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Factors Influencing Acrylamide Content and Color in Rye Crisp Bread

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An industrial baking procedure for yeast-leavened whole-grain rye crisp bread was adapted to local laboratory conditions to study the effect of time and temperature of baking and the addition of fructose, asparagine, and oat-bran concentrate on the acrylamide content and color of the bread. Baking time and temperature affected acrylamide content that increased from 10 to 30 μg/kg of bread at the combination of a long time and high temperature, with a significant interaction between the two factors ($p < 0.008$). Added asparagine had a significant effect ($p < 0.001$) on the formation of acrylamide, but fructose did not. There was a correlation between acrylamide content and color of the milled bread in the time−temperature experiment, but this correlation was not observed in the experiment with added precursors. Added oat-bran concentrate with high content of mixed-linkage β-glucan did not influence the acrylamide content in the breads.

KEYWORDS: Acrylamide; asparagine; baking; color; rye crisp bread; fructose; oat-bran concentrate; time; temperature

INTRODUCTION

The presence of acrylamide (AA) in foods has caught a worldwide attention since it was announced by a group of Swedish researchers in 2002 (*1*). AA is known to be a neurotoxin, a carcinogen in animals, and a probable carcinogen to humans as defined by IARC (*2*, *3*). It has been reported in numerous studies that AA is formed in many heat-treated foods, e.g., potato products, different cereal-based products, and coffee. The chemistry, biochemistry, analytical methods, occurrence, metabolism, and toxicology of AA were described in recent reviews (*4*−*7*).

The formation of AA in foods takes place during Maillard reactions involving free asparagine (ASN) and reducing sugars under high temperature (*8*, *9*). It was confirmed that ASN is the limiting factor for the formation of AA in yeast-leavened wheat bread and ginger bread (*10*, *11*). However, added fructose did not show any effect. Studies in model systems suggested that the Maillard pathway for the generation of flavor and color under thermal processing conditions might be linked to the formation of AA (*9*, *12*−*14*). Similarly, Pedreschi et al. (*15*) found a linear correlation between the AA content of potato chips and color. However, in the study by Surdyk et al. (*10*), the addition of ASN increased the content of AA but did not affect the color. The surface color of the bread is controlled by many factors including water content, pH, reducing sugars, amino acids, temperature, air speed, relative humidity, and modes of heat transfer during baking (*16*). The Swedish National Food Administration has reported the average daily intake of AA as 35 μg corresponding to 0.5 μg of AA/kg of body weight and the mean contribution of AA from soft and crisp bread as 6 and 11%, respectively (*17*). The concentration of AA in soft and crisp breads ranged between <30−160 and <30−1900 μg/kg, respectively.

Nutrition recommendations advise increased consumption of whole-grain products. In many European countries, rye is especially important and is consumed as soft or crisp breads, which contain up to 15 g of fiber/100 g. Rye contribution to dietary fiber intake may reach up to 40% (in Finland), which makes it an important component in the diet (*18*). Nutritional importance is not the only factor that governs the preference of consumers to bread, along with it come, e.g., texture, flavor, and color.

In a previous study on yeast-leavened wheat bread, Surdyk et al. (*10*) found a strong correlation between AA content and color with varying heat treatments. However, although ASN was the limiting factor for the increase of AA, it did not influence color significantly. The aim of this study was to investigate, using designed experiments, the effect of time and temperature and the addition of AA precursors and oat-bran concentrate (OBC) on AA content and color in whole-grain rye crisp bread.

