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Influence of Agronomic Factors and Extraction Rate on the Acrylamide Contents in Yeast-Leavened Breads

ACHIM CLAUS,[†] PAT SCHREITER,[‡] ALBRECHT WEBER,[§] SIMONE GRAEFF,[§] WILFRIED HERRMANN,[#] WILHELM CLAUPEIN,[§] ANDREAS SCHIEBER,*[,†] AND REINHOLD CARLE[†]

Institute of Food Technology, Section Plant Foodstuff Technology, Hohenheim University, August-von-Hartmann-Strasse 3D-70599 Stuttgart, Germany, Chemisches und Veterinäruntersuchungsamt Stuttgart, Schaflandstrasse 3/2, D-70736 Fellbach, Germany, Institute of Crop Farming and Grassland Research, Hohenheim University, Fruwirthstrasse 23, D-70599 Stuttgart, Germany, and Experimental Station Ihinger Hof, Hohenheim University, D-71272 Renningen, Germany

Because the impact of agronomical factors on bakery products quality is still an insufficiently studied field, acrylamide contents of breads produced from flours of nine wheat, two rye, and two spelt varieties harvested in 2003 and 2004 were investigated. It could be demonstrated that acrylamide content in bread strongly depends on the cultivar, with extremes differing by a factor of 5.4 due to marked differences in free asparagine and crude protein contents. Nitrogen fertilization also resulted in elevated amino acid and protein contents, thus increasing acrylamide levels from 10.6 to 55.6 μg/kg. Independent of fertilization, harvest year turned out to be another factor influencing acrylamide formation. Breads produced from 2003 flours showed significantly higher acrylamide contents than those of 2004, which was ascribed to favorable light and temperature conditions during the cultivation period, thus enhancing amino acid and protein contents. Sprouting of the grain also resulted in significantly higher acrylamide levels, which was attributed to elevated enzyme activities and the formation of precursors from protein and starch. Furthermore, bakery products made from flours with higher extraction rates were shown to contain higher acrylamide levels resulting from extracted free asparagine and protein from the aleuron layers of the cereal grain.

KEYWORDS: Acrylamide; cereal; variety; harvest year; bread; flour; fertilization; extraction rate; sprouting; enzyme activity, reducing sugars; asparagine

INTRODUCTION

Because of the potentially carcinogenic properties of acrylamide (*1*−*4*), the announcement of the Swedish National Food Authority of April 2002 and the University of Stockholm (*1*) concerning acrylamide in foodstuff initiated considerable research efforts. Elucidation of the reaction pathways pointed to the formation of acrylamide through reaction of reducing sugars with asparagine. Hence, the Maillard reaction was found to be the key mechanism of acrylamide formation during food preparation (*5*−*9*). Subsequent studies revealed additional mechanisms, mainly based on peptides and without carbohydrate participation (*10*−*13*). Current investigations focus on metabolism and toxicology of acrylamide (*14*, *15*) as well as strategies to minimize the levels in heat-treated foodstuffs. So far, many technological measures for acrylamide reduction have been considered. In order to decrease acrylamide contents, the heat impact has been minimized, citric acid or sulfur-containing amino acids have been used as additives, and precursor contents were lowered through fermentation during bread making and blanching of potato chips, respectively (*16*−*25*). However, all technological efforts to reduce acrylamide are questionable if unsuitable raw material is used. Consequently, inherent factors of the raw material affecting the acrylamide content of deep-fried potatoes have been extensively studied (*26*−*34*). Acrylamide contents of potato chips and French fries prepared under identical conditions were found to vary depending on potato cultivar. Furthermore, a reverse correlation between amount of fertilizer applied in potato cultivation and acrylamide content in the edible products has been established, since reducing sugar contents were elevated while crude protein and free amino acids decreased when less N-fertilizer was given (*31*). Climatic conditions during cultivation were shown to be another impact factor (*27*, *35*). Dry and hot weather seems to increase acrylamide formation as a result of higher contents of reducing sugars.

* Corresponding author. Phone: +49-(0) 711-459-3125. Fax: +49-(0) 711-459-4110. E-mail: schieber@uni-hohenheim.de.
[†] Institute of Food Technology.
[‡] Chemisches und Veterinäruntersuchungsamt Stuttgart.
[§] Institute of Crop Farming and Grassland Research.
[#] Experimental Station Ihinger Hof.

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To the best of our knowledge, comparable studies on the effect of cereal varieties and their growing conditions on acrylamide in bakery products are limited to one single study (*36*). Therefore, the content of potential precursors in grains and the influence of postharvest processing including storage and milling are of fundamental interest for bakery products. In view of the considerable economic value of bakery products, filling this gap is essential. In contrast, consumption of French fries and potato chips is much lower. In Germany, per capita consumption of bread and bread rolls amounted to 86.3 kg in 2005, which is equivalent to an average daily intake of about 236 g (*37*), while it is only 24 g for potato chips and French fries (*38*, *39*). Due to customary consumption habits, bakery products contribute about 25% of the total acrylamide intake through the diet (*3*, *40*, *41*). Therefore, the objective of this study was to investigate the influence of wheat, rye, and spelt varieties, nitrogen and sulfur fertilization, impact of the harvest year, grain sprouting, and extraction rate of the flours on the acrylamide content of yeast-leavened breads.

MATERIALS AND METHODS

Sample Material. Wheat (*Triticum aestivum* L.), rye (*Secale cereale* L.), and spelt (*Triticum aestivum* subsp. *spelta* (L.) Thell) were cultivated at Ihinger Hof, Germany, an experimental station of Hohenheim University, in 2003 and 2004. To prevent enzymatic reactions cereals were stored at 6 °C until further treatment. The influence of variety was studied using nine wheat, two rye, and two spelt cultivars. Unless otherwise noted all samples were fertilized using 190 kg N/ha. Furthermore, the effect of sprouting on the acrylamide content of bread was studied using the varieties Thasos and Terrier (2004 harvest). To mimic the effect of sprouting, grains were moisturized in water for 30 min and then stored at room temperature for 24 h until several germ buds occurred. Subsequently, the wheat was dried to 14% moisture in a drying chamber at 30 °C for an additional 24 h. The influence of N-fertilizer amounts was studied using the wheat varieties Enorm with 0, 140, 180, and 220 kg N/ha (CAN, calcium ammonium nitrate, 27% N, 13.5% NH_4-N, 13.5% NO_3-N, 12% CaO, amounts based on nitrogen) and Tommi with 0 and 220 kg N/ha. Additionally, using a basic nitrogen level of 180 kg N/ha the influence of a supplementary sulfur application (20 kg S/ha, applied as kieserite: 25% MgO, 20% S) was studied. Seasonal effects were determined using three wheat and one rye cultivar, respectively. Flours were prepared in a Quadrumat Junior laboratory mill (Brabender, Duisburg, Germany) yielding an ash content of about 0.55%. To study the influence of extraction rate on the acrylamide content of bread, flours were prepared on a Newtronic pilot plant roller mill (Bühler, Uzwil, Switzerland). Cultivars used in this trial were Thasos and 331 for wheat and rye, respectively.

Chemicals and Materials. Acrylamide (99%) was purchased from ICN Biomedicals (Eschwege, Germany) and 2,3,3-d_3-labeled acrylamide (98%) was from Cambridge Isotope Laboratories (Andover, MA). All other chemicals and reagents were provided by VWR (Darmstadt, Germany). Deionized water was used throughout. All buffer solutions used for the determination of enzymatic activities were autoclaved. Fermipan red dry yeast was used for bread dough fermentation (Uniferm, Werne, Germany).

Preparation of Yeast-Leavened Bread. Yeast-leavened bread was prepared according to common manufacturing practices. In detail, doughs were prepared on a Maestro TM 18 CEA spiral kneader (System Catering Industries, Italy) from 1600 g of flour (14% moisture content, adjusted by water addition), 960 mL of water (plus correction for 14% flour moisture), 22 g of dry yeast, and 24 g of NaCl. After fermentation at 70% relative humidity and 35 °C for 20 min, three loaves were manually formed (800 g), and a dough rest for additional 20 min in bread baskets was kept. The pH values were determined using a model 691 pH meter (Metrohm, Filderstadt, Germany) after dough preparation and after each fermentation step. An aliquot of each dough was frozen with liquid nitrogen to inhibit further enzymatic activity of yeast and subsequently freeze-dried for the determination of reducing sugars and amino acids immediately before baking. Breads were baked at 230 °C for 30 min in a model Monsun City 680-C convection oven (DEBAG, Bautzen, Germany). Baked breads were allowed to cool at room temperature and then separated into crusts and crumbs containing only minor contents of acrylamide (*42*) after determination of the $L^*a^*b^*$ color values. Lyophilized crusts were ground to a fine powder and stored at −25 °C under vacuum until analysis.

