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Influence of Sulfur Fertilization on the Amounts of Free Amino Acids in Wheat. Correlation with Baking Properties as well as with 3-Aminopropionamide and Acrylamide Generation during Baking

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Sulfur (S) fertilization has been long-known to influence the amounts of total free amino acids in plants. To determine the impact of S deficiency in wheat on the concentration of, in particular, free asparagine, the spring wheat cultivar ‘Star’ was grown in a laboratory scale (5 L pot) at five different levels of S fertilization. After maturity, the kernels were milled into white flours (1−5) and analyzed for their contents of total S and total nitrogen as well as for free amino acids and glucose, fructose, maltose, and sucrose. Extremely high concentrations of free asparagine (Asn; 3.9−5.7 g/kg) were determined in flours 1 and 2 (30 and 60 mg of S), whereas much lower amounts (0.03−0.4 g/kg) were present in flours grown at higher S levels. The amounts of the reducing carbohydrates were, however, scarcely affected by S fertilization. In agreement with the high amount of Asn in flours 1 and 2, heating of both flours led to the generation of very high amounts of acrylamide (1.7−3.1 mg/kg) as well as of 3-aminopropionamide (40−76 mg/kg). Similar concentrations were measured in crispbread prepared from both flours. Application of rheological measurements on doughs prepared from each flour and a determination of the loaf volume of bread baked therefrom clearly indicated that flours 1 and 2 would be excluded from commercial bread processing due to their poor technological properties. Two commercial flours showed relatively low concentrations of acrylamide after a thermal treatment.

KEYWORDS: **Acrylamide; 3-aminopropionamide; asparagine; baking; rheological properties; sulfur fertilization; wheat flour**

INTRODUCTION

Shortly after acrylamide had been characterized as a thermally generated constituent of many processed foods (*1*), it became a major challenge for the food industry to determine how to reduce the concentration of this potential carcinogen in foods such as fried potatoes or bread. Because Maillard-type reactions of the amino acid asparagine (Asn) and reducing sugars, or degradation products thereof, have previously been identified as the key step in acrylamide formation (*2*, *3*), the reduction of free Asn concentration in the raw materials, for example, by hydrolysis of the amide into aspartic acid by a pretreatment with asparaginase prior to the thermal treatment (*4*−*7*), or the use of potato cultivars low in carbohydrates (*8*) was suggested. In addition, some empirically determined changes in the respective recipes, such as the addition of calcium chloride (*9*), other free amino acids (*10*, *11*), or acids (*4*, *12*−*14*), or the reduction of ammonium hydrogen carbonate in the recipe of sweet baked goods, for example, gingerbread (*4*−*6*, *15*), have been proposed for mitigation.

In baked cereal products free Asn has been elucidated as the limiting factor in acrylamide formation (*4*, *5*, *16*−*18*). Thus, reduction of the free Asn concentration in flours should produce lower acrylamide concentrations after heat treatment of the flours during baking. In an earlier study, Karmoker et al. (*19*) have demonstrated that free Asn in the root tissue of young barley plants strongly increased during the first 4 days after the removal of sulfate. In addition, in a recent study, Claus et al. (*20*) have shown a significant influence of nitrogen (N) fertilization on the concentration of free Asn in different wheat and rye flours. The authors reported that, compared to the zero control, free Asn was increased by a factor of 4 when 220 kg of N/ha was

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applied. In bread produced with both flours, the concentrations of acrylamide also increased from ∼10 to 50 μg/kg. In that study (*20*), however, no influence of an additional sulfur (S) fertilization on the content of free Asn in the flour as well as on acrylamide formation in the heated flour was observed. In another, very recent, investigation, the influence of S fertilization on the amount of free Asn in different wheat cultivars was reinvestigated (*21*). In contrast, these authors found that in whole flours prepared from wheat kernels grown under conditions of S depletion, up to 30 times higher concentrations of free Asn were present as compared to control samples grown at normal S levels. Consequently, heating of such flours at 160 °C for 20 min in a model system led to an increase in the acrylamide level from about 720 to 5300 μg/kg (*21*). Although the influence on the acrylamide levels in bread prepared by using these flours was not studied, these data suggest that the N as well as the S fertilization is crucial for the accumulation of free Asn in wheat.

In previous studies (*22*−*24*), we have established 3-aminopropionamide as a potent intermediate in acrylamide formation. On the basis of studies using isotopically labeled Asn, we also showed that 3-aminopropionamide can be regarded as a transient, but also permanent, precursor of acrylamide in foods, such as cocoa or Gouda cheese (*24, 25*).

