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Analysis of free amino acids in cereal products

Arwa Mustafa *, Per Åman, Roger Andersson, Afaf Kamal-Eldin

Department of Food Science, Swedish University of Agricultural Sciences (SLU), P.O. Box 7051, S-750 07 Uppsala, Sweden

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Abstract

Free amino acids were extracted from cereal products using 50% ethanol to prevent solubilization of polysaccharides and other viscous polymers and to avoid starch gelatinization. The extracts were analyzed by GC after ion-exchange solid phase extraction and chloroformate derivatization using Ez-Faast technology (Phenomenex). Free amino acids in cereal products could be analyzed within 1 h of extraction and determination, with good separation between peaks and repeatable retention times. Relative correction factor for each amino acid was established. The matrix did not affect the results and the method was repeatable for most of the amino acids (coefficient of variation was in the order of 10%). Different fractions and products of wheat, rye, oats and barely were analyzed. The bran contained more free amino acids than did the other analysed fractions of cereals. Fermentation seemed to consume free asparagine and aspartic acid and to use or release other amino acids.

Keywords: Free amino acids analysis; Cereal fractions; Cereal products; Fermentation; Baking; Gas chromatography

1. Introduction

Cereals and cereal products contain variable amounts of free amino acids, depending largely on the species, cultivar and growing conditions (Abdel-Aal & Hucl, 2002). Amino acids serve as important substrates for dough microorganisms and are important from the sensory point of view as they contribute to bread flavour (Benedito De Barber, Prieto, & Collar, 1989; Collar, Mascarods, & Benedito De Barber, 1992). Free amino acids in raw materials of heat-treated foods take part in the Maillard reaction, which is important for cereal food quality. Recently, acrylamide was proven to be formed by a reaction between free Asn and reducing sugars (Mottram, Wedzicha, & Dodson, 2002; Stadler et al., 2002; Taeymans et al., 2004). In a recent study, it was shown that whole grain wheat flour contained 0.5 g/kg of Asn, while whole grain rye contained 1.1 g/kg (Fredriksson, Tallving, Rosen, & Åman, 2004). The study also showed that sifted fractions of the flours contained the least amount of Asn, which was mainly concentrated in the germ and the bran.

Free amino acids can be analyzed by both liquid and gas chromatography. Some of the liquid chromatography methods, e.g. the standard method using the amino acid analyzer, have drawbacks, such as the lengthy cleanup and preparation steps. Gas chromatography is also used after derivatization of both functional groups in the amino acid to suitable volatile derivatives (Davies, 2002; Molnar-Perl, 2000). For this purpose, Husêk (1998) recommended derivatization of amino acids with propyl chloroformate (Husêk, 1998; Husêk & Sweeley, 1991). Phenomenex (Phenomenex, 2001) released an analytical kit based on this method for analysing a range of free amino acids in physiological fluids. This method involves a simple solid phase extraction (SPE) step, followed by a rapid derivatization reaction and analysis by gas chromatography (GC) with internal standard (Farkas & Toulouee, 2003). This method has recently been applied to the analysis of amino acids in potato, wheat and rye products but no validation of the method for these type of matrixes has been reported (Elmore, Koutsidis, Dodson, Mottram, & Wedzicha, 2005a).

* Corresponding author. Tel.: +46 18672048; fax: +46 18 2995.
E-mail address: Arwa.Mustafa@lmv.slu.se (A. Mustafa).

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The aim of this study was to develop an extraction procedure for cereal products that gives stable extracts that can readily be used at the SPE step. The Phenomenex method was tested for validity with respect to repeatability and recovery of standards added to different cereal matrices. The method was used to analyze the free amino acid contents in cereals and cereal products and to test the effect of fermentation on free amino acid content.

2. Materials and methods

2.1. Cereal materials

Flour samples of rye bran, sifted rye flour, whole grain wheat, wheat bran, sifted wheat flour, low fibre oat flour, oat bran and oat grouts were supplied by Lantmännen (Stockholm, Sweden) and whole grain rye was from Wasabröd AB (Filipstad, Sweden). Soft wheat bread was baked from sifted wheat flour using dry yeast (*Saccharomyces cerevisiae*) Kronjäst original (Jästbolaget AB, Sweden) according to the recipe described by Surdyk, Rosén, Andersson, and Åman (2004) and dough from the same baking batches was also used in the analysis. Whole grain rye crisp bread was baked using the same yeast according to the recipe described by Mustafa, Andersson, Rosen, Kamal-Eldin, and Åman (2005).