Chemical Characterization of Flour, Dough, and Bread. *Ash Content.* Ash contents of flours were determined according to ICC standard no. 104/1. Briefly, 2−3 g of sample material was weighed in porcelain crucibles and 2 mL of ethanol was added for preincineration. Subsequently, samples were kept at 900 °C for 2 h and weighed again after cooling in a desiccator.

Moisture Content. Moisture contents of flours were determined using an MA51 moisture analyzer (Sartorius, Göttingen, Germany). For this purpose, a 3−5 g sample was weighed and dried at 130 °C until a constant weight was obtained.

Crude Protein Content. Crude protein of flours was determined according to the Dumas method using a Vario Max CNS analyzer (Elementar, Hanau, Germany) (*43*). This method is based on oxidative decomposition of the samples under controlled oxygen supply at high temperatures (900 °C). The residual nitrogen in the gas phase was detected in a thermal conductivity cell, and crude protein content was calculated using conversion factors (wheat and spelt 5.7, rye 6.25). For this purpose, samples (∼250 mg) were weighed in steel crucibles and transferred to the analyzer. Determination of nitrogen and calculation of crude protein concentration were performed using the supplier's software.

Amylase Activity. The α-amylase activity in flours was determined according to ICC rapid method no. 303 using a Ceralpha (α-amylase) assay kit (Megazyme, Wicklow, Ireland) and reported as Ceralpha units (CU). One CU is defined as the amount of enzyme, in the presence of excess thermostable α-glucosidase, required to release one μmol of *p*-nitrophenol from the substrate nonreducing-end blocked *p*-nitrophenyl maltoheptaoside in 1 min under defined assay conditions. For this purpose, 3 g of flour was extracted with 20 mL of extraction buffer (1 M sodium malate, 1 M sodium chloride, 40 mM calcium chloride, and 0.005% sodium azide) at 40 °C for 20 min under constant stirring and filtered through a glass fiber filter. A 0.2 mL aliquot was incubated with 0.2 mL of amylase HR reagent (blocked *p*-nitrophenyl maltoheptaoidase and thermostable α-glucosidase) at 40 °C for exactly 20 min, and the reaction was stopped with 3 mL of trisodium phosphate solution (10 g/L). The absorbance was measured at 400 nm, and the activity was calculated according to the supplier's formula and expressed as Ceralpha units (CU).

Protease Activity. Protease activity in flour was determined using 2% azocasein as substrate according to the endoprotease assay described by Sarath (*44*) with the following modifications: about 3 g of wheat or spelt flour or 1.5 g of rye flour, respectively, was extracted with 12 mL of 50 mM sodium acetate buffer, pH = 5.0, at room temperature for 20 min and filtered through a glass fiber filter (Whatman GF/A). The assay was performed after incubation at 37 °C for 20 h, with protease activity expressed as ΔE_{440nm}/h/g.

Asparagine Content. Amino acids in flours and doughs were determined by gas chromatography and flame ionization detection (GC-FID) using an EZ:faast cleanup and derivatization kit (Phenomenex, Torrance, CA) (*10*). For this purpose, 12.5 g of flour or freeze-dried dough (rye, 6.25 g) was weighed in a 100 mL Erlenmeyer flask and extracted with 50 mL of 45% ethanol for 30 min. After centrifugation for 15 min at 4000 rpm, 3 mL of the supernatant was filtered through a 0.45 μm syringe filter and 400 μL of the filtrate was subjected to cleanup and derivatization. For correction of analyte losses during sample preparation, 100 μL of the internal standard norvaline was added to the effluent in a glass sample preparation vial and slowly passed over a sorbent tip filled with ion exchanger. Subsequently, washing solution (200 μL of 2-propanol/water) was passed over the tip, and the sorbent material was eluted with 200 μL of the eluting medium (NaOH/water/2-propanol/methylpyridine) into the sample vial. After completion of the cleanup, derivatization was performed with 50 μL of chloroform/2,2,4-trimethylpentane/alkylchloroformiate for 2 min. Additionally, 100 μL of chloroform/2,2,4-trimethylpentane was added and allowed

Table 1. Chemical Composition of Flours, Doughs, and Breads as Affected by Variety

					asparagine [mg/100 g]		reducing sugars [g/100 g]		acrylamide [μg/kg]	
sample[a]	crude protein [%]	ash [%]	protease [ΔE/h/g]	amylase [CU]	flour	dough	flour	dough	heated flour	bread
Enorm (1, E)	14.45	0.542	0.149 ± 0.018	0.177 ± 0.009	16.99 ± 0.76	4.70 ± 0.05	0.79 ± 0.04	1.32 ± 0.00	491.1 ± 6.4	36.1 ± 0.5
Monopol (1, E)	15.26	0.493	0.066 ± 0.041	0.117 ± 0.007	9.93 ± 0.37	4.10 ± 0.02	0.39 ± 0.00	1.41 ± 0.03	305.8 ± 22.7	30.7 ± 0.5
Ellvis (1, A)	12.19	0.627	0.106 ± 0.013	0.172 ± 0.010	19.05 ± 0.14	4.23 ± 0.08	0.87 ± 0.02	1.67 ± 0.03	461.0 ± 72.4	30.1 ± 1.4
Tommi (1, A)	12.60	0.596	0.156 ± 0.145	0.149 ± 0.010	12.98 ± 0.70	3.21 ± 0.08	0.80 ± 0.02	1.48 ± 0.03	379.8 ± 38.0	20.5 ± 1.0
Transit (1, A)	12.81	0.690	0.134 ± 0.000	0.182 ± 0.010	24.95 ± 0.13	9.58 ± 0.13	0.93 ± 0.01	0.48 ± 0.01	624.1 ± 39.3	74.4 ± 6.6
Magnus (1, A)	12.36	0.572	0.093 ± 0.001	0.120 ± 0.010	15.91 ± 0.17	5.41 ± 0.17	0.86 ± 0.01	1.92 ± 0.00	354.5 ± 23.1	40.8 ± 0.0
Terrier (1, B)	7.17	0.513	0.045 ± 0.040	0.106 ± 0.008	4.90 ± 0.07	1.74 ± 0.03	0.66 ± 0.00	1.80 ± 0.00	153.7 ± 17.0	13.8 ± 1.0
Manhattan (1, C_k)	9.72	0.510	0.156 ± 0.058	0.123 ± 0.007	11.62 ± 0.12	2.97 ± 0.05	0.71 ± 0.01	1.28 ± 0.02	347.7 ± 59.8	28.8 ± 1.2
Wasmo (1, C_k)	9.36	0.488	0.119 ± 0.001	0.100 ± 0.007	8.72 ± 0.16	1.88 ± 0.01	0.63 ± 0.04	0.94 ± 0.05	273.1 ± 16.9	15.5 ± 0.3
Schwabenkorn (2)	15.28	0.681	0.054 ± 0.001	0.165 ± 0.009	12.17 ± 0.19	2.25 ± 0.05	0.97 ± 0.00	0.77 ± 0.01	369.6 ± 19.5	21.0 ± 0.2
Frankenkorn (2)	13.51	0.615	0.094 ± 0.071	0.136 ± 0.009	6.46 ± 0.17	2.26 ± 0.15	0.56 ± 0.02	0.69 ± 0.00	243.1 ± 2.3	21.3 ± 1.0
Picasso (3)	8.25	0.733	0.130 ± 0.047	0.079 ± 0.020	41.37 ± 0.48	17.80 ± 0.17	1.15 ± 0.00	1.84 ± 0.03	962.7 ± 26.5	118.3 ± 5.6
Nikita (3)	8.97	0.732	0.112 ± 0.023	0.105 ± 0.019	44.10 ± 0.43	25.59 ± 0.08	1.06 ± 0.00	2.16 ± 0.29	930.3 ± 121.8	161.0 ± 0.3

[a] Varieties: 1, wheat; 2, spelt; 3, rye. Quality levels: E, high protein; A, normal protein; B, low protein; C_k, very low protein.

to react for 1 min. After washing with 0.1 M HCl (100 μL), the upper layer was transferred to a conical amber glass autosampler vial, and 2 μL aliquots were injected into the gas chromatograph. GC analysis was carried out on a 10 m × 0.25 mm i.d. Zebron-AAA column (Phenomenex, Torrance, CA) with an HP 6890 GC (Agilent, Waldbronn, Germany) equipped with a flame ionization detector operated at 320 °C with an injection volume of 2 μL (250 °C) and a split ratio of 1:20. The oven temperature was increased from 110 to 320 °C within 7 min followed by a 1 min isothermal hold. The software Chromeleon 6.6 (Dionex, Sunnyvale, CA) was used for operating the instrument, data acquisition, and processing. All analyses were performed in duplicate. External standards were used for quantification.