In Germany, as in many other countries, the consumption of bread and bread rolls is about 10 times higher as compared to that of French fries and potato chips (*20*, *26*−*28*). Therefore, it is of crucial interest to reduce the amounts of acrylamide, and also of its precursor 3-aminopropionamide, in baked goods. Therefore, the purpose of this study was (i) to reinvestigate the role of S depletion in growing wheat on the concentration of free Asn in the kernel; (ii) to correlate the amounts of 3-aminopropionamide and acrylamide formed during heating of the flours or during crispbread baking with S fertilization, respectively; (iii) to correlate the technofunctional properties of the flours with S supply; and (iv) to compare these results with data of commercial flour samples.

MATERIALS AND METHODS

Chemicals. [$^{13}C_3$]Acrylamide (99%) was obtained from CIL (Andover, MA), and 3-aminopropionamide hydrochloride was obtained from Chemos (Regenstauf, Germany). Acrylamide (99.9%), asparagine monohydrate, glucose, fructose, sucrose, and maltose were from VWR International (Darmstadt, Germany). 2-Mercaptobenzoic acid, 5-(dimethylamino)-1-naphthalene sulfonyl chloride (dansyl chloride), glycinamide hydrochloride, and norleucine were from Aldrich (Sigma-Aldrich, Steinheim, Germany). The enzymatic test kits were obtained from R-Biopharm (Darmstadt, Germany). All other reagents were of analytical grade.

Flour Samples. The German spring wheat cultivar ‘Star’ was grown in pots (5 L, 25 plants/pot; 10 replicates per treatment) supplied before sowing with either 30 mg (flour 1), 60 mg (flours 2 and 4), or 90 mg (flours 3 and 5) of S (applied as an aqueous solution of K_2SO_4). Potassium sulfate was uniformly mixed with the upper 6 cm layer of the soil (mixture of a loamy soil and a sandy soil in a ratio of 2:1). The S content in the soil was 2.6 mg/kg of soil (= 15 mg of SO_4^{2-}-S/pot). After 6 weeks of growth, flours 4 and 5 received an additional 60 mg of S. The supply of nitrogen, phosphorus, potassium (K compensation by KCl), and magnesium was optimal for normal development. Plants were cultivated under natural temperature and light conditions (11° 44′ eastern longitude, 48° 24′ northern latitude), protected only against rainfall, and regularly watered at 70% of the soil’s total water-holding capacity (200 g of water/kg of soil). The mature grains were milled (Quadrumat Junior; Brabender, Duisburg, Germany) into white flours with an ash content of 0.55% in dry mass. Samples were stored at room temperature prior to analysis. Commercial wheat flours with an ash content of 0.405% or 0.55% in dry mass, respectively, were purchased in a local supermarket. All flours were analyzed for free Asn and other amino acids as well as for glucose, fructose, maltose, and sucrose. The water content of the flours was determined gravimetrically after heating at 130 °C for 2 h.

Quantitation of Total Sulfur and Total Nitrogen. The S content of the flours [0.3 g of dry sample with nitric acid (3.5 mL) and hydrogen peroxide (2.5 mL)] was determined after microwave digestion (45 min, 1200 psi; MDS-2100, CEM Corp., Matthews, NC) in closed vessels by means of inductively coupled plasma atomic emission spectrometry at 182.034 nm (ICP-AES Liberty RL, Varian, Mulgrave, Australia). For quantitation, an international certified S standard was used (Community Bureau of Reference, BCR 129; Brussels, Belgium) containing 3.16 ± 0.04 mg of S/g. The S content of the standard measured by ICP-AES was 3.16 ± 0.13 mg of S/g. The N content of the flours was determined according to the Dumas method using a protein-nitrogen analyzer Leco FP-328 (Leco Corp., St. Joseph, MI).

