Fermentation Reduces Free Asparagine in Dough and Acrylamide Content in Bread

H. Fredriksson,[1,2] J. Tallving,[3] J. Rosén,[4] and P. Åman[3]

Cereal Chem. 81(5):650–653

ABSTRACT

Free asparagine is an important precursor for acrylamide in cereal products. The content of free asparagine was determined in 11 milling fractions from wheat and rye. Whole grain wheat flour contained 0.5 g/kg and whole grain rye flour 1.1 g/kg. The lowest content was found in sifted wheat flour (0.2 g/kg). Wheat germ had the highest content (4.9 g/kg). Fermentation (baker's yeast or baker's yeast and sourdough) of doughs made with the different milling fractions was performed to investigate whether the content of free asparagine was reduced by this process. In general, most of the asparagine was utilized after 2 hr of fermentation with yeast. Sourdough fermentation, on the other hand, did not reduce the content of free asparagine as efficiently but had a strong negative impact on asparagine utilization by yeast. This indicates that this type of fermentation may result in breads with higher acrylamide content than in breads fermented with yeast only. The effect of fermentation time on acrylamide formation in yeast-leavened bread was studied in a model system. Doughs (sifted wheat flour with whole grain wheat flour or rye bran) were fermented for a short (15+15 min) or a long time (180+180 min). Compared with short fermentation time, longer fermentation reduced acrylamide content in bread made with whole grain wheat 87%. For breads made with rye bran, the corresponding reduction was 77%. Hence, extensive fermentation with yeast may be one possible way to reduce acrylamide content in bread.

It was recently discovered that heat-treated starch-rich foods may contain high levels of acrylamide (Tareke et al 2002). Later, a link between acrylamide formation in food and the Maillard reaction was suggested (Mottram et al 2002; Stadler et al 2002; Friedman 2003). Acrylamide is a known neurotoxin, a carcinogen in animals, and a probable carcinogen in humans (IARC 1994; Lingnert et al 2002; Friedman 2003).

It is now well established that free amino acids, mainly asparagine, and reducing sugars are important precursors for acrylamide in foods and that raw materials and processing conditions such as time, temperature, pH, water activity, additives, and matrix will influence its formation and degradation (Rydberg et al 2003). During baking of yeast-leavened wheat bread, the effects of asparagine and fructose, added to the dough, were studied in a designed experiment (Surdyk et al 2004). Added asparagine dramatically increased the acrylamide content while added fructose did not, indicating that in this system, reducing sugars not were a limiting factor.

Little is known about the content of free asparagine in cereals and cereal fractions (Friedman 2003). During bread dough fermentation, amino acids are assimilated by yeast or lactic acid bacteria and metabolized as a source of nitrogen for growth (Benedito De Barber et al 1989). Yeast fermentation of wheat bread dough reduced the content of free asparagine by 92%. Fermentation of wheat doughs with lactic acid bacteria decreased the content of free asparagine with ≈60% (Collar et al 1991).

The aim of this study was to investigate the content of free asparagine in milling fractions of wheat and rye and to study the possibility to reduce the content by fermentation in different dough systems. A baking experiment was also performed to evaluate whether doughs with long fermentation time resulted in breads with reduced content of acrylamide.

[1] Cerealia R & D AB, SE-153 81 Järna, Sweden.

[2] Corresponding author. Phone: +46851978950. Fax: +46851978965. E-mail: helena.fredriksson@cerealia.se

[3] Swedish University of Agricultural Sciences, Department of Food Science, PO Box 7051, SE-750 07 Uppsala, Sweden.

[4] National Food Administration, PO Box 622, SE-751 26 Uppsala, Sweden.

Publication no. C-2004-0805-03R.

MATERIALS AND METHODS

Cereal Fractions

Eleven different commercial milling fractions of wheat (sifted flour, fine shorts, coarse shorts, bran, germ, whole grain flour) and rye (sifted flour, coarse shorts, bran, whole grain flour) were obtained from Cerealia, Järna, Sweden, and Wasabröd, Filipstad, Sweden (Table I). A number of fractions were collected twice, they are referred to as batch A and batch B. All milling fractions were analyzed for content of ash and free asparagine.