Reducing Sugar Content. Reducing sugars in flour and dough were determined using liquid chromatography with refractive index detection (HPLC-RI) (*45*). After grinding of the samples, 10 g quantities were weighed in a 250 mL Erlenmeyer flask together with 100 mL of 60% ethanol, homogenized with an Ultra Turrax, and sonicated in a water bath at 70 °C for 20 min. Hot ethanolic extraction was performed to prevent sugar metabolization by yeast and for starch precipitation. HPLC analysis was carried out using an Agilent HPLC series 1100 (Agilent, Waldbronn, Germany) equipped with a refractive index detector. The separation was performed using a 250 mm × 4.6 mm i.d., 5 μm Polyamine II column (YMC Europe, Schermbeck, Germany) equipped with a 4.0 mm × 3.0 mm i.d. NH_2 guard column (Phenomenex, Torrance, CA) operated at 35 °C. The injection volume was 20 μL. Isocratic elution was performed within 15 min using a mobile phase of 60% aqueous acetonitrile at a flow rate of 1 mL/min. Fructose, glucose, sucrose, and maltose were detected with a refractive index detector operated at 35 °C. Individual data acquisition and processing was performed using ChemStation software. Compounds were quantified using a calibration curve of the corresponding standard compounds ranging from 5 μg/L to 5 g/L.

Acrylamide Formation Potential in Flour. To determine the potential of acrylamide formation in flour a sample of 4 g was weighed in open flasks and heated at 170 °C for 20 min. After cooling at room temperature the acrylamide content was determined as described below.

Determination of Acrylamide in Heated Flour and Breads. Acrylamide in heated flour and bread crusts was determined using LC−MS/MS according to Gutsche et al. (*46*). Briefly, together with acrylamide-d_3 as an internal standard, heated flour (4 g) or lyophilized and homogenized crust (5 g) was extracted with 100 mL of water in an ultrasonic water bath at 40 °C for 10 min. After Carrez precipitation the filtrate was cleaned up over Chromabond ABC18 cartridges (Macherey-Nagel, Düren, Germany) preconditioned with methanol and water to remove interfering matrix compounds. Chromatographic separation of acrylamide was carried out via HPLC (Agilent 1100 series) on a 100 mm × 2.1 mm i.d., 5 μm Hypercarb column (Thermo Hypersil, Dreieich, Germany) with a 4.0 mm × 3.0 mm i.d. C18 guard precolumn (Phenomenex, Torrance, CA) at 30 °C. The sample (20 μL) was separated at a flow rate of 0.2 mL/min isocratically with mobile phase composed of 1% acetonitrile/0.05% formic acid (v/v) in water. Mass spectra were recorded by means of a triple quadrupole tandem mass spectrometer (Quattro Ultima, Micromass, Altrincham, Cheshire, U.K.) operated in the positive electrospray ionization mode (ESI+). The precursor/product ion transitions m/z 72 → 55 (quantifier) as well as 72 → 54 and 72 → 44 (qualifier) were monitored for acrylamide, and 75 → 58 (quantifier) and 75 → 44 (qualifier) were monitored for acrylamide-d_3. Masslynx software (Micromass) was used for data acquisition and processing. All analyses were performed at least in duplicate.

*Determination of L*a*b* Values.* $L^*a^*b^*$ values, describing the color of the samples in a three axis color space (*L* lightness, *a* red−green offset, *b* blue−yellow offset) were determined using a Chromameter CR-300 (Minolta, Osaka, Japan) with D65 light. The measuring head was placed on top of the breads and six reading points were recorded automatically by the instruments software. Medians were calculated using the supplier's software and transformed to the $L^*a^*b^*$ system.

RESULTS AND DISCUSSION

Influence of Variety on Acrylamide Formation. In addition to the main acrylamide precursors asparagine and reducing sugars, also moisture, crude protein, ash, as well as protease and amylase activities were determined for all samples, and their impact on acrylamide formation during bread making or in heated flour was assessed. As previously shown, reducing sugars and consequently acrylamide are significantly dependent on potato variety (*26*−*34*), while there is still only insufficient knowledge concerning the inherent agronomical factors affecting the acrylamide levels of cereal products like bread. As can be seen from **Table 1**, reducing sugars in flour and dough produced therefrom are also cultivar dependent. They vary between 0.39 and 0.93 g/100 g for wheat, whereas only a minor impact of the variety was observed for rye. No correlation could be established between reducing sugar and acrylamide contents of heated flour or breads since asparagine was found to be the crucial precursor in bakery products (*22*, *41*). In contrast, asparagine contents of the flour and asparagine in dough significantly affected acrylamide contents (**Figure 1**). They vary in the range from 8.7 to 24.9 mg/100 g, which is comparable to the results of Taeymans et al. (*17*) reporting a range of 7.4−66 mg/100 g for wheat mainly from the United Kingdom. Benedito De Barber et al. (*47*) reported a decrease in asparagine from 8.21 to 0.64 mg/100 g during dough fermentation. This is more than in our experiments (around 60%, dough corrected to dry matter), but fermentation time was a factor of 4 longer, so results are not totally comparable. Consequently, acrylamide in wheat bread varied in a wide range (by a factor of 5.4) from about 14 μg/kg

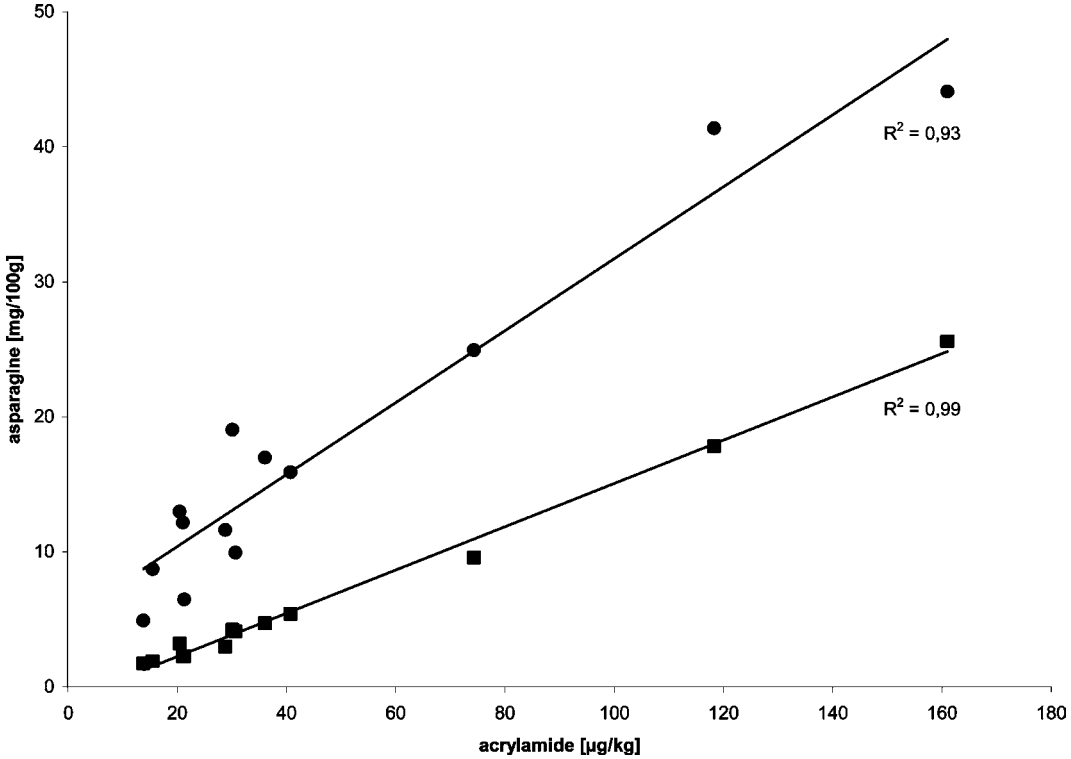

Figure 1. Correlation of asparagine concentrations in flours (●) and doughs (■) of different wheat, spelt, and rye varieties with the resulting acrylamide contents in breads produced thereof.