Quantitation of Asparagine. Aqueous buffer (5 mL) containing the internal standard norleucine (0.1−0.5 μg, depending on the amount of Asn determined in a preliminary experiment) was added to the flour sample (100−400 mg), and the suspension was homogenized with an Ultraturrax (Jahnke & Kunkel, IKA-Labortechnik, Staufen, Germany) for 2 min. After centrifugation (10000 rpm, 30 min, at 20 °C; Beckman J2-HS, Munich, Germany), the supernatant was filtered through a 0.45 μm Spartan filter 13/0.45RC (Schleicher & Schuell, Dassel, Germany), and the Asn concentration was analyzed by means of an LC 3000 amino acid analyzer (Onken, Gründau, Germany) as reported recently (*29*). For calibration, defined mixtures of Asn and norleucine were analyzed (mixtures ranging from 3:1 to 1:3 in a molar ratio). Buffer solution was prepared as follows: Lithium acetate dihydrate (16.3 g), formic acid (7.5 mL), thiodiglycol (20 mL; 25%, w/w in water), and octanoic acid (0.1 mL) were dissolved in water (900 mL), and the pH was adjusted to 2.20 with trifluoroacetic acid and made up to 1000 mL with water.

Quantitation of Carbohydrates. The amounts of glucose, fructose, sucrose, and maltose were analyzed by means of enzymatic test kits (R-Biopharm).

Thermal Processing of Samples. *Model System.* Binary mixtures of Asn and either glucose or fructose (100 μmol each) were homogenized with silica gel 60, 0.063−0.200 mm (3 g, containing different amounts of water; VWR International) and heated for 30 min at 170 °C in closed glass vessels. After cooling, the internal standard [$^2H_3$]-acrylamide and water (20 mL) were added, and the mixture was stirred for 15 min. After ultrasonification (4 min), the suspension was centrifuged (4000 rpm; 10 min at 10 °C). The supernatant was filtered, and an aliquot of the reaction mixture (5 mL) was applied onto an Extrelut NT 20 column (VWR International); after equilibration for 15 min, elution of the analyte and the internal standard was performed using ethyl acetate. The solution was dried over anhydrous sodium sulfate and concentrated to about 5 mL at 20 kPa and 40 °C. This extract was used for GC-MS analysis (*24*).

Flour. Flour (400 mg) was filled into a glass vessel and heated for 20 min at 170 °C. The amount of 3-aminopropionamide was determined after derivatization with dansyl chloride (*23*). Acrylamide was quantified by LC-MS-MS after derivatization with 2-mercaptobenzoic acid (*30*).

Microscale Baking of Crispbread. Wheat flour (1.5 g) and an aqueous sodium chloride solution (2 mL; 10 g/L) were filled into a plastic baking dish (40 × 18 × 10 mm; width, length, height) and mixed for 3 min at room temperature using a metal spatula. The baking dish was part of a special device containing 15 slots in total. The resulting dough was stored for 30 min at room temperature and then baked at 250 °C for 10 min. The crispbread was cooled to room temperature, and the acrylamide formed was quantified as reported in ref *30*.

Rheological Measurements and Baking Tests. Rheological measurements and baking tests were carried out according to the methods of ref *31* with some modifications. For dough rheology, flour (10 g) and sodium chloride (0.2 g) were mixed in a micro-Farinograph (Brabender) for 1 min at 60 rpm and 22 °C. Distilled water was added, and the dough was mixed until a maximum consistency of 550 Brabender units (BU) was reached. The dough was removed, shaped to an ellipsoid form, and pressed into PVDF (Teflon) forms to give strands of 53 × 4 × 4 mm. After 40 min of resting in a desiccator at

Table 1. Content of Nitrogen (N) and Sulfur (S) in Unheated White Wheat Flours from Grains Differing in Sulfur Fertilization and in Unheated Commercial Flours

flour	amount of S added[a] (mg)	grain yield (g/pot)	N[b,c] (%)	S[b,c] (%)	ratio N/S	moisture[b] (%)
1	30	30.3	2.09	0.066	31.7	14.5
2	60	41.3	2.44	0.084	29.0	14.5
3	90	42.0	2.75	0.128	21.5	14.8
4	60 + 60	38.3	2.79	0.143	19.5	15.1
5	90 + 60	44.3	2.74	0.158	17.3	14.9
C1[d]	nd[e]	nd	2.09	0.135	15.5	13.1
C2[d]	nd	nd	2.19	0.142	15.4	13.4

[a] The original S content in the soil was 15 mg/pot. [b] Analyses were performed in triplicates. [c] Amount based on dry mass. The relative standard deviation was below 2%. [d] Commercial wheat flours: C1, ash content 0.405% in dry mass; C2, ash content 0.55% in dry mass. [e] Not defined.

22 °C in a water-saturated atmosphere, the strands were measured with a texture analyzer (Stable Microsystems, Godalming, U.K.).