2.2. Chemicals and reagents

Standard solutions of the amino acids Ala, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr and Val, in addition to the eluting and derivatization agents, were all provided in an inclusive kit (EZ-Faast GC-FID for amino acid analysis) purchased from Phenomenex (Torrance, CA, USA). The amino acids Asn, Gly, Val, Ser, Ala, Asp, Trp and Glu, used for spiking, were purchased from Pierce (Amino Acid Standard kit, St Louis, Il, USA). Additionally, Trp (98% by TLC) and the internal standard l-norvaline were purchased from Sigma Chemicals Co. (St Louis, MO, USA). Ethanol was purchased from Solveco Chemicals AB (Stockholm, Sweden).

2.3. Extraction and derivatization

All samples were milled using an ultra centrifuge mill type ZM1 with a 0.5 mm ring sieve (Retsch, Hann, Germany). Fermented dough and soft wheat bread were freeze-dried before milling. Samples were weighed in test tubes with screw caps, 0.2 g for whole grain and bran samples and 0.5 g for sifted flours and breads. Samples were first subjected to alcohol extraction. Ethanol (50% at 50 °C) and internal standard l-norvaline (70 mg/ml water) were added in volumes of 14 and 1 ml, respectively. Samples were mixed in a rotatory mixer at 50 °C for 20 min inside an incubator. After mixing, samples were subjected to centrifugation for 20 min at 1350*g*. An aliquot of 500 μl of the supernatant was subjected to solid phase extraction (SPE) and derivatization steps using the EZ-Faast technology and kit (Phenomenex, 2001).

2.4. Gas chromatography analysis

The derivatized amino acids were analyzed using a GC-FID instrument (Hewlett Packard; HP, 5890 Series II) equipped with an auto-sampler (Avondale, PA, USA). Aliquots of the derivatized amino acids (2 μl) were injected at 1:15 split ratio at 250 °C into a Zebron column (ZB – AAA, 10 m and 0.25 mm in diameter) programmed from 110–320 °C at 32 °C/min. Helium was used as a carrier gas at 60 kPa and nitrogen was used as a make-up gas. The detector temperature was 320 °C.

2.5. Calibration

Six different standard solutions with different concentrations of both the free amino acid standards and the internal standard were prepared. Concentrations ranged from 25 to 200 nmol/500 μl. Samples were analyzed in triplicate. Relative correction factors (RCF) were obtained as $(A_{IS} \times C_{aa})/(A_{aa} \times C_{IS})$ where IS = internal standard, aa = amino acid, C = concentration, A = peak area.

2.6. Analytical method validation

Within a day and day-to-day variations were determined using samples of rye bran, whole grain rye, sifted rye flour, rye crisp bread, wheat bran, sifted wheat flour and wheat bread run in triplicates for three different days. Relative standard deviation, expressed as coefficient of variation (CV), was used as a measure of precision. To check matrix

Table 1
Relative retention times (RRT) and relative correction factor (RCF) for individual amino acids

Amino acid	RRT[a]	RCF[a] (CV $n = 6$)
Ala	0.80	1.27 (8.8)
Gly	0.85	1.38 (8.2)
Val	0.94	1.05 (7.4)
Ile	1.04	0.85 (7.7)
Leu	1.06	0.97 (7.2)
Thr	1.16	1.49 (12.0)
Ser	1.18	1.87 (11.6)
Pro	1.22	1.07 (7.0)
Asn	1.26	1.63 (11.3)
Asp	1.50	1.17 (10.3)
Met	1.52	0.94 (8.9)
Glu	1.66	2.23 (10.5)
Phe	1.68	0.62 (8.6)
Gln	1.95	2.79 (8.8)
Lys	2.23	0.72 (12.5)
His	2.32	0.98 (23.2)
Tyr	2.44	0.58 (8.7)
Trp	2.59	0.60 (11.8)

Calibration range is 25–200 nmol/500 μl for individual amino acids.
[a] RRT and RCF are relative to internal standard IS (l-norvaline).