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MATERIAL AND METHODS

Baking Procedure. The recipe of baking the rye crisp bread was obtained from Wasabröd AB (Filipstad, Sweden) and was modified for its applicability to laboratory conditions. Whole-grain rye flour (500 g, containing ca. 1 g of asparagine/kg) (*19*) and about 4 g of reducing sugars/kg flour, sodium chloride solution (60 mL, 10% weight/volume), fresh yeast solution (100 mL of 15%, weight/volume Original kronjäst, Jästbolaget, Sweden), and tap water (350 mL) were all mixed in a kitchen dough maker (Electolux Assistant, Sweden) for 10 min. The dough was then transferred to a leavening cupboard and allowed to ferment at 30 °C and 85% relative humidity for 90 min. After this fermentation, the dough was kneaded and 90 g of the dough was spread out with a roller pin on a silicone baking sheet (Silpat, Åsö AB, Åtvidaberg, Sweden) placed within a square-shaped form (1.5 mm height) for controlling the thickness of the bread. A circular form (170 mm in diameter) was used to shape the bread, and a spiked pattern rolling pin was used to give the crisp bread its characteristic pattern. This bread was taken for a second fermentation in the same leavening cupboard for 50 min. Bread was baked at 250 °C for 8 min (standard procedure), except for the time−temperature experiment, in a rotating laboratory oven (Simon, Greenfield, U.K.). After baking, the bread was dried in an upright position for 25 min at a temperature of 105 °C.

Effect of Time and Temperature. A randomized circumscribed central composite (CCC) design of 11 experiments was made including the central point repeated 3 times. From each dough, three replicates of bread were baked then, analyzed separately, and averaged before statistical evaluation. The levels for the baking time and temperature were 3−15 min and 207−282.5 °C, respectively.

Effect of Asparagine and Fructose. A CCC design of 10 experiments was made including two central points. The levels for this design were 0.88−6.16 g/kg of ASN (Merck, Darmstadt, Germany) and 0.7−5.1 g/kg of fructose (Merck, Darmstadt, Germany). The design was duplicated, and the resulting experiments were run in a randomized order. Results were evaluated by regression analysis with a total of 19 degrees of freedom.

Effect of OBC and Lichenase. A duplicated randomized full factorial design of 5 experiments including one central point was used in this study. The range of the added OBC (CreaNutrition, Sweden) was 0−10% per 500 g of flour, and that of the lichenase (endo-1,3-(4)-β-D-glucanase) (*Bacillus* sp.) EC 3.2.1.73, (1000 units/mL, Megazyme, Co. Wicklow, Ireland) was 0−200 μL/500 g of flour. Results were evaluated by regression analysis with a total of 9 degrees of freedom.

Analysis of Physical Parameters of the Bread. After the bread had been baked and dried, fresh weight was obtained. The thickness of the bread was measured using a vernier caliper at five different positions at the circumference of the bread, selected randomly. The edge of the bread was placed all of the way through the knob of the caliper (5 cm) to obtain the thickness, which was then averaged. Bread was milled using an ultracentrifuge mill type ZM1 with a 0.5 mm ring sieve (Retsch, Hann, Germany). Dry matter of the milled bread was obtained by drying the samples for 16 h at a temperature of 105 °C.

Color was measured with a Chroma Meter (Minolta, Milton Keynes, England) in the L^*, a^*, and b^* modes, which provides uniform color difference in relation to visual differences; L^*, a^*, and b^* are chromaticity coordinates, where L^* = the lightness of the color, positive a^* = red, negative a^* = green, positive b^* = yellow, and negative b^* = blue. Because the surface of the crisp bread is uniform in neither color nor shape, color measurements were obtained after milling the whole piece of bread. The milled material was mixed by tumbling to avoid particle separation, and the optical probe was placed over the sample in a central position. Results are reported as an average of 3 measurements.

Analysis of AA Content. AA was analyzed by liquid chromatography−tandem mass spectrometry (LC−MS/MS) as described before (*19*−*21*). Briefly, dried and milled samples were extracted with water, and deuterium-labeled AA (obtained from Polymer Source Inc., Dorval, Quebec, Canada) was added as an internal standard. AA was purified and concentrated using two types of solid-phase extraction columns (Isolute Multimode at 1 g and ENV+ also at 1 g, both purchased from IST, Hengoed, Mid Glamorgan, U.K.). The extract was then analyzed twice using LC−MS/MS with electrospray ionization. The method was further developed to be able to quantify down to 2 μg/kg (solid matrixes) and 0.5 μg/kg (liquid matrixes). Validation data in the interval 5−1000 μg/kg using spiked samples of mashed raw potatoes to represent solid matrixes were excellent with a relative standard deviation from 2 to 9% and a bias of ±3%. The laboratory has also participated in several proficiency tests for acrylamide in food. The majority of the samples (n = 9) representing cereals or bread contained AA in the range of 4.9−711 μg/kg. The obtained z scores ranged from −0.55 to 0.60 (the limits for acceptable results of z scores are ±2.0.), indicating a relevant working range and applicability of the method for the present work. Results are reported on fresh weight basis.