For baking, flour (10 g), distilled water (5 mL), a solution of sodium chloride and sucrose (1 mL; sodium chloride, 200 mg/mL; sucrose, 100 mg/mL), a solution of L-ascorbic acid (0.05 mL; 4 mg/mL), and yeast (0.7 g) were added and mixed for 1 min at 1250 rpm and 15 °C. After the dough was removed from the mixer, it was allowed to rest for 20 min at 30 °C in a water-saturated atmosphere. Then, the dough was reshaped on a dough rounder for eight cycles. The resulting dough ball was passed through the rolls of a pasta machine to form an oval dough piece. This was folded and reshaped on the dough rounder for 15 cycles. The resulting spherical dough piece was proofed for 45 min at 30 °C in a water-saturated atmosphere and baked for 10 min at 230 °C. The volume of the bread was determined after wax coating by measuring the amount of water displaced by the bread at room temperature (*31*).

RESULTS AND DISCUSSION

To grow wheat under defined conditions, pots were filled with the same soil and increasing amounts of K_2SO_4 were added (30−150 mg of S). After ripening of the kernels, first, the influence of the increasing S fertilization on the total amount of N and S in the samples was determined. The total S content in the wheat flours rose with increasing the amounts of S administered to the soil (**Table 1**). Low S dosages (flours 1 and 2) also affected the total N content, but when amounts of ≥90 mg were applied, the N content remained constant. Wheat with an S content of <0.12% is regarded as S-deficient (*32*), and, as to be expected, S dosages below 90 mg resulted in S depletion compared to common agricultural practices. The ratio of N to S, which is another indicator for S deficiency (*32*), indicated that only flour 5 was provided with an optimal amount of S (ratio of N/S ≤ 17). Both commercial flours met these conditions, very well indicating a sufficient S supply during wheat growth.

Because free Asn is generally recognized as the most important precursor in acrylamide formation, its concentration was determined next in the five flours (**Table 2**). The results showed that flours 1 and 2, which were produced from the wheat samples most deficient in S, contained very high amounts of free Asn. However, at an S dosage of ≥90 mg (flours 3−5), the concentration of free Asn dropped dramatically from 5688 (flour 1) to 35 mg/kg of flour (flour 5). The commercial flour with an ash content of 0.55% in dry mass showed an Asn concentration similar to that of flour 4 (**Table 2**). This finding was not in agreement with the results of Claus et al. (*20*), who found no influence of S fertilization on the amounts of free Asn in wheat. However, our data agreed very well with results reported by Muttucumaru et al. (*21*), who recently reported an extreme influence of S depletion on the concentration of free Asn in wheat, confirming that only at higher S deficiencies did wheat produce very huge amounts of free Asn.

Table 2. Concentrations of Asparagine and Other Free Amino Acids in Unheated White Wheat Flours from Grains Differing in Sulfur Fertilization and in Commercial Flours[a]

	concn (mg/kg)					
flour	asparagine	glutamine	aspartic acid	glutamic acid	serine	threonine
1	5688 (163)[b]	2440	1259	927	499	209
2	3920 (112)	1510	1182	616	276	153
3	426 (12.2)	122	422	151	42	28
4	142 (4.1)	45	110	46	7.6	7.1
5	35 (1.0)	11	72	26	5.0	4.7
C1[c]	112 (3.2)	23	212	70	14	9.7
C2[c]	130 (3.7)	30	259	89	20	11

[a] Analyses were performed in triplicates; relative standard deviation below 9%. [b] Relative concentrations calculated by assigning the concentration in flour 5 as 1.0. [c] Commercial wheat flours: C1, ash content of 0.405% in dry mass; C2, ash content of 0.55% in dry mass.

Table 3. Concentrations of Glucose, Fructose, Maltose, and Sucrose in Unheated White Wheat Flours from Grains Differing in Sulfur Fertilization and in Commercial Flours[a]

	concn (g/kg)			
flour	glucose	fructose	maltose	sucrose
1	0.51	0.39	21.04	3.44
2	0.32	0.20	15.31	3.39
3	0.33	0.25	7.74	3.16
4	0.39	0.29	9.73	3.10
5	0.45	0.32	9.83	3.12
C1[b]	0.47	0.25	6.68	4.72
C2[b]	0.49	0.30	3.63	5.50

[a] Analyses were performed in triplicates; relative standard deviation below 2%. [b] Commercial wheat flours: C1, ash content of 0.405% in dry mass; C2, ash content of 0.55% in dry mass.