for Terrier to 74 μg/kg for Transit, while contents in rye differed by 30%. Due to elevated asparagine contents, acrylamide levels in rye were generally higher than in wheat or spelt. Acrylamide contents in breads produced from spelt were similar to those from wheat, confirming their close relationship. These data are in agreement with the results of Springer et al. (*36*) and Elmore et al. (*48*). There was no influence of pH values because they were almost constant for all samples decreasing from 5.75 after dough preparation to 5.5 prior to baking. This decrease can be ascribed to the fermentation. In Germany, different quality categories have been established for wheat, where E is premium, due to their high crude protein contents. Flours derived from such wheat varieties (e.g., cultivars Monopol and Enorm) are suitable for quality improvement of flours of lower categories. This group is followed by A (cultivars Tommi, Ellvis, Magnus, Transit) and B (Terrier) which are commonly used as bread flour, whereas C_k is only suitable for cookies where low gluten content is sufficient (cultivars Wasmo and Manhattan). Because of the different varieties the quality of the resulting breads was not fully comparable, higher protein concentrations resulted in firmer doughs. But changes in the recipe would have a negative effect on the data. Furthermore, the differences were not very big, so the recipe was considered as appropriate for scientific studies. In general, wheat varieties belonging to the E and A category show higher acrylamide levels than B and C_k. Surprisingly, as shown by the cultivar Tommi, despite their higher crude protein content (category A) some varieties may provide relatively low acrylamide concentrations with 20.5 μg/kg acrylamide in bread produced therefrom. Consequently, the use of low-category wheat is not essential in minimizing acrylamide levels.

Influence of Harvest Year on Acrylamide Formation. As reported by Olsson et al. (*28*), harvest year significantly influences asparagine and reducing sugars in potato. Acrylamide was not analyzed in this study. Therefore, precursor contents in flours and doughs and acrylamide contents in bakery products derived therefrom were assessed. In the present study, the wheat varieties Terrier, Enorm control, and Enorm CAN and the rye cultivar Nikita of harvest years 2003 and 2004 were included. While reducing sugars were not significantly influenced by

Table 2. Chemical Composition of Flours, Doughs, and Breads as Affected by Harvest Year

					asparagine [mg/100 g]		reducing sugars [g/100 g]		acrylamide [μg/kg]	
sample[a]	crude protein [%]	ash [%]	protease [ΔE/h/g]	amylase [CU]	flour	dough	flour	dough	heated flour	bread
Terrier 03 (1)	9.35	0.508	0.007 ± 0.010	0.093 ± 0.006	5.37 ± 0.09	1.82 ± 0.01	0.61 ± 0.02	1.57 ± 0.02	231.8 ± 12.6	13.9 ± 0.5
Terrier 04 (1)	7.17	0.513	0.045 ± 0.040	0.106 ± 0.008	4.90 ± 0.07	1.74 ± 0.03	0.66 ± 0.00	1.80 ± 0.00	153.7 ± 17.0	13.8 ± 1.0
Enorm CAN 03 (1)	14.95	0.595	0.063 ± 0.062	0.161 ± 0.011	19.61 ± 0.31	5.94 ± 0.11	0.75 ± 0.03	1.23 ± 0.00	472.0 ± 36.5	48.9 ± 0.8
Enorm CAN 04 (1)	14.16	0.503	0.077 ± 0.049	0.164 ± 0.011	13.29 ± 0.12	1.77 ± 0.03	0.69 ± 0.02	1.10 ± 0.00	397.8 ± 30.6	18.7 ± 0.2
Enorm cont 03 (1)	9.50	0.540	0.037 ± 0.052	0.148 ± 0.009	9.76 ± 0.21	3.15 ± 0.07	0.67 ± 0.01	1.32 ± 0.03	287.5 ± 0.8	21.1 ± 0.8
Enorm cont 04 (1)	7.90	0.494	0.020 ± 0.006	0.124 ± 0.011	3.84 ± 0.02	1.25 ± 0.01	0.62 ± 0.02	1.39 ± 0.05	166.8 ± 23.3	10.0 ± 0.5
Nikita 03 (2)	9.38	0.592	0.082 ± 0.034	0.085 ± 0.019	48.54 ± 0.79	27.13 ± 0.15	0.95 ± 0.00	2.16 ± 0.14	1026.4 ± 8.0	175.2 ± 5.0
Nikita 04 (2)	8.97	0.732	0.112 ± 0.023	0.105 ± 0.019	44.10 ± 0.43	25.59 ± 0.08	1.06 ± 0.00	2.16 ± 0.29	930.3 ± 121.8	161.0 ± 0.3

[a] Varieties: 1, wheat; 2, rye.

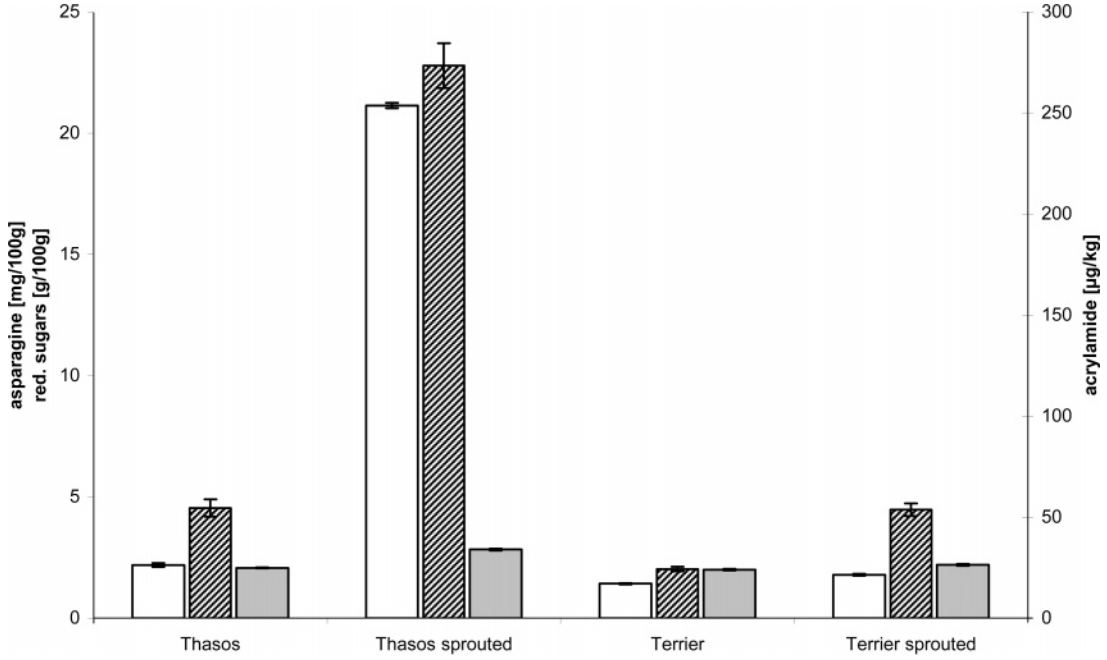

Figure 2. Effect of sprouting on asparagine (white), acrylamide (black), and reducing sugar (gray) contents in wheat flours and breads produced thereof.

harvest year (**Table 2**), asparagine and crude protein were higher in 2003, resulting in elevated acrylamide contents in the heated flours and breads. Because the samples were harvested from the same plots in 2003 and 2004 using identical fertilization, increasing precursor concentrations were ascribed to differing weather conditions. In 2003, sunshine duration and temperature during the vegetation period as recorded from February to August were higher than in 2004 (1580 h compared to 1296 h and daily average temperature 15.2 versus 13.2 °C). As reported earlier (*49*, *50*), crude protein content of rye and wheat increased with elevated growth temperatures. In potato tubers the amino acid content was also found to depend on growth temperatures (*51*). Very recently, industrial quality of durum wheat was shown to be enhanced by dry weather conditions (*35*). In the present study, both precursor and acrylamide contents were shown to be dependent on year and especially growing temperature. Since both parameters cannot be influenced by cultivation measures, their variability is unavoidable. More detailed long-term studies including a broader range of varieties are currently undergoing.