General Analyses

Dry matter content was determined by drying samples at 105°C for 6 hr. All results are given on dry matter basis, if not otherwise stated. Ash content was determined by placing samples in an oven at 600°C for 2 hr, followed by cooling, moistening with distilled water, and another 1 hr in the oven at 600°C. The content of free asparagine was analyzed according to Davies (2002). Free amino acids were extracted with sulfosalicylic acid. A synthetic amino acid, norleucine, was used as internal standard. Amino acids were separated by cation exchange chromatography and quantified by postcolumn derivatization with ninhydrine using a Biochrom Analysator (Pharmacia Biotech, Uppsala, Sweden).

Dough Preparation and Yeast Fermentation

Doughs were prepared with sifted wheat flour as base and cereal fractions as outlined in Table II. The disintegrated coarse shorts fraction from wheat was obtained by impact milling in a pilot system from Alpine AG (Augsburg, Germany) as described by Andersson et al (2000). The disintegrated wheat germ was obtained by treatment in a food blender. Particle size of the two milling fractions was determined by stack-sieving (Bühler Miag, model 300/B, Milano, Italy) for 30 min, using sieves with the openings of 50, 75, 150, 340, 670, and 1,110 µm.

Doughs were prepared in a farinograph (Brabender, Duisburg, Germany) using a modified version of Approved Method 54-21 (AACC 2000) without sugar to peak of the curve at 500 BU instead of 400 BU. The basic recipe included 180 g of sifted wheat flour, 2.2 g of dry yeast (*Saccharomyces cerevisiae,* Jästbolaget AB, Sweden), 1.8 g of sodium chloride, and 125 mL of tap water. After mixing, the dough was divided into six equal pieces. Fermentation then took place at 33°C and 60% rh. Samples were taken for analysis of free asparagine after 0, 0.5, 1.0, 2.0, 4.0, and

6.0 hr of fermentation from doughs made with the base flour mixed with whole grain wheat flour or rye bran from batch A. For doughs made from other flour mixtures, samples were only taken after 0 and 6 hr of fermentation. After withdrawal, samples were immediately put in a freezer at –40°C, lyophilized, and milled (Retch, model ZM1, Haan, Germany) to pass a 0.5-mm screen.

Sourdough Preparation and Fermentation with Sourdough and Yeast

A spontaneous sourdough was prepared by mixing 0.5 kg of whole grain rye flour (Wasabröd) and tap water (40°C) at a ratio of 1:1, followed by fermentation at 28°C for 72 hr. Refreshment was then made by mixing one part of sourdough (200 g) with nine parts of the flour and water (1:1). Thereafter, the dough was fermented for 24 hr as above (96-hr sample). To facilitate bacterial growth, the dough was mixed twice a day. Two samples were taken during fermentation after 0, 72, and 96 hr. One sample was used for pH determination and the other for analysis of free asparagine content. Samples collected for analysis of free asparagine were immediately frozen, lyophilized, and milled as described above. Doughs were also prepared, fermented, and sampled as above, with the different milling fractions, sourdough (96 hr), and yeast (Table II).

Baking and Analysis of Acrylamide

Doughs were essentially prepared as above and breads baked as previously described by Surdyk et al (2004) with the base flour mixed with whole grain wheat flour or rye bran from batch B. In this experiment, the doughs were fermented in two steps, first at 33°C and 60% rh and then at 39°C and 85% rh. Short (15+15 min) and long (180+180 min) fermentation times were used. The doughs were baked at 270°C for 15 min and acrylamide was analyzed in the lyophilized and milled crust.

Lyophilized crust (4 g) was mixed with water (40 mL) and internal standard (400 µL of water solution with deuterium-labeled acrylamide 1.0 µg/mL). The sample was extracted by means of an

TABLE I
Extraction Rates and Content of Ash and Free Asparagine in Milling Fractions of Wheat and Rye

Milling Fraction	Extraction Rate (%)	Ash Content (g/kg, dmb)	Free Asparagine (g/kg, dmb)
Wheat			
Whole grain flour (batch A)	100	17.0	0.51
Whole grain flour (batch B)	100	14.2	0.48
Sifted flour, base flour (batch A)	73–78	6.5	0.17
Sifted flour, base flour (batch B)	73–78	5.8	0.14
Fine shorts	6	43.4	1.93
Coarse shorts	<5	30.0	1.59
Bran	14	61.0	1.48
Germ (batch A)	0.3	55.5	4.88
Germ (batch B)	0.3	57.4	4.99
Rye			
Whole grain flour	100	16.2	1.07
Sifted flour (batch A, Cerealia)	80	7.9	0.53
Sifted flour (batch B, Wasabröd)	80	7.9	0.68
Coarse shorts	2	31.8	2.39
Bran (batch A)	18	55.0	3.18
Bran (batch B)	18	53.6	2.61