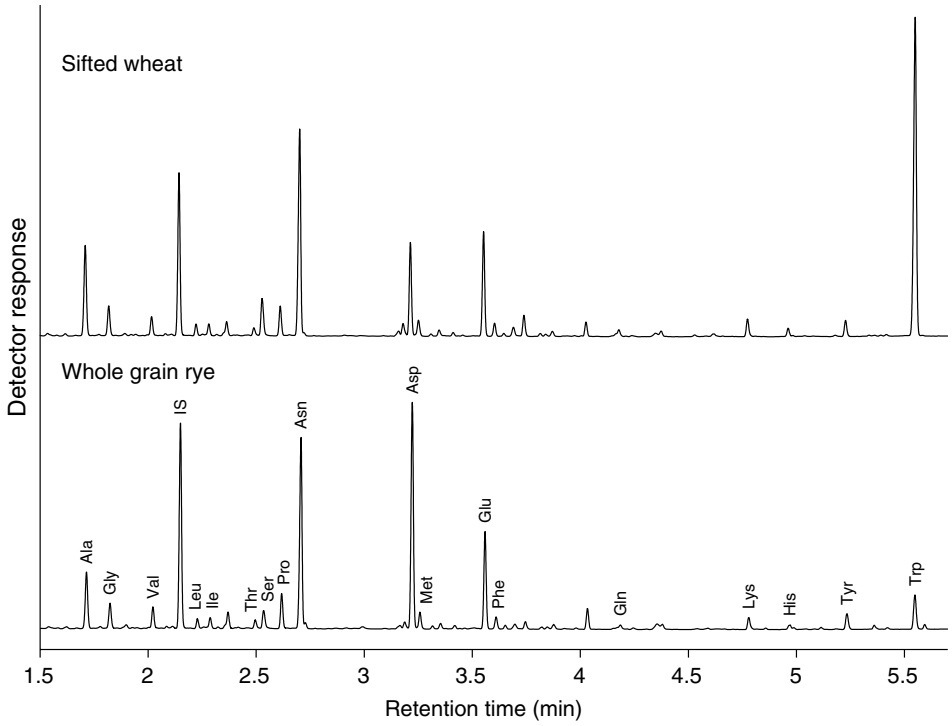

Fig. 1. Chromatogram showing the amino acid profiles in sifted wheat and whole grain flour (IS = internal standard:norvaline).

effect on recovery, four different samples (rye bran, sifted rye flour, rye crisp bread and soft wheat bread) and a blank sample were spiked with eight standard amino acids (Asn, Ala, Asp, Glu, Gly, Ser, Trp, Val). The spiking concentration used for each amino acid was an average of the amino acid concentration in the different samples. A blank sample was subjected to the same experimental conditions as the test samples.

2.7. Biochrom method

This is a cation-exchange chromatography method that analyzes amino acids using the amino acid analyzer (Davies, 2002). Samples were extracted using 5-sulphosalicylic acid with norleucine as internal standard. The method involves a post-column derivatization of the amino acids with ninhydrin that produces coloured amino acid derivatives that can be determined spectrophotometerically at 570 and 440 nm.

3. Results and discussion

3.1. General

In this study, we used alcohol extraction, followed by the EZ-Faast method, to analyze the levels of amino acids in cereals and cereal fractions. Amino acids were extracted from the cereal using 50% ethanol at 50 °C in order to partially inactivate enzymes, and prevent the extraction of polysaccharides and other viscous polymers, in addition to avoiding starch gelatinization. The alcohol extraction gave a stable extract that was readily used for solid phase extraction (SPE) using an ion-exchange sorbent provided in the analysis kit.

The calibration solutions had six different concentrations for each of the amino acid standards and internal standard (l-norvaline). The chromatographic separation of peaks was good, which made the identification of the amino acids easy. All amino acids were separated within

Table 2
Sample weight and recovery of free amino acids (%) after spiking: means ± SD ($n = 3$)[a]

	Sample weight (mg)	Recovery percent							
		Asn	Gly	Val	Ser	Ala	Asp	Trp	Glu
Blank	0	96 ± 1	97 ± 0	112 ± 0	108 ± 0	95 ± 0	104 ± 1	75 ± 0	111 ± 1
Rye bran	200	109 ± 12	96 ± 3	104 ± 1	105 ± 5	94 ± 1	108 ± 2	77 ± 5	130 ± 133
Sifted rye flour	500	97 ± 4	91 ± 0	100 ± 1	104 ± 1	94 ± 1	118 ± 3	82 ± 0	182 ± 1
Rye crisp bread	500	89 ± 2	93 ± 1	99 ± 0	98 ± 1	93 ± 3	105 ± 2	78 ± 2	185 ± 18
Wheat bread	500	89 ± 5	91 ± 1	98 ± 0	96 ± 4	94 ± 0	105 ± 1	74 ± 1	178 ± 19

[a] The amount used for spiking was the average content of each of free amino acids in the four samples.