In addition, the concentrations of other free amino acids were also determined. They were significantly increased in S-depleted flours and, in particular, glutamine increased in the same order of magnitude as Asn, whereas other amino acids, such as serine and threonine, increased less (**Table 2**).

Reducing sugars and their degradation products as well as carbohydrates in general (e.g., sucrose) are proposed as important reaction partners of Asn in acrylamide generation (*2*, *3*, *22*). However, as shown in **Table 3**, the concentrations of glucose, fructose, and sucrose were hardly affected by the S fertilization, except that a variation by a factor of 2.7 was found for maltose.

To check the influence of S fertilization on acrylamide formation, aliquots of the five flours were then heated in glass vessels. As expected, heating of flours 1 and 2, which are high in Asn, generated very high amounts of 3124 and 1703 μg of acrylamide/kg of flour, respectively (**Table 4**). These concentrations were well in line with the high amounts of Asn in these flours produced from S-depleted wheat (**Table 2**). By contrast, thermal treatment of flours 3−5 produced from wheat grown at elevated S levels generated much lower amounts of acrylamide. From two commercial wheat flours (C1 and C2), the acrylamide levels formed after thermal processing were in the same order of magnitude as found for flour 4 produced from kernels grown at an S level of 120 mg (**Table 4**). These findings

Table 4. Concentrations of Acrylamide (AA) and 3-Aminopropionamide (3-APA) Generated from the Five Flours and from Commercial Flours during Heat Processing[a]

	AA		3-APA	
flour	concn (μg/kg)	rel concn[b]	concn (mg/kg)	rel concn[b]
1	3124 (4.0)[c]	33.3	76.01 (7.2)[c]	200.0
2	1703 (0.6)	18.1	39.58 (5.7)	104.2
3	460 (0.4)	4.9	4.84 (2.0)	12.7
4	155 (3.8)	1.7	1.42 (12.0)	3.7
5	94 (6.1)	1.0	0.38 (1.9)	1.0
C1[d]	157 (12.1)	1.7	1.47 (3.4)	3.9
C2[d]	213 (4.1)	2.3	1.74 (4.1)	4.6

[a] Analyses were performed in triplicates. Heating of the flour (400 mg) was performed for 20 min at 170 °C. [b] Relative concentrations calculated by assigning flour 5 as 1.0. [c] Relative standard deviation. [d] Commercial wheat flours: C1, ash content of 0.405% in dry mass; C2, ash content of 0.55% in dry mass.

Table 5. Concentrations of Acrylamide (AA) Generated in Crispbread Produced from the Five Flours and from Commercial Flours[a]

bread prepared from flour	AA (μg/kg)	RSD[b] (%)
1	2534	6.2
2	3010	4.9
3	401	12.1
4	145	12.4
5	89	15.2
C1[c]	162	11.2
C2[c]	175	9.2

[a] Analyses were performed in duplicates. [b] Relative standard deviation. [c] Commercial wheat flours: C1, ash content of 0.405% in dry mass; C2, ash content of 0.55% in dry mass.

Table 6. Influence of the Water Content on the Formation of Acrylamide (AA) from Asparagine (Asn) in the Presence of Glucose or Fructose[a]

	AA (mmol/mol of Asn) formed in the presence of	
water content (%)	glucose	fructose
0	1.9	1.5
2.5	2.3	1.7
5	2.8	1.9
10	6.2	4.8
25	9.4	7.6

[a] Analyses were performed in triplicates. Asparagine (0.1 mmol) and the respective carbohydrate (0.1 mmol) were mixed with silica gel (3 g, water content given in table) and reacted for 30 min at 170 °C in a closed glass vial.

are in good agreement with results published by Muttucumaru et al. (*21*), who found an increase in the amounts of the amide from about 720 to 5300 μg/kg after heating (160 °C; 20 min) of a flour from grains differing in S fertilization (from 40 to 0 kg of S/ha).