TABLE II
Asparagine Content in Doughs Made from Sifted Wheat Flour and Wheat or Rye Milling Fractions, Before and After Fermentation (6 hr)

	Asparagine Content		
Milling Fractions[a]	Before Fermentation (g/kg, dmb)	After Fermentation (g/kg, dmb)	Reduction (%)
Wheat			
Sifted flour (100% base flour)	0.17	0.01	96
Fine shorts	0.93	0.12	87
Coarse shorts	0.82	0.13	84
Disintegrated coarse shorts	0.82	0.05	94
Bran	0.73	0.06	92
Germ (batch A)	2.25	1.02	55
Disintegrated germ (batch B)	2.65	0.60	77
Whole grain (batch A)	0.34	0.05	85
Whole grain (batch B)	0.29[b]	0.05[c]	82
Rye			
Sifted flour (Cerealia)	0.31	0.03	90
Sifted flour (Wasabröd)	0.41	0.10	75
Coarse shorts	0.99	0.05	95
Bran (batch A)	1.43	0.07	95
Bran (batch B)	1.33[b]	0.05[c]	96
Whole grain	0.56	0.03	94
Whole grain (100%)[d]	0.99	0.04	96
Whole grain rye sourdough (96 hr)			
Sourdough	0.66	0.55	17
Whole grain rye (25%) and sourdough (25%)	0.74	0.34	54
Sifted rye flour (37.5%), whole grain rye (37.5%) and sourdough (25%)[d]	0.94	0.47	50

[a] 50% of different wheat or rye fractions and 50% base flour, if not otherwise stated.
[b] Dough fermented 15+15 min.
[c] Dough fermented 180+180 min.
[d] In this mixture, no base flour was included.

Ultra Turrax homogenizer (2 min, 9,500 rpm) and centrifuged in a cooling centrifuge (10°C, 4,000 rpm, 20 min). Supernatant (10 mL) was filtered through a solid-phase extraction (SPE) column (multimode, 1 g) and pretreated with acetonitrile (3 mL) and water (2 × 6 mL) in a vacuum manifold. The filtered extract (≈10 mL) was then loaded onto a second SPE column (ENV+, 1 g) that had been pretreated with methanol (5 mL) and water (5 mL), all in a vacuum manifold. The column was rinsed with water (2 × 5 mL) and then eluted with 60%, v/v, methanol in water. The first fraction of the eluate (1.7 mL) was discarded and only the eluate fraction at 1.7–3.7 mL was collected. The collected extract was evaporated by means of a vortex evaporator under vacuum (40°C, 20–25 min) until ≈1 mL of the extract remained. The final extract (10 µL) was analyzed with liquid chromatography tandem mass spectrometry (LC-MS-MS) according to the method developed by Rosén and Hellenäs (2002).

Statistical Analysis

Analysis of linear regression was performed using Microsoft Excel 97.

RESULTS AND DISCUSSION

Flour Fractions

Content of ash and free asparagine was determined in all the collected milling fractions (Table I). Ash content was within the specification given by the millers. Free asparagine content was 0.5 g/kg in wheat and 1.1 g/kg in rye. This is higher than the values previously reported both for wheat (0.2 and 0.3 g/kg) and for rye (0.2g/kg) (Tkachuk 1979; Mottram et al 2002). Sifted flour from wheat (0.2 g/kg) also contained lower amounts of free asparagine compared with the sifted rye flour (0.6 g/kg). Shorts and bran fractions had intermediate values and wheat germ the highest content of free asparagine (4.9 g/kg). The rye germ was not separated in a discrete fraction during the milling. In general, the higher content of free asparagine in rye endosperm fractions was a result of the initially higher content in the rye grain but probably also a consequence of less efficient separation of starchy endosperm from bran and germ during milling.

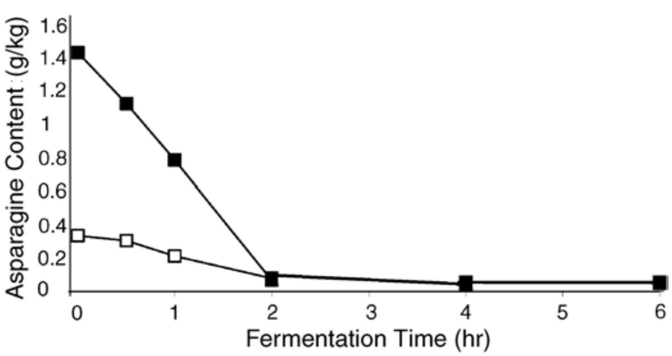

Fig. 1. Free asparagine content before and after different fermentation times in doughs made from a 1:1 mixture of base flour and whole grain wheat flour (□) or rye bran (■) from batch A.