Influence of Sprouting on Acrylamide Formation. Due to unfavorable climatic conditions shortly before harvest sprouting of the grains can occur. As a consequence, enzyme activities are considerably increased, leading to a degradation of starch and proteins and a release of the precursors of acrylamide. Therefore, sprouting was artificially generated to study its impact on acrylamide formation using wheat varieties Thasos and Terrier. As expected, amylase activity ascended from 0.092 to 0.104 CU and from 0.218 to 0.463 CU for Terrier and Thasos, respectively. Protease activity was increased from 0.144 to 0.304 ΔE/h/g (Terrier) and from 0.182 to 0.405 ΔE/h/g (Thasos). Therefore, precursor contents, especially asparagine, were significantly elevated in flour from sprouted wheat grains, as can be seen in **Figure 2**. This increase in precursors caused significantly higher acrylamide contents in breads. In Thasos acrylamide increased from 54.5 to 273.4 μg/kg, corresponding to almost 500%. In Terrier the change in asparagine was about one-third, but acrylamide was almost doubled. This might be ascribed to an increase in other acrylamide-forming amino acids like methionine, which was not observed for Thasos. Therefore, sprouted wheat or rye should not be used for bakery products even in combination with other flours because of the enhancement of acrylamide formation.

Influence of N-Fertilization on Acrylamide Formation. While climatic conditions can only be influenced in greenhouse experiments, fertilization is a key factor in large-scale crop farming. As recently demonstrated by De Wilde et al. (*31*), lowered amounts of nitrogen increased reducing sugars and decreased free asparagine in potato tubers. Because sugars were shown to be the crucial precursors in this commodity, acrylamide levels in products were higher when less nitrogen was applied. In our study, the impact of fertilizer rate on acrylamide formation in heated wheat flour and baked bread produced therefrom were assessed using the wheat cultivars Enorm and Tommi. In contrast to earlier results (*31*), reducing sugars in flour remained constant around 0.7 g/100 g when higher amounts of N-fertilizer were applied and did not diminish. These findings are in good agreement with those of Zahedi et al. (*52*) reporting that N-fertilization of wheat neither influenced starch nor soluble carbohydrates like fructose or glucose. However, N-fertilization had a strong impact on crude protein and free asparagine contents in flour. For the variety Enorm asparagine rose from 5.4 mg/100 g in the control samples (0 kg N/ha) to 22.0 mg/100 g when 220 kg N/ha were applied (**Table 3**), while crude protein contents were almost doubled, comparable to cultivar Tommi, where both asparagine and crude protein contents increased from 7.3 mg/100 g and 7.6% to 18.7 mg/100 g and 15.4%, respectively. This can primarily be ascribed to improved nitrogen utilization and is in full agreement with the observations of Lerner et al. (*35*). Due to the elevated levels of the crucial precursors, acrylamide in bread produced from flours derived from cultivar Enorm increased from 10.6 μg/kg for the samples without fertilization to 55.6 μg/kg at maximum N-dosage (220 kg N/ha). Accordingly, acrylamide in breads from cultivar Tommi increased from 12.1 to 47.9 μg/kg. Since N-fertilization is a prerequisite to increase crop yields, protein contents, and baking quality of the flours, elevated acrylamide contents resulting from this measure seem to be inevitable. Therefore, to minimize acrylamide levels lower flour qualities would have to be accepted. Consequently, N-fertilization should

Table 3. Chemical Composition of Flours, Doughs, and Breads as Affected by Fertilization

					asparagine [mg/100 g]		reducing sugars [g/100 g]		acrylamide [μg/kg]	
sample	crude protein [%]	ash [%]	protease [ΔE/h/g]	amylase [CU]	flour	dough	flour	dough	heated flour	bread
					Influence of N-Fertilizer					
Enorm 0 kg N	8.50	0.596	0.068 ± 0.004	0.135 ± 0.011	5.40 ± 0.05	1.33 ± 0.01	0.75 ± 0.01	1.55 ± 0.00	151.1 ± 4.9	10.6 ± 0.8
Enorm 140 kg N	13.02	0.438	0.075 ± 0.060	0.165 ± 0.010	11.30 ± 0.02	1.98 ± 0.04	0.71 ± 0.00	1.23 ± 0.02	344.3 ± 20.1	15.8 ± 0.5
Enorm 180 kg N	15.07	0.521	0.067 ± 0.003	0.169 ± 0.013	19.93 ± 0.11	5.39 ± 0.07	0.74 ± 0.02	1.26 ± 0.00	495.9 ± 2.4	39.5 ± 0.3
Enorm 220 kg N	15.53	0.528	0.066 ± 0.016	0.171 ± 0.011	22.03 ± 0.27	5.50 ± 0.22	0.71 ± 0.01	1.15 ± 0.04	579.4 ± 8.1	55.6 ± 1.0
Tommi 0 kg N	7.55	0.600	0.056 ± 0.001	0.120 ± 0.012	7.28 ± 0.21	1.63 ± 0.01	0.69 ± 0.01	1.63 ± 0.02	196.7 ± 27.2	12.1 ± 0.3
Tommi 220 kg N	15.43	0.557	0.065 ± 0.004	0.163 ± 0.010	18.73 ± 0.12	7.06 ± 0.05	0.77 ± 0.02	1.45 ± 0.05	478.0 ± 57.4	47.9 ± 1.0
					Influence of S-Fertilizer					
Enorm cont	7.90	0.494	0.020 ± 0.006	0.124 ± 0.011	3.84 ± 0.02	1.25 ± 0.01	0.62 ± 0.02	1.39 ± 0.05	166.8 ± 23.3	10.0 ± 0.5
Enorm CAN	14.16	0.503	0.077 ± 0.049	0.164 ± 0.011	13.29 ± 0.12	1.77 ± 0.03	0.69 ± 0.02	1.10 ± 0.00	397.8 ± 30.6	18.7 ± 0.2
Enorm CAN + S	14.42	0.499	0.078 ± 0.061	0.164 ± 0.007	13.49 ± 0.33	1.82 ± 0.08	0.67 ± 0.01	1.21 ± 0.09	382.4 ± 12.2	36.3 ± 0.2
Tommi cont	6.97	0.569	0.037 ± 0.025	0.114 ± 0.011	5.90 ± 0.10	2.14 ± 0.03	0.64 ± 0.00	1.67 ± 0.01	178.5 ± 13.0	15.4 ± 0.0
Tommi CAN + S	12.85	0.513	0.073 ± 0.103	0.141 ± 0.009	9.90 ± 0.14	4.04 ± 0.00	0.68 ± 0.02	1.48 ± 0.03	300.5 ± 6.5	14.9 ± 0.5

Table 4. Chemical Composition of Flours, Doughs, and Breads as Affected by Extraction Rate

					asparagine [mg/100 g]		reducing sugars [g/100 g]		acrylamide [μg/kg]	
sample[a]	crude protein [%]	ash [%]	protease [ΔE/h/g]	amylase [CU]	flour	dough	flour	dough	heated flour	bread
Thasos low (1)	15.73	0.631	0.011 ± 0.015	0.156 ± 0.006	13.76 ± 0.39	5.38 ± 0.08	0.64 ± 0.01	1.22 ± 0.01	375.5 ± 25.4	45.5 ± 0.9
Thasos normal (1)	16.23	0.743	0.023 ± 0.002	0.171 ± 0.010	19.62 ± 0.24	7.14 ± 0.11	0.75 ± 0.01	1.37 ± 0.04	440.4 ± 129.8	49.5 ± 0.0
Thasos high (1)	18.07	1.352	0.183 ± 0.030	0.209 ± 0.024	48.51 ± 1.83	22.46 ± 0.76	1.44 ± 0.01	1.76 ± 0.02	954.3 ± 35.6	115.3 ± 0.0
331 low (2)	9.36	0.900	0.165 ± 0.001	0.088 ± 0.021	59.47 ± 3.43	32.40 ± 0.72	1.17 ± 0.01	1.76 ± 0.59	1287.6 ± 153.5	167.4 ± 2.0
331 normal (2)	9.96	1.184	0.332 ± 0.034	0.108 ± 0.034	63.50 ± 0.49	33.84 ± 1.03	1.35 ± 0.05	2.15 ± 0.00	1498.0 ± 49.4	183.4 ± 2.0
331 high (2)	13.13	1.932	0.586 ± 0.022	0.127 ± 0.047	83.17 ± 2.05	52.01 ± 0.19	1.92 ± 0.11	2.10 ± 0.03	1746.1 ± 109.0	233.9 ± 2.3

[a] Varieties: 1,wheat; 2, rye.

be adjusted to the minimum requirement of the crops which would also meet the standard of environmental protection.