In particular for flours 1 and 2, Asn concentrations were not perfectly correlated with acrylamide formation (**Tables 2** and **4**). This may be because in flours obtained from kernels grown under sufficient S supply (flours 4 and 5), the molar amount of free Asn (1.1 and 0.3 mmol/kg) was considerably lower than the concentrations of reducing carbohydrates (32 and 33 mmol/kg) (**Tables 2** and **3**). Therefore, in these flours, acrylamide formation was limited by the concentration of available free Asn. However, in the S-deficient flours 1 and 2 the molar amounts of Asn (43 and 30 mmol/kg), and also of other free amino acids, were considerably higher (up to a factor of about 140), whereas the molar amounts of reducing carbohydrates were only doubled at most (66 and 48 mmol/kg) (**Tables 2** and **3**). Thus, lower amounts of reducing carbohydrates were available as partners to react with Asn in the generation of acrylamide. Therefore, a comparatively lower amount of acrylamide was formed from flours 1 and 2 despite the higher concentrations of Asn as compared to flours 3−5 (**Tables 2** and **4**).

In previous studies, we have estalished 3-aminopropionamide as a transient intermediate in acrylamide formation, but quantitative studies on its occurrence in foods showed that 3-aminopropionamide might also be regarded as a direct precursor of acrylamide, for example, when foods are reheated (*23*−*25*). To get an insight into the role of 3-aminopropionamide in acrylamide formation from wheat flours, its concentrations were determined after thermal processing of the five flours as well as of the two commercial wheat flours. The results showed extremely high concentrations of 3-aminopropionamide of 76 and 40 mg/kg in flours 1 and 2 produced from the S-depleted wheat, respectively (**Table 4**). These amounts were the highest ever determined in a food before (*23*−*25*). As found for acrylamide, also much lower amounts of 3-aminopropionamide were generated from flours grown at an S level of ≥90 mg and, thus, containing lower amounts of Asn. Also, the amounts of 3-aminopropionamide formed in heated flours were correlated very well with S fertilization, which can be regarded as further proof of the key role of 3-aminopropionamide in acrylamide formation. The two commercial flours also generated 3-aminopropionamide (C1 and C2; **Table 4**), but the much lower amounts clearly indicated that these flours were manufactured from wheat grown under suitable S conditions (compare to flour 4).

In a last series of experiments the five flours were used in crispbread making. Because only low amounts of flours were available, a microscale baking test with 1.5 g of flour was used. Doughs were baked for 10 min at 250 °C to obtain crispbread with good texture and color, and the amounts of acrylamide formed were determined using the entire crispbread. As found for the processed flours, the amounts of acrylamide were highest in crispbread produced from flours 1 and 2 (**Table 5**), which contained the highest amounts of free Asn. Crispbread prepared from flours 3−5 contained significantly lower amounts of acrylamide, as was to be expected due to the lower amounts of free Asn present (**Table 2**). In agreement with their low Asn content, crispbreads prepared from the two commercial flours also contained lower amounts of acrylamide.

Except in crispbread from flour 2, the amounts of acrylamide formed during baking were quite in line with the results obtained by heating these flours alone. The higher amount of acrylamide formed in the crispbread made from flour 2 (3010 μg/kg) (**Table 5**) compared to the heated flour 2 (1703 μg/kg) (**Table 4**) might be explained by the different water content of the crispbread (water content at the beginning of baking was 63%) as compared to the heated flours (water content of 13.1−15.1%) (**Table 1**).

Thus, to study the effect of the water content, model experiments were performed, in which Asn and glucose or fructose, respectively, were reacted in the presence of increasing amounts of water. As shown in **Table 6**, when this binary mixture was reacted in a completely dry system, the yields dropped to about one-third of those formed in a matrix with 10% of water. Interestingly, the yields of acrylamide were further increased when the reaction was per-

Table 7. Technological Properties of Doughs Made from Wheat Flours Out of Grains Differing in Sulfur Fertilization

flour	loaf vol[a] (mL)	dough development time[b] (min)	resistance to extension[b] (mN)	extensibility[b] (mm)	extension energy[b] (N × mm)
1	19.8	2.0	138	29	1.5
2	24.8	2.0	169	38	2.1
3	46.7	16.5	331	61	11.3
4	47.5	15.5	293	84	14.2
5	55.3	14.5	316	92	17.1

[a] Analyses were performed in a single experiment. [b] Analyses were performed in quintuplicates; relative standard deviation below 9%.

formed at a water content of 25% (**Table 6**). These results indicated that the formation of acrylamide needs at least a certain amount of water to generate distinct transient intermediates, such as 3-aminopropionamide, able to release acrylamide upon further thermal treatment (*22*). The influence of the water content on acrylamide formation was also investigated and confirmed in potato model systems (*33*, *34*). The slight decrease of acrylamide in the crispbread made from flour 1 compared to crispbread made from flour 2 (**Table 5**) can be similarly explained by the results obtained for the heated flours. The enormous increase of the other free amino acids competing with free Asn for the reaction partners (e.g., reducing carbohydrates), which remained nearly constant, diminished the generation of acrylamide from Asn. First, an increase of Asn led to an increase of acrylamide, but a further increase of Asn could not form acrylamide to the same degree.