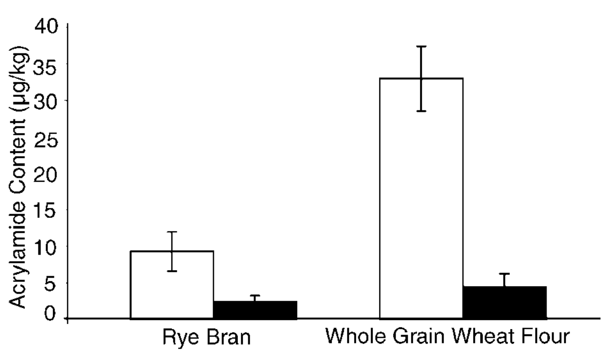

Fig. 2. Calculated acrylamide content in fresh bread (n = 3) made from a mixture (1:1) of base flour with rye bran or whole grain wheat flour from batch B. Doughs were fermented for 15+15 min (□) or 180+180 min (■). Bars show standard deviations.

Yeast Fermentation

Doughs were prepared with base flour and milling fractions, (see Table II). Two of the fractions, rye bran and whole grain wheat flour from batch A, were selected to study the effects of fermentation more thoroughly (Fig. 1). Initially, the asparagine content was higher in the dough made with rye bran (1.4 g/kg) than in the dough made with whole grain wheat flour (0.3 g/kg). The content of free asparagine decreased dramatically on fermentation in both doughs; after 1 hr the content had decreased ≈40%. During the next hour, nearly all asparagine was utilized by the yeast, and <0.1 g/kg remained. Thereafter, the asparagine content remained at the low level throughout the 6-hr fermentation period studied. These results are in accordance with the findings by Benedito De Barber et al (1989) that free amino acids such as asparagine are important sources of nitrogen for yeast growth.

The effect of yeast fermentation was also studied in doughs containing a number of different milling fractions from wheat and rye (Table II). In general, 6 hr of fermentation reduced the content of free asparagine more in the doughs with rye fractions (>90%) than in doughs with wheat fractions. One possible explanation of this difference in utilization could be due to a higher endogenous enzyme activity in the rye material. The effect of particle size reduction was studied in doughs made with disintegrated coarse shorts of wheat (before 15% < 150 µm, after 95% < 150 µm), and disintegrated wheat germ (before 5% < 1,110 µm, after 100% < 1,110 µm and 77% < 340 µm). During fermentation, the content of free asparagine was reduced more after disintegration of both coarse shorts and germ, 10 and 22%, respectively. Therefore, particle size seems to be an important factor for the asparagine availability during fermentation.

Sourdough and Yeast Fermentation

Spontaneous sourdough was made from whole grain rye flour and water. Fermentation for 72 hr decreased free asparagine from 1.0 g/kg to 0.7 g/kg. In the final sample (96 hr), after refreshment, the asparagine content had increased to 1.2 g/kg. Compared with the content in the original dough, this increase may have been a result of endogenous proteolytic activity in the flour or lactic acid bacteria (Benedito De Barber et al 1989; Collar et al 1991).

Fermentation experiments were also performed with different milling fractions, sourdough, and yeast (Table II). Sourdough seemed to have a strong negative impact on yeast activity. In dough made with 50% sourdough and batch A base flour, the asparagine content was only reduced 17%. Using less sourdough (25%), ≈50% of the asparagine was utilized by the yeast. However, what seemed to be a smaller decrease in asparagine content in the doughs made with 50% sourdough may have been a combination of reduced yeast activity due to low pH (3.7) and increased proteolytic activity, mainly caused by lactic acid bacteria (Benedito De Barber et al 1989). These results indicate that bread with this type of dough fermentation may result in breads containing more acrylamide than similar breads fermented with only yeast.