Influence of S-Fertilization on Acrylamide Formation. Analogous observations were made when studying the influence of S-fertilization. For this purpose, the wheat cultivars Enorm and Tommi were fertilized with CAN (except Tommi) and CAN with additional sulfur (kieserite, 25% MgO, 20% S), using an unfertilized sample as control. As can be seen from **Table 3**, crude protein, asparagine, and enzyme activities increased when CAN was added, while reducing sugars remained constant like in the N-fertilization experiment. Consequently, acrylamide levels in the breads produced from these flours rose from 10.0 to 18.7 μg/kg. Additional sulfur fertilization did not increase the precursor contents. Asparagine, reducing sugars, crude protein, and enzyme activities remained constant when compared to those from CAN fertilization. This is in good agreement with a recent report where additional sulfur application did not affect various quality indices and grain protein concentration in durum wheat (*53*). Unexpectedly, in our study acrylamide contents in the bread differed when CAN and CAN + S were compared. In Enorm, acrylamide contents were nearly doubled by additional S-fertilization from 18.7 to 36.3 μg/kg. Since the concentrations of asparagine were found to be almost constant, the full set of amino acids was evaluated. A slight increase in methionine may point to the involvement of this amino acid in acrylamide formation as described by Stadler et al. (*5*). Therefore, methionine as well as glutathione and cysteine, which can also be affected by sulfur fertilization, were allowed to react in a model system (300 mg of amino acid and 50 μL of water) with and without the addition of glucose (300 mg) at 180 °C for 15 min. After redissolving in water and subsequent filtration, acrylamide was determined as described above but could not be detected in any of the samples. This is in agreement with Stadler et al. (*5*) reporting acrylamide release from methionine or glutamine is considerably lower than from asparagine by a factor of 1000. Hence, the elevated acrylamide contents following additional sulfur fertilization cannot necessarily be ascribed to an increase in methionine and remain to be elucidated especially because acrylamide in heated flour was not affected. Furthermore, the cultivar Tommi does not show such an effect, so the observed increase might also be ascribed to measurement problems.

Influence of Extraction Rate on Acrylamide Content. Beside crop fertilization technological measures also influence the quality of cereal flours. Extraction rate, indicated by the ash content, was assumed to be the most relevant factor directly affecting acrylamide levels in bakery products. Therefore, the effect of extraction rate on the concentrations of precursors and acrylamide was assessed as exemplified with the wheat variety Thasos and rye 331. Both were milled on a pilot plant roller mill, and each fraction yielded in the milling process was collected separately. From these fractions three flours per cultivar were prepared representing low, medium, and high ash content (**Table 4**). As can be seen from **Table 4**, ash contents ranged from 0.63% to 1.35% and 0.90% to 1.93% for wheat and rye, respectively, corresponding to concentrations commonly used in bread making. Besides the ash content, other relevant precursor concentrations were also influenced by the extraction rate. Expectedly, protease and amylase activities increased with higher extraction rates, resulting in higher concentrations of asparagine and reducing sugars in the flour. Higher precursor concentrations may be transferred from the aleurone layer or result from higher protease activities degrading proteins from this layer. Accordingly, asparagine rose from 13.7 to 48.5 mg/100 g and 59.5 to 83.1 mg/100 g for wheat and rye, respectively. Reducing sugars were elevated in the same order (**Table 4**). As a result, acrylamide levels in bread were also significantly higher when flours of higher extraction rate were used. In wheat,

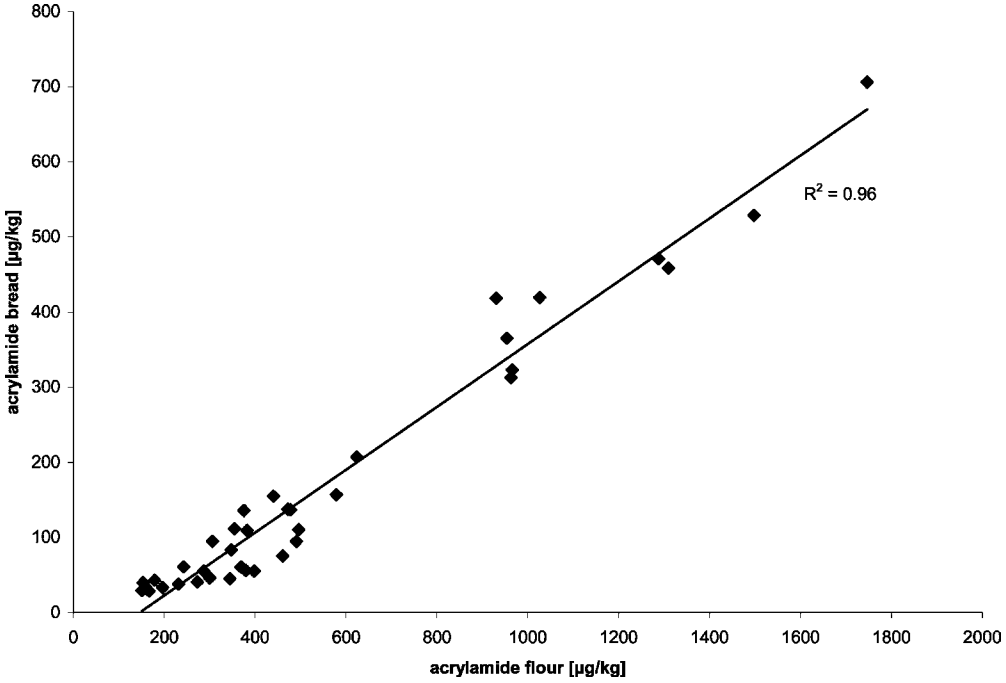

Figure 3. Estimation of acrylamide-forming potential by correlation of acrylamide concentrations in heated flour and breads produced thereof.

acrylamide content rose by 250% to 115.3 μg/kg. In rye, corresponding to the lower increase in asparagine, acrylamide rose by only 140% which is in good agreement with previous publications (*22*) reporting a three-fold increase of asparagine in wheat when extraction rate was increased from 80% to 100%, while contents in rye were only doubled within the same cummulation. Thus, the increase in acrylamide can be ascribed to elevated levels of asparagine and possibly crude protein, whereas it is unlikely that the acrylamide content is strongly influenced by reducing sugars within this product group as previously reported (*54*). While flours containing higher amounts of dietary fiber and ash are high-valued from a nutritional point of view, they cause elevated acrylamide levels in bread. Therefore, minimization of acrylamide in breads avoiding high extraction rate flours would be contradictory.

Evaluation of the Acrylamide-Forming Potential. All flours used in this study were not only applied for breadmaking but also heated as described above to assess the acrylamide-forming potential of the crop varieties and flours thereof. As can be seen from **Figure 3**, a good correlation of acrylamide contents in heated flours and acrylamide contents in breads produced therefrom could be observed. Hence, dry heating of flour was found to be a feasible measure to evaluate the suitability of a variety for breadmaking without tedious baking studies.