The data clearly showed that growing wheat under S-deficient conditions may lead to crispbread with high concentrations of acrylamide and, in addition, extremely high concentrations of its precursor, 3-aminopropionamide. Because the latter is able to generate acrylamide without the need of carbohydrates (*22*−*24*), it may easily generate an additional amount of acrylamide, for example, upon reheating or toasting of bread.

S deficiency was also reported to influence the protein composition (*35*) of wheat flour as well as the technological properties of the dough made of it, such as a decreased extensibility, a decrease of the resistance to extension, a decrease of the loaf volume, and a poorer texture (*36*−*42*). These changes are caused by a modification of the protein composition, and it has been shown that the concentration of S-poor proteins, such as ω-gliadins, increased, whereas the concentration of S-rich proteins such as α- and γ-gliadins, as well as of low molecular weight (LMW) glutenin subunits, decreased by S deficiency (*35*). These results were also confirmed in the present study (data not shown).

Studies performed so far report either on the influence of S fertilization on the technological properties of wheat (*35*−*42*) or on the effect of free amino acids, or on acrylamide formation (*19*−*21*). However, up to now, a correlation of technofunctional properties and acrylamide formation has not been performed. To close this gap, mixing experiments on a Farinograph and extension tests on a texture analyzer were performed to check the technofunctional properties of the five flours. Flours 1 and 2 had poor textural properties (**Table 7**), such as short dough development time, low resistance to extension, low extension energy, and low extensibility, which may be due to the low glutenin content of these flours (*35*). Flours 3−5 showed much better properties, but the effect of S fertilization was still obvious. In most parameters, the rheological data correlated well with the different S contents and N/S ratios of the flours (**Tables 1** and **7**).

Finally, microscale baking tests using 10 g of flour were carried out. Bread made with flour 5 had by far the highest loaf volume (55.3 mL) followed by flour 4 (47.5 mL) and flour 3 (46.7 mL), whereas the loaf volumes of the bread prepared from S-deficient flours 1 and 2 were substantially lower (19.8 and 24.8 mL, respectively; **Table 7**).

Due to flue gas desulfurization, SO_2 emission in Germany has been reduced from >5000 kt/year at the end of the 1980s to about 700 kt in 2003 (*43*). Consequently, S deposition to arable land decreased from about 80 kg of S/ha and year to 5−10 kg of S/ha and year (*43*). As a result, S deficiency may occur under unfavorable site conditions (especially shallow, coarse textured soils with low contents of organic matter and a low groundwater table). It is, therefore, recommended to evaluate the site-specific S availability and to supply sufficient amounts of S by fertilization, if necessary. However, taking into account the influence of S depletion on the chemical and technological data, it can be assumed that S-deficient wheat might not cause a high risk for the consumer, because, on the one hand, the poor technological properties of flours 1 and 2 would exclude such highly S-deficient samples from application in breadmaking; on the other hand, even flour from wheat kernels grown under slight S-deficient conditions can be used for breadmaking due to its technological properties (flour 3; **Table 7**) and with respect to only moderate acrylamide concentration. Furthermore, both good technological properties and a very low potential for acrylamide formation of <160 μg/kg during thermal processing (**Tables 4** and **5**) were obtained using flours 4 and 5. The chemical data of the commercial flour samples were in good agreement with these flours (**Tables 2**, **4**, and **5**). Thus, due to missing data for commercial flours in already published studies (*20*, *21*), the data of the present study suggest for the first time that commercial wheat flours can be used in baking without a high risk of producing high amounts of acrylamide, despite the low number of commercial flours investigated. Nevertheless, an S content of at least 0.13% and an N/S ratio lower than 20 is recommended for a high-quality baking performance, on the one hand, and a low content of free Asn on the other hand. This approach will help to reduce the contribution of cereals to the overall consumption of acrylamide in the human diet.

SAFETY

CAUTION: Acrylamide and [$^{13}C_3$]acrylamide as well as dansyl chloride are hazardous and must be carefully handled.

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