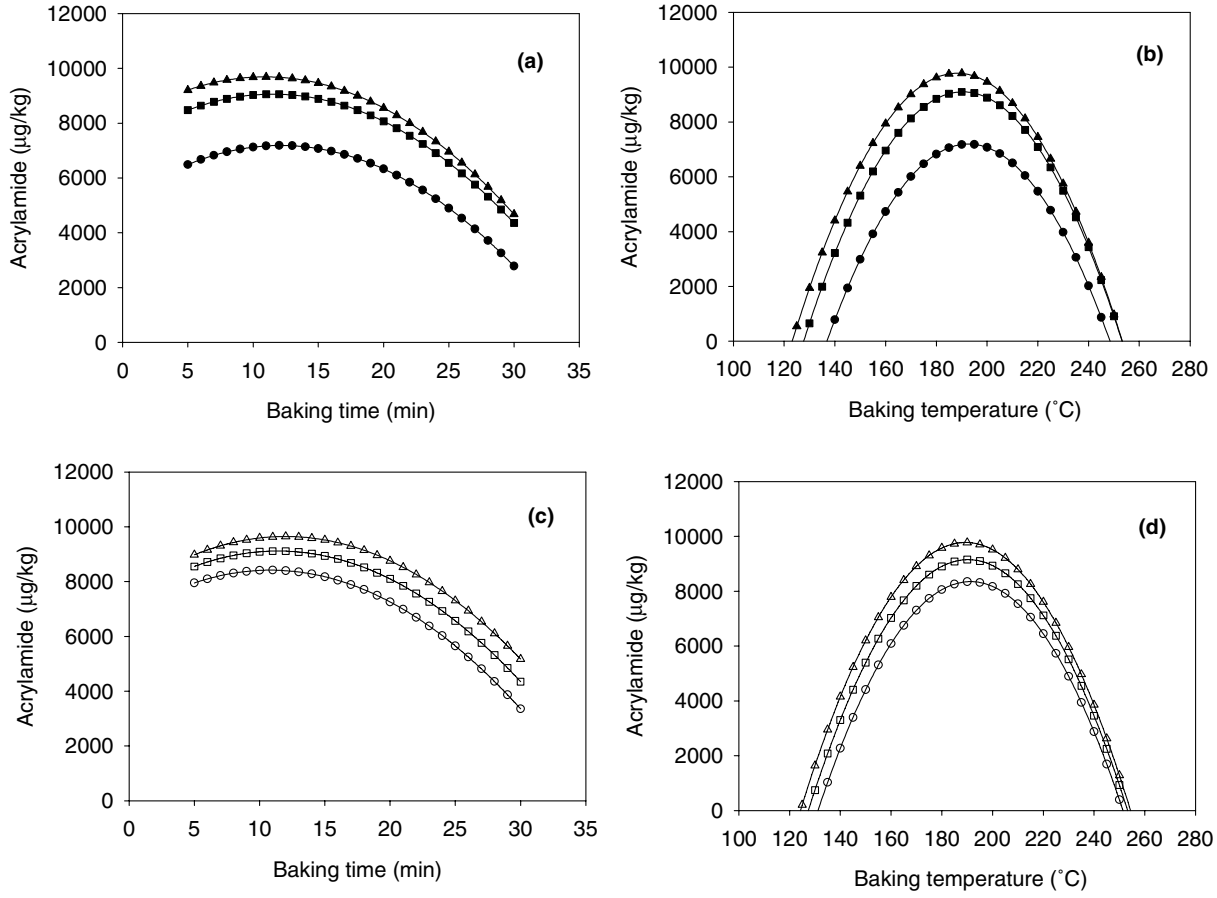

Fig. 3. The effects of time and temperature at varying levels of asparagine and glucose, calculated from the response surface equations: effect of time (a) and temperature (b) at different levels of asparagine (glucose = 1550 µg/g starch; ●, asparagine 1000 mg/g starch; ■, asparagine 1500 mg/g starch; ▲, asparagine 2000 mg/g starch). Effect of time (c) and temperature (d) at different levels of glucose (asparagine = 1550 µg/g starch; ○, glucose 1000 mg/g starch; □, glucose 1500 mg/g starch; Δ, glucose 2000 mg/g starch).