Asparagine contents of the flours may be taken as another indicator of the acrylamide-forming potential. As can be seen from **Figure 1**, asparagine in flour and acrylamide in breads, respectively, did show a significant correlation while crude protein only showed ascending tendency. Including all samples used in this study, the regression factor even exceeds 0.94. This is in excellent agreement with previous publications (*23*, *25*, *36*, *55*) reporting similar correlations. This relationship is of particular interest for manufacturers because laboratory equipment needed for a simple evaluation of the acrylamide-forming potential using asparagine as an indicator is usually available.

Correlation of *L*a*b and Acrylamide in Bread.** Since acrylamide is formed during Maillard reaction, its concentration and the color of the processed products were reported to show good correlation (*23*, *55*). However, in contrast to these previous studies this could not be confirmed by our data. Color values were determined for each bread and compared with corresponding acrylamide levels. Neither *a* or *b* values showed any correlation; only the brightness *L* and ΔE, which was previously shown to correlate very good with acrylamide in bread (*23*), displayed a decreasing trend, reflecting that darker products showed higher acrylamide contents, but the regression coefficient was very poor ($R^2 = 0.3$). This must mainly be attributed to problems during color characterization. While the measurement head may easily be placed on a flat surface like chips or toast, the bent crust of breads might result in scattered light and therefore in imprecise data acquisition. Moreover, the proposed novel formation mechanism recently proposed (*10*, *11*) might potentially also contribute to the different findings because acrylamide is formed from peptides and proteins without noticeable changes in product color. The extent of its contribution to acrylamide formation is not yet known. Furthermore, previous studies reporting good correlations had more variables under control, e.g., toasting a single type bread with variable toasting times, leading to more precise data. This is in agreement to previous results (*55*) reporting that after precursor addition to crisp bread color measurement was also insufficient what was ascribed to an much higher effect of temperature on color than that of asparagine/sugars.

The objective of this study was to investigate the influence of crop variety, N− and S-fertilization, harvest year, grain sprouting, and flour extraction rate on the acrylamide content of yeast-leavened bread. It could be shown that the variety strongly affects acrylamide concentration of breads, mainly due to varying asparagine contents. Furthermore, higher nitrogen fertilization enhances acrylamide levels by the same mechanism, whereas the role of S-fertilization remains to be elucidated. Among agronomical parameters the harvest year also is an important factor for acrylamide formation because higher growing temperature and longer sunshine duration results in elevated protein and amino acid contents in grain and flour. Heavy rainfall shortly before harvest can result in grain sprouting. Due to the elevated enzyme activities and the release of precursors from protein and starch, acrylamide levels were significantly

higher. Whereas climatic conditions cannot be influenced by the producer, the extraction rate of the flours is of technological importance. As expected, higher extraction rates were shown to result in higher asparagine contents in the flour, and consequently higher acrylamide levels in the bread.

The data presented here has to be further substantiated by long-term trials. The cultivar screening especially should be continued over a longer period to clearly understand climatic influences so that recommendations for plant breeders and manufacturers regarding selection criteria of varieties aiming at lower acrylamide levels can be given. Furthermore, the influence of a long-term storage of flour on precursors and acrylamide should be assessed in a separate study.

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LITERATURE CITED

(1) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Acrylamide: A cooking carcinogen? *Chem. Res. Toxicol.* **2000**, *13*, 517−522.

(2) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998−5006.

(3) Konings, E. J. M.; Baars, A. J.; van Klaveren, J. D.; Spanjer, M. C.; Rensen, P. M.; Hiemestra, M.; van Kooij, J. A.; Peters, P. W. J. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Food Chem. Toxicol.* **2003**, *41*, 1569−1579.

(4) Erdreich, L. S.; Friedman, M. A. Epidemiological evidence for assessing the carcinogenicity of acrylamide. *Regul. Toxicol. Pharmacol.* **2004**, *39*, 150−157.

(5) Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P. A.; Robert, M.-C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002**, *419*, 449−450.

(6) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448.

(7) Zyzak, D. V.; Sanders, R. A.; Stojanovic, M.; Tallmadge, D. H.; Eberhardt, B. L.; Ewald, D. K.; Gruber, D. C.; Morsch, T. R.; Strothers, M. A.; Rizzi, G. P.; Villagran, M. D. Acrylamide formation mechanism in heated foods. *J. Agric. Food Chem.* **2003**, *51*, 4782−4787.

(8) Weisshaar, R.; Gutsche, B. Formation of acrylamide in heated potato products—model experiments pointing to asparagine as precursor. *Dtsch. Lebensm.—Rundsch.* **2002**, *98*, 397−400.

(9) Yaylayan, V. A.; Wnorowski, A.; Perez-Locas, C. Why asparagine needs carbohydrates to generate acrylamide. *J. Agric. Food Chem.* **2003**, *51*, 1753−1757.

(10) Claus, A.; Weisz, G. M.; Schieber, A.; Carle, R. Pyrolytic acrylamide formation from purified wheat gluten and gluten-supplemented wheat bread rolls. *Mol. Nutr. Food Res.* **2006**, *49*, 87−93.

(11) Buhlert, J.; Carle, R.; Majer, Z.; Spitzner, D. Thermal degradation of peptides and formation of acrylamide. *Lett. Org. Chem.* **2006**, *3*, 356−357.

(12) Gertz, C.; Klostermann, S. Analysis of acrylamide and mechanisms of its formation in deep-fried products. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 762−771.

(13) Yaylayan, V. A.; Perez, L. C.; Wnorowski, A.; O'Brien, J. The role of creatine in the generation of *N*-methylacrylamide: a new toxicant in cooked meat. *J. Agric. Food Chem.* **2004**, *52*, 5559−5565.

(14) Friedman, M. Chemistry, biochemistry, and safety of acrylamide. A review. *J. Agric. Food Chem.* **2003**, *51*, 4504−4526.

(15) Rudén, C. Acrylamide and cancer risk-expert risk assessments and the public debate. *Food Chem. Toxicol.* **2004**, *42*, 335−349.

(16) Biederman, M.; Noti, A.; Biederman-Brem, S.; Mozzetti, V.; Grob, K. Experiments on acrylamide formation and possibilities to decrease the potential of acrylamide formation in potatoes. *Mitt. Lebensmittelunters. Hyg.* **2002**, *93*, 668−687.

(17) Taeymans, D.; Wood, J.; Ashby, P.; Blank, I.; Studer, A.; Stadler, R. H.; Gondé, P.; Van Eijck, P.; Lalljie, S.; Lingnert, H.; Lindblom, M.; Matissek, R.; Müller, D.; Tallmadge, D.; O'Brien, J.; Thompson, S.; Silvian, D.; Whitmore, T. A review of acrylamide: an industry perspective on research, analysis, formation, and control. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 323−347.

(18) Biedermann, M.; Grob, K. Model studies on acrylamide formation in potato, wheat flour and corn starch; ways to reduce acrylamide content in bakery ware. *Mitt. Lebensmittelunters. Hyg.* **2003**, *94*, 406−422.

(19) Graf, M.; Amrein, T. M.; Graf, S.; Szalay, R.; Escher, F.; Amado, R. Reducing the acrylamide content of a semi-finished biscuit on industrial scale. *Food Sci. Technol.* **2006**, *39*, 724−728.

(20) Biedermann-Brem, S.; Noti, A.; Grob, K.; Imhof, D.; Bazzocco, D.; Pfefferle, A. How much reducing sugar may potato contain to avoid excessive acrylamide formation during roasting and baking? *Eur. Food Res. Technol.* **2003**, *217*, 369−373.

(21) Jung, M. Y.; Choi, D. S.; Ju, J. W. A novel technique for limitation of acrylamide formation in fried and baked corn chips and French fries. *J. Food Sci.* **2003**, *68*, 1287−1290.

(22) Fredriksson, H.; Tallving, J.; Rosén, J.; Åman, P. Fermentation reduces free asparagine in dough and acrylamide content in bread. *Cereal Chem.* **2004**, *81*, 650−653.

(23) Surdyk, N.; Rosén, J.; Andersson, R.; Åman, P. Effects of asparagine, fructose and baking conditions on acrylamide content in yeast-leavened wheat bread. *J. Agric. Food Chem.* **2004**, *52*, 2047−2051.