Statistics. All experiments were designed and evaluated by regression analysis using Minitab software (Minitab Inc., State Collage, PA).

Table 1. Response Surface Design of Experiments with Rye Crisp Bread Baked at Different Oven Times and Temperatures[a]

time (min)	temperature (°C)	height (mm)	L^*	a^*	b^*	acrylamide (μg/kg of bread)
3	245	7.2	68.2	4.6	16.7	8.4
5	220	6.4	69.0	4.5	17.1	8.5
5	270	5.5	68.9	4.6	17.4	10.2
9	207.5	4.8	66.2	5.1	17.9	6.5
9	245	5.4	69.1	4.8	18.2	9.2
9	245	4.5	67.8	4.9	18.3	7.7
9	245	4.8	68.3	4.8	18.5	6.9
9	282.5	5.1	66.6	6.0	20.9	15.4
13	220	4.7	70.3	4.6	18.5	9.3
13	270	5.0	65.7	6.6	22.2	30.7
15	245	5.8	68.2	5.9	22.2	20.0

[a] Height, color (L^*, a^*, and b^* values), and acrylamide levels are presented.

RESULTS

Yeast-fermented rye crisp bread made at our laboratory standard conditions (8 min and 250 °C for baking) had a dry matter of 93% (after the drying step) and a low content of AA with a mean value of about 10 μg/kg. The recipe used was provided by a major producer of rye crisp bread in Sweden. The optimized procedure provided breads with similar characteristics to a commercial product.

Effect of Baking Time and Temperature. A CCC design was chosen to test the effect of baking time and temperature on AA content and color of the bread. This design was used to avoid the extreme combinations of times and temperatures during baking. A prior baking experiment was made to determine the upper and lower baking levels given bread that was neither burnt nor too under-baked. The lower and upper levels in the design were 220 °C for 4 min and 270 °C for 13 min, respectively. The height range of these breads was 4.5−7.2 mm (**Table 1**). Time, as well as its interaction with temperature, had significant effects on the height of the bread ($p < 0.01$) (**Table 2**).

In the color analysis, the degree of lightness (L^* value) varied between 66 and 70 (**Table 1**), and none of the factors had a significant effect on the lightness of the color (**Table 2**). The degrees of redness (a^* value) and yellowness (b^* value) were significantly affected by both time and temperature of baking. There was also an interaction indicating that the increase in color was accentuated by the combined effect of time and temperature. The level of AA in the breads ranged between 6.5 μg/kg at the mildest and 30.7 μg/kg at the most severe baking conditions. Both time and temperature and their interaction significantly affected the AA content of the bread.

Effect of Asparagine and Fructose. In this study, an experiment was designed to test the effect of added asparagine

Table 2. *P* Values for the Effect of Time and Temperature of Baking and Their Interaction on Acrylamide Content from Response Surface Regression

factors	height	*L**	*a**	*b**	acrylamide
p value					
time	0.009	0.658	0.006	<0.001	<0.001
temperature	0.878	0.346	0.011	0.002	0.001
time × time	NI[a]	NI	NI	0.001	0.051
temperature × temperature	NI	NI	NI	0.002	0.056
time × temperature	0.002	NI	0.032	<0.001	0.008
R^2 [b] (%)	76.2	0.0	75.6	93.5	95.8

[a] NI = not included; nonsignificant squares and interaction factors had been excluded from the model. [b] Explained variances by the model are given as R^2.