Thus, the effects of time and temperature are not influenced by the levels of reactants, as expected.

Becalski et al. (2003) found that, in dry systems, the amount of acrylamide was reduced when the temperature was increased from 155 to 185 °C, at least when heating for more than 10 min. Almost the exact same dependence on temperature was found by Mottram et al. (2002) in aqueous solutions, showing that the presence of water does not hinder the formation of acrylamide. On the other hand, several investigations have found an increase in acrylamide with increasing temperature in products such as hamburgers and french fries (Grob et al., 2003; Rydberg et al., 2003; Tareke et al., 2002). This might be explained by water evaporation keeping the effective temperature down when water is still present. This also explains why the amount of acryl-

amide in flat breads increased only when the moisture content was below approximately 4% (Fig. 2) and why the amount of acrylamide was lower in flat breads than in the starch system.

Prolonged heating tended to reduce the amount of acrylamide in the dry systems but not in bread crusts (Fig. 1). Similar dependence on time has been reported in several other systems (Becalski et al., 2003; Grob et al., 2003; Rydberg et al., 2003; Stadler et al., 2002; Tareke et al., 2002; Yasuhara et al., 2003). This clearly indicates that acrylamide reacts further and/or is eliminated through evaporation, as was also concluded by Rydberg et al. (2003). However, at low temperatures, the decrease in acrylamide with baking time did not occur even in the dry systems, in agreement with the results of Becalski et al. (2003). This strengthens the possibility that the differences between dry systems and systems containing residual water are due to evaporation decreasing the effective temperature. Furthermore, the elimination of acrylamide is implied to be slower than the formation at low temperature but faster at higher temperatures.

The level of reducing sugars is more important than the level of asparagine for the formation of acrylamide in french fries and potato chips (Amrein et al., 2003; Grob et al., 2003). However, in cereal systems, the opposite seems to be true. In a bread model system, addition of asparagine dramatically increased the amount of acrylamide, but no effect of glucose addition was found. Similarly, addition of asparaginase gave a large reduction while no effect of glucose oxidase was found (Knutsen, Bråthen, & Syversen, 2003). Thus, it was of interest to study the effect of different levels of glucose and asparagine on the formation of acrylamide.

The amount of acrylamide increased with increasing concentration of asparagine at low concentrations, but at higher concentrations a decrease was observed. Becalski et al. (2003) found that the amount of acrylamide varied with the molar ratio between asparagine and glucose. However, they did not state whether they varied the amount of glucose, asparagine or both. Provided glucose was kept constant and asparagine varied our result was in agreement with theirs.

Our findings indicate that one of the acrylamide elimination mechanisms might be reaction with amino acids. This is in agreement with the results of Rydberg et al. (2003), showing that addition of amino acids or protein-rich ingredients reduced the amount of acrylamide. No similar decrease at high concentrations was found for glucose, indicating that reaction with carbohydrates is insignificant in the removal of acrylamide. Thus, the reason for the low level of acrylamide found in protein-rich foodstuffs (Tareke et al., 2002) might be a more effective removal rather than less formation.

5. Conclusion

The formation of acrylamide showed a strong dependence on temperature. In dry systems, a maximum in the level of acrylamide is found at approximately 190–210 °C, depending on the system. The composition of the system does not influence the time–temperature dependence of the acrylamide level. In systems containing residual water, the formation of acrylamide is reduced because evaporation reduces the effective temperature, even in putatively dry areas of the product, i.e., the crust. After formation, acrylamide is eliminated by further reaction, probably with amino acids or protein, and/or evaporation. The presence of carbohydrates seems to be of less importance in this respect. In starch-based and cereal systems, asparagine is more important than reducing sugars for the formation of acrylamide.

Acknowledgements

This work was supported by the Norwegian Research Council (grant no 155839/130).

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