(24) Vattem, D. A.; Shetty, K. Acrylamide in food: A model for mechanism of formation and its reduction. *Innovative Food Sci. Emerging Technol.* **2003**, *4*, 331−338.

(25) Amrein, T. M.; Schönbächler, B.; Escher, F.; Amadò, R. Acrylamide in gingerbread: Critical factors for formation and possible ways for reduction. *J. Agric. Food Chem.* **2004**, *52*, 4282−4288.

(26) Amrein, T. M.; Bachman, S.; Noti, A.; Biedermann, M.; Ferraz Barbosa, M.; Biederman-Brem, S.; Grob, K.; Keiser, A.; Realini, P.; Escher, F.; Amadó, R. Potential of acrylamide formation, sugars, and free asparagine in potatoes: A comparison of cultivars and farming systems. *J. Agric. Food Chem.* **2003**, *51*, 5556−5560.

(27) Amrein, T. M.; Schönbächler, B.; Rohner, F.; Lukac, H.; Schneider, H.; Keiser, A.; Escher, F.; Amadó, R. Potential for acrylamide formation in potatoes: Data from the 2003 harvest. *Eur. Food Res. Technol.* **2004**, *219*, 572−578.

(28) Olsson, K.; Svensson, R.; Roslund, C.-A. Tuber components affecting acrylamide formation and colour in fried potato: Variation by variety, year, storage temperature and storage time. *J. Sci. Food. Agric.* **2004**, *84*, 447−458.

(29) Williams, J. S. E. Influence of variety and processing conditions on acrylamide levels in fried potato crisps. *Food Chem.* **2005**, *90*, 875−881.

(30) De Wilde, T.; De Meulenaer, B.; Mestdagh, F.; Govaert, Y.; Vandeburie, S.; Ooghe, W.; Fraselle, S.; Demuelemeester, K.; Van Petegheim, C.; Calus, A.; Degroodt, J.-M.; Verhé, R. Influence of storage practices on acrylamide formation during potato frying. *J. Agric. Food Chem.* **2005**, *53*, 404−408.

(31) De Wilde, T.; De Meulenaer, B.; Mestdagh, F.; Govaret, Y.; Vanderburie, S.; Ooghe, W.; Fraselle, S.; Demeulemeester, K.; Van Peteghem, C.; Calus, A.; Degroodt, J.-M.; Verhé, R. Influence of fertilization on acrylamide formation during frying of potatoes harvested in 2003. *J. Agric. Food Chem.* **2006**, *54*, 404−408.

(32) De Wilde, T.; De Meulenaer, B.; Mestdagh, F.; Govaert, Y.; Ooghe, W.; Fraselle, S.; Demeulemeester, K.; Van Peteghem, C.; Calus, A.; Degroodt, J.-M.; Verhé, R. Selection criteria for potato tubers to minimize acrylamide formation during frying. *J. Agric. Food Chem.* **2006**, *54*, 2199−2205.

(33) Wicklund, T.; Ostlie, H.; Lothe, O.; Knutsen, S.; Brathen, E.; Kita, A. Acrylamide in potato crisp—the effect of raw material and processing. *Food Sci. Technol.* **2006**, *39*, 571−575.
(34) Vivanti, V.; Finotti, E.; Friedman, M. Levels of acrylamide precursors asparagine, fructose, glucose, and sucrose in potatoes sold at retail in Italy and in the United States. *J. Food Sci.* **2006**, *71*, 81−85.
(35) Lerner, S. E.; Seghezzo, M. L.; Molfese, E. R.; Pozio, N. R.; Cogliatti, M.; Rogers, W. J. N- and S-fertiliser effects on grain composition, industrial quality and end-use in durum wheat. *J. Cereal Sci.* **2006**, *44*, 2−11.
(36) Springer, M.; Fischer, T.; Lehrack, A.; Freund, W. Acrylamidbildung in Backwaren. *Getreide, Mehl Brot* **2003**, *57*, 274−278.
(37) GMF, 2005. Bread consumption in Germany. http://www.gmf-info.de (accessed April 2006).
(38) ESA, 2004. Chips sales in the EU. http://www.esa.org.uk/europe_statistics.php (accessed June 2000).
(39) Meat and More, 2006. Verzehrsmengen Pommes frites. http://meat-n-more.info/portal/news/news_buntes.php?we_objectID=4754 (accessed June 2006).
(40) Svensson, K.; Abramsson, L.; Becker, W.; Glynn, A.; Hellenas, K.-E.; Lind, Y.; Rosen, J. Dietary intake of acrylamide in Sweden. *Food Chem. Toxicol.* **2003**, *41*, 1581−1586.
(41) Weisshaar, R.; Acrylamid in Backwaren—Ergebnisse von Modellversuchen. *Dtsch. Lebensm.—Rundsch*. **2004**, *100*, 92−97.
(42) Claus, A.; Weisz, G. M.; Kammerer, D. R.; Carle, R.; Schieber, A. A method for the determination of acrylamide in bakery products using ion trap LC-ESI-MS/MS. *Mol. Nutr. Food Res.* **2005**, *49*, 918−925.
(43) Winkler, R.; Botterbrodt, S.; Rabe, E.; Lindhauer, M. G. Stickstoff-/Proteinbestimmung mit der Dumas-Methode in Getreide und Getreideprodukten. *Getreide, Mehl Brot* **2000**, *54*, 86−91.
(44) Sarath, G. Protease assay methods. In *Proteolytic Enzymes: A Practical Approach*, 2nd ed.; Beynon, R. J., Bond, J. S., Eds.; Oxford University Press: Oxford, 2001; pp 45−75.
(45) Langemeier, J. M.; Rogers, D. E. Rapid method for sugar analysis of doughs and baked products. *Cereal Chem.* **1995**, *72*, 349−351.
(46) Gutsche, B.; Weisshaar, R.; Buhlert, J. Acrylamide in food—screening results from food controlled in Baden-Württemberg. *Dtsch. Lebensm.—Rundsch.* **2002**, *98*, 437−443.
(47) Benedito De Barber, C.; Prieto, J. A.; Collar, C. Reversed-phase high-performance liquid chromatography analysis of changes in free amino acids during wheat bread dough fermentation. *Cereal Chem.* **1989**, *66*, 283−288.
(48) Elmore, J. S.; Koutsidis, G.; Dodson, A. T.; Mottram, D. S.; Wedzicha, B. L. Measurement of acrylamide and its precursors in potato, wheat, and rye model systems. *J. Agric. Food Chem.* **2005**, *53*, 1286−1293.
(49) Hansen, H. B.; Møller, B.; Andersen, S. B.; Jørgensen, J. R.; Hansen, Å. Grain characteristics, chemical composition, and functional properties of rye (*Secale cereale* L.) as influenced by genotype and harvest year. *J. Agric. Food Chem.* **2004**, *52*, 2282−2291.
(50) Sosulski, F. W.; Paul, E. A.; Hutcheson, W. L. The influence of soil moisture, nitrogen fertilization, and temperature on quality and amino acid composition of Thatcher wheat. *Can. J. Soil Sci.* **1963**, *43*, 219−228.
(51) Vigue, J. T.; Li, P. H. The influence of growing temperature on the amino acid and nucleic acid concentrations of potato tubers. *J. Am. Soc. Hortic. Sci.* **1976**, *101*, 341−344.
(52) Zahedi, M.; McDonald, G.; Jenner, C. F. Nitrogen supply to the grain modifies the effects of temperature on starch and protein accumulation during grain filling in wheat. *Aust. J. Agric. Res.* **2004**, *55*, 551−564.
(53) Garrido-Lestache, E.; López-Bellido, R. J.; López-Bellido, L. Durum wheat quality under Mediterranean conditions as affected by N rate, timing and splitting, N form and S fertilization. *Eur. J. Agron.* **2005**, *23*, 265−278.
(54) Haase, N. U.; Matthäus, B.; Vosmann, K. Acrylamid in Backwaren—ein Sachstandsbericht. *Getreide, Mehl Brot* **2003**, *57*, 180−184.
(55) Mustafa, A.; Andersson, R.; Rosén, J.; Kamal-Eldin, A.; Aman, P. Factors influencing acrylamide content and color in rye crisp bread. *J. Agric. Food Chem.* **2005**, *53*, 5985−5989.

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