Table 3. Response Surface Design of Experiments with Crisp Bread Baked for 8 min at 250 °C with Different Amounts of Added Free Asparagine and Fructose to 500 g of Flour[a]

ASN (g/500 g of flour)	fructose (g)	height (mm)	*L**	*a**	*b**	acrylamide (μg/kg of bread)
0.44	1.45	4.4	66.4	5.7	19.4	31
0.88	0.72	4.8	65.7	5.9	20.1	70
0.88	2.18	4.2	68.4	5.4	19.2	84
1.76	0.35	4.5	66.6	5.8	19.8	208
1.76	1.45	4.2	66.6	5.9	20.0	203
1.76	1.45	4.3	63.4	6.7	20.9	152
1.76	1.45	4.2	69.2	5.0	19.7	179
1.76	1.45	4.2	66.7	5.9	19.6	222
1.76	2.55	4.9	65.9	5.9	20.2	97
2.64	0.72	4.4	68.1	5.4	19.3	273
2.64	2.18	4.5	66.7	5.9	20.0	329
3.08	1.45	4.5	67.6	5.5	19.5	349

[a] Bread height, color (*L**, *a**, and *b** values), and acrylamide levels are presented.

Table 4. *P* Values for the Effect of Addition of Free ASN and Fructose and Their Interaction on AA Content from Response Surface Regression

factors	height	*L**	*a**	*b**	acrylamide
p value					
fructose	0.836	0.933	0.871	0.518	0.357
ASN	0.945	0.460	0.696	0.877	<0.001
fructose × fructose	0.005	NI[a]	NI	NI	NI
ASN × ASN	NI	NI	NI	0.012	NI
fructose × ASN	0.008	NI	NI	0.004	NI
R^2 [b] (%)	45.9	0.0	0.0	45.9	92.6

[a] NI = not included; nonsignificant squares and interaction factors had been excluded from the model. [b] Explained variances by the model are given as R^2.

(ASN) and fructose on the content of AA in rye crisp bread. Heights of the resulting breads (**Table 3**) did not vary much but were significantly affected by fructose and the interaction between fructose and ASN (**Table 4**). The variation observed in color was relatively small, although the degree of yellowness could be partly explained by the model.

The AA content of the bread ranged between 31 μg/kg for the lowest and 349 μg/kg for the highest amount of added ASN, which ranged from 0.44 to 3.08 g/500 g of flour (**Table 3**). The model, explaining 92.6% of the variation, showed that added ASN but not fructose significantly affected AA content in the bread (**Table 4**).

Effect of OBC. OBC was used for its documented water-binding capacity, and the enzyme lichenase was used to selectively degrade β-glucan into fragments that were expected

Table 5. *P* Values for the Effect of Addition of OBC and Lichenase Enzyme and Their Interaction on AA Content from Full Factorial Design

factors	height	*L**	*a**	*b**	acrylamide
p value					
OBC	0.020	0.188	0.222	0.133	0.892
enzyme	0.033	0.995	0.743	0.181	0.685
OBC × enzyme	NI[a]	NI	NI	NI	NI
R^2 [b] (%)	68.5	0.0	0.0	22.5	0.0

[a] NI = not included; nonsignificant squares and interaction factors had been excluded from the model. [b] Explained variances by the model are given as R^2.

Table 6. Factorial Design of Experiment with Rye Crisp Bread Baked (8 min at 250 °C) with Different Concentration of OBC and Lichenase in Flour Mix[a]

OBC (%)	lichinase (μL)	height (mm)	*L**	*a**	*b**	acrylamide (μg/kg of bread)
0	0	4.6	65.6	6.1	19.9	7.7
0	200	4.9	64.2	6.4	20.3	6.6
5	100	4.9	66.2	6.0	20.1	7.9
10	0	4.2	66.0	6.0	20.3	8.2
10	200	4.5	67.4	5.8	20.7	7.6

[a] Bread height, color (*L**, *a**, and *b** values), and acrylamide levels are presented.

to influence viscosity and water-binding capacity (*22*). Thus, using lichenase would enable us to separate the effect of β-glucan from the effect of other components of OBC. Both OBC and lichenase significantly increased the height of the resulting bread (**Tables 5** and **6**). The color of the bread was not affected by OBC and lichenase addition. The AA content in this experiment varied between 6.6 and 8.2 μg/kg, compared to a value of 7.7 μg/kg for our standard baking conditions, indicating that the AA content was not affected by the addition of either the OBC or lichenase.