Acrylamide Content in Bread

Doughs were made with sifted wheat flour and whole grain wheat flour or rye bran from batch B that contained relatively high amounts of free asparagine. The doughs were yeast fermented for a short or a long period of time. During the short fermentation time, little utilization of asparagine occurred, while the long fermentation time resulted in extensive utilization as shown above (Table II). The doughs were baked under standard conditions and resulted in breads with reduced volume due to the high content of added fiber-rich ingredients, especially the bran. Crusts were separated from the breads and analyzed for acrylamide content. Crusts from the breads with whole grain wheat contained 180 μg of acrylamide/kg when fermented for a short period of time but only 24 μg of acrylamide/kg when fermented for the longer time. For the crusts from bread with rye bran, the corresponding figures were 50 and 12 μg of acrylamide/kg, respectively. Because essentially all acrylamide is found in the crust of yeast-leavened wheat bread (>94%) (Surduk et al 2004), acrylamide content in bread can be calculated on a fresh weight basis when weights of crust and crumb are known. Taking this into account, the bread with whole grain wheat would contain 33 μg of acrylamide/kg of fresh bread when fermented for a short time and 4 μg of acrylamide/kg (reduction 87%, $P < 0.001$) after the long fermentation time (Fig. 2). For the bread with bran, the corresponding values were 9 and 2 μg of acrylamide/kg (reduction 77%, $P < 0.001$), respectively.

Normal yeast-leavened wheat bread contains relatively low contents of acrylamide (Surduk et al 2004). Because the consumption of this type of bread is high in many countries, however, their contribution to total intake of acrylamide from food is significant (Svensson et al 2003). Therefore, it may be desirable to lower the content of acrylamide and extensive fermentation with yeast would be one possible way to do this.

CONCLUSIONS

During breadmaking, a reduction of the free asparagine content in dough resulted in breads with reduced acrylamide. Raw material, fermentation time, flour particle size, and sourdough content in the doughs seem to be important factors for the reduction of free asparagine during dough making. In this study, all doughs and breads were made in a model system. Pilot and full-scale studies are therefore needed to further explore the results from this study.

ACKNOWLEDGMENTS

This work was carried out with financial support from The Cerealia Foundation R&D, Wasabröd AB, and the Commission of the European Communities, STREP project, "HEATOX": heat-generated food toxicants, identification, characterization and risk minimization. It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

LITERATURE CITED

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th Ed. Method 54-21. The Association: St. Paul, MN.

Andersson, A. A. M., Andersson, R., and Åman, P. 2000. Air classification of barley flours. Cereal Chem. 77:463-467.

Benedito De Barber, C., Preito, J. A., and Collar, C. 1989. Reversed-phase high-performance liquid chromatography analysis of changes in free amino acids during wheat bread dough fermentation. Cereal Chem. 66:283-288.

Collar, C., Mascaros, A. F., Preito, J. A., and Benedito De Barber, C. 1991. Changes in free amino acids during fermentation of wheat doughs started with pure culture of lactic acid bacteria. Cereal Chem. 68:66-72.

Davies, M. 2002. The Biochrom Handbook of Amino Acids. Pages 51-56 and 69-77. Biochrom: Cambridge, UK.

Friedman, M. 2003. Chemistry, biochemistry, and safety of acrylamide. A review. J. Agric. Food Chem. 51:4504-4526.

IARC. 1994. Monographs on the evaluation of carcinogen risk to humans: Some industrial chemicals. 60:389-433. International Agency for Research on Cancer: Lyon, France.

Lingnert, H., Grivas, S., Jägerstad, M., Skog, K., Törnqvist, M., and Åman, P. 2002. Acrylamide in food: Mechanisms of formation and influencing factors during heating of foods. Scand. J. Nutr. 46:159-172.

Mottram, D. S., Wedzicha, B. I., and Dodson, A. T. 2002. Acrylamide is formed in the Maillard reaction. Nature 419:448.

Rosén, J., and Hellenäs, K.-E. 2002. Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. Analyst 127:880-882.

Rydberg, P., Eriksson, S., Tareke, E., Karlsson, P., Ehrenberg, L., and Törnquist, M. 2003. Investigations of factors that will influence the acrylamide content of heated foodstuffs. J. Agric. Food Chem. 51:7012-7018.

Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., Robert, M.-C., and Riediker, S. 2002. Acrylamide from Maillard reaction products. Nature 419:449.

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

Svensson, K., Abramsson, L., Becker, W., Glynn, A., Hellenäs, K.-E., Lind, Y., and Rosén, J. 2003. Dietary intake of acrylamide in Sweden. Food Chem. Toxicol. 41:1581-1586.

Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., and Törnqvist, M. 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J. Agric. Food Chem. 50:4998-5006.

Tkachuk, R. 1979. Free amino acids in germinated wheat. J. Sci. Food Agric. 30:53-58.

[Received February 4, 2004. Accepted March 24, 2004.]