DISCUSSION

Yeast-leavened whole-grain rye crisp bread produced at the standard conditions contained about 10 μg of AA/kg. These values are low compared to the reported AA contents (<30−1900 μg/kg) in commercial rye crisp bread (*17*). These low levels could at least be partly explained by the long fermentation times used during this bread-making procedure, because it has been reported that long fermentation compared to short fermentation times decreased AA content by 77% in soft bread made with rye bran (*19*). Another partial explanation could be the moderate moisture content of these breads, which was about 7%, because it has been reported that moisture content affects AA content in biscuits. (*7*). That study reported different contents of AA at different moisture contents; at 10%, no AA was observed, and at 6%, AA was detected at high baking temperatures. The highest amount was detected at the lowest moisture content of 2%, which is the normal moisture content for commercial biscuits.

Baking rye crisp bread at different combinations of time and temperature showed that both factors favored the browning reactions (**Figure 1**). The intensity of the color remained constant at lower time−temperature combinations, limited by 10 min at a temperature of 220 °C and 6 min at 270 °C, according to the surface response model.

AA content increased with time and temperature of baking, with higher effects perceived at higher temperatures and longer times in an accelerating slope. The AA content remained below

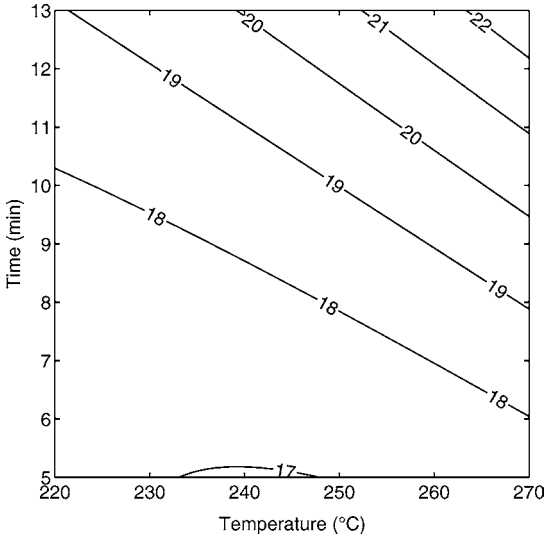

Figure 1. Graph of response surface model of color development (*b** value) for the time–temperature experiment.

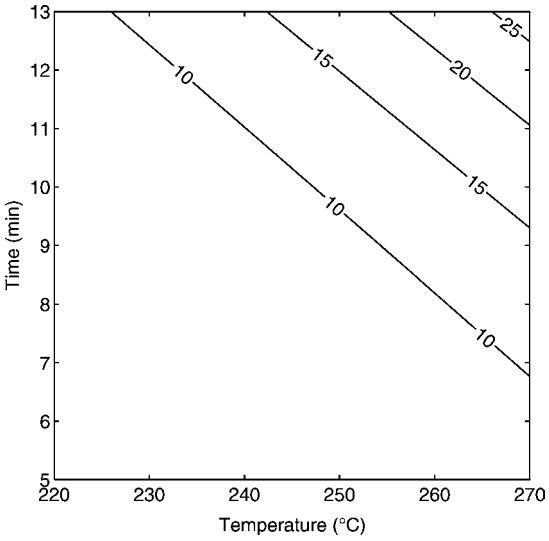

Figure 2. Graph of response surface model of acrylamide content (μg/kg) in rye crisp bread for the time–temperature experiment.

10 μg/kg of bread at a lower time−temperature combination, limited by 13 min at 230 °C and 7 min at 270 °C, according to the surface response model (**Figure 2**).

During mixing and fermentation, free ASN is likely to be consumed by the yeast (*19*) and reducing sugars might have been formed by enzymatic activity. Thus, the situation in the fermented dough might have been very different from the situation in the flour mixture. In the ASN−fructose experiment, the highest level of added ASN corresponded to about 7 times more than what is generally found in whole-grain rye flour. However, certain bread ingredients, such as germ, might contain high levels of free ASN that would increase the content significantly if added to the dough (*19*). On the other hand, when free ASN and fructose were added, the increase in AA content was proportional to the ASN addition regardless of the fructose level (**Figure 3**), leading to the conclusion that ASN is the main limiting precursor in AA formation in yeast-leavened rye crisp bread.

When color development and AA content were correlated, it was shown that the chromaticity coordinates (*a** and *b** values) and AA content had the same pattern of increase in the time−temperature experiment (**Figure 4a**, shown for the *b** value

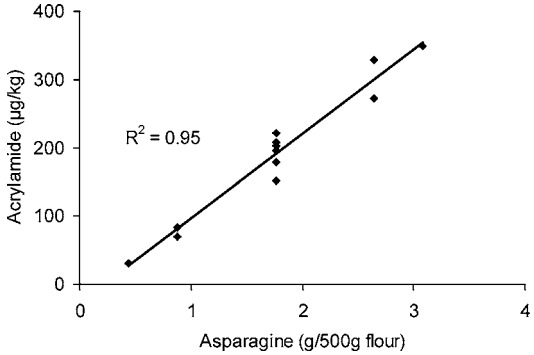

Figure 3. Relationship between acrylamide content in rye crisp bread and added asparagine in the asparagine–fructose experiment. Breads were baked at 250 °C for 8 min.

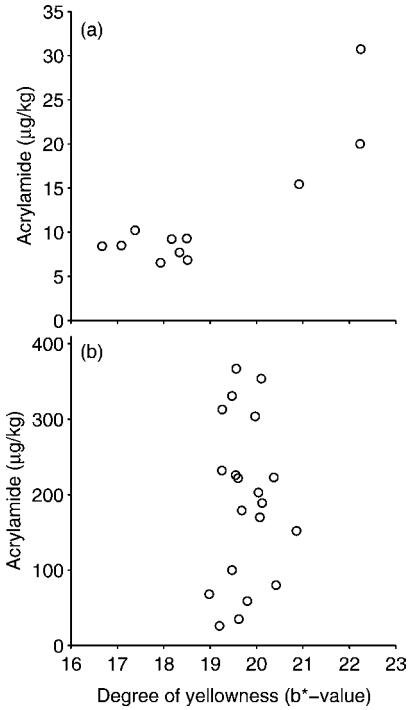

Figure 4. Relationship between degree of yellowness (*b** value) and acrylamide content (μg/kg) in rye crisp bread, with (a) a constant amount of asparagine and varying times and temperature of baking and (b) varying concentrations of asparagine (0.44–3.08 g/500 kg of flour) and a constant time and temperature of baking (250 °C for 8 min).

only). On the other hand, in the ASN−fructose experiment, the effect on the color was very limited. Time and temperature of baking had a higher effect than added fructose and ASN in the variation of the *b** value in relation to the AA content (**Figure 4b**). The addition of ASN resulted in formation of more AA and little variation in the *b** value. This shows that ASN is contributing to the pathway leading to AA, but its effect on color formation is limited.

The addition of OBC increased soluble dietary fiber content in the dough and bread and was expected to influence the water activity in the system. Results indicated that the level of dietary fiber can be increased by the addition of an ingredient such as OBC without influencing the AA content, because OBC is an alcohol-extracted product that does not contain free amino acids. This is in contrast with the results by Rydberg et al. (*23*), which showed that components that bind water may reduce the AA level. It was also reported that the addition of whole wheat flour

and bran to biscuit formulas increased the AA content when compared to those made of wheat flour (*7*).

The acrylamide content in yeast-leavened whole-grain rye crisp breads in this study was low, unless ASN was added. The extensive fermentation times used in this recipe might be a reason for the low acrylamide values compared to many other types of rye crisp bread. The time−temperature experiment showed that it was possible to produce breads with low AA content (<10 μg/kg) by adapting the time and temperature of baking.

ABBREVIATIONS USED

AA, acrylamide; ASN, asparagine; CCC, circumscribed central composite; OBC, oat-bran concentrate.

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