Table 3
Content (μg/g) of free amino acids in cereal products using (E) EZ-Faast method ($n = 3$) and (B) Biochrom method ($n = 1$)

Amino acid	Whole grain rye	Rye bran	Sifted rye	Rye crisp bread	Whole grain wheat	Wheat bran	Sifted wheat	Soft wheat bread	Wheat dough	Barely flour	Low fibre oat	Oat bran	Oat grouts
Ala													
E	98 ± 1	167 ± 2	74 ± 2	80 ± 0.4	61 ± 1	197 ± 1	25 ± 0.2	68 ± 0.2	47 ± 1	49 ± 0.3	31 ± 0.4	116 ± 4	30 ± 0.4
B	105	181	79	79	66	222	26	73	48	58	32	122	32
Gly													
E	39 ± 2	45 ± 0.3	18 ± 0.6	56 ± 1	32 ± 0.4	58 ± 0.5	10 ± 0.3	23 ± 0.3	22 ± 1	27 ±0.5	15 ± 0.4	32 ± 1	11 ± 0.3
B	45	51	20	64	42	72	11	25	24	34	16	37	13
Val													
E	38 ± 1	66 ± 1	29 ± 1	10 ± 0.3	21 ± 1	42 ± 0.3	9 ± 0.7	15 ± 1	6 ± 0.5	38 ± 0.3	19 ± 0.5	35 ± 0.8	19 ± 0.1
B	63	101	58	35	26	58	13	17	35	45	39	63	44
Ile													
E	20 ± 1	27 ± 0.3	14 ± 1	5 ± 1	16 ± 1	27 ± 0.5	7 ± 0.2	8 ± 0.04	4 ± 0.5	21 ± 1	9 ± 0.1	18 ± 1	8 ± 0.3
B	15	21	10	NR[a]	10	23	5	7	NR	20	5	12	5
Leu													
E	15 ± 0.4	25 ± 1	13 ± 0.1	8 ± 0.3	14 ± 0.8	23 ± 0.8	7 ± 0.3	12 ± 0.2	4 ± 0.7	28 ± 0.8	10 ± 0.0	13 ± 0.3	7 ±0.2
B	15	25	12	NR	14	29	8	10	NR	28	10	11	7
Thr													
E	24 ± 2	35 ± 4	15 ± 1	16 ± 1	14 ± 0.3	28 ± 1	6 ± 1	12 ± 1	8 ± 0.1	23 ± 2	14 ± 0.5	27 ± 0.4	13 ± 0.6
B	20	38	16	20	11	26	NR	11	NR	23	14	22	NR
Ser													
E	62 ± 1	120 ± 4	40 ± 2	166 ± 4	99 ± 3	149 ± 3	16 ± 2	65 ± 2	71 ± 3	100 ± 6	27 ± 0.4	93 ± 2	27 ± 0.5
B	17	25	13	14	20	31	6	15	7	20	10	29	11
Pro													
E	62 ± 1	97 ± 4	46 ± 1	79 ± 1	31 ± 1	67 ± 2	10 ± 0.2	31 ± 0.4	30 ± 1	54 ± 1	26 ± 0.4	55 ± 1	71 ± 1
B	NA[b]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Asn													
E	615 ± 19	1070 ± 16	365 ± 7	21 ± 1	290 ± 8	856 ± 8	88 ± 1	13 ± 0.1	9 ± 0.7	293 ± 0.4	277 ± 8	783 ±17	437 ± 6
B	656	1140	389	NR	347	931	99	3	7	315	298	819	525
Asp													
E	503 ± 20	472 ± 19	351 ± 30	159 ± 11	227 ± 16	252 ± 2	148 ± 5	17 ± 1	12 ± 2	159 ± 1	118 ± 3	127 ± 2	100 ± 3
B	455	492	365	141	219	237	151	25	32	174	114	118	101
Met													
E	38 ± 5	39 ± 3	18 ± 3	16 ± 1	37 ± 3	40 ± 1	17 ± 1	19 ± 3	16 ± 0.5	18 ± 0.7	16 ± 2	37 ± 6	17 ± 1
B	NA	NA	NR	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Phe													
E	18 ± 0.2	28 ± 1	16 ± 0.2	5 ± 0.1	14 ± 1	24 ± 1	8 ± 0.4	7 ± 0.1	2 ± 1	39 ± 0.4	11 ± 0.1	16 ± 0.1	10 ± 0.3
B	NR	17	NR	NR	7	24	NR	NR	NR	33	NR	NR	NR
Gln													
E	49 ± 1	68 ± 7	35 ± 4	26 ± 2	37 ± 6	71 ± 3	19 ± 3	14 ± 2	19 ± 3	28 ±1	10 ±2.5	23 ± 9	18 ± 5
B	31	44	29	22	25	49	23	9	23	21	17	18	17

Lys													
E	20 ± 0.6	36 ± 2	15 ± 1	30 ± 0.4	20 ± 1	37 ± 1	8 ± 0.6	28 ± 1	26 ± 19	36 ± 0.6	18 ± 0.4	35 ± 0.5	20 ± 1
B	29	51	18	34	28	63	8	32	27	46	18	56	30
His													
E	12 ± 0.3	21 ± 6	8 ± 1	8 ± 1	10 ± 1	23 ± 0.2	3 ± 0.5	5 ± 0.4	4 ± 1.3	23 ± 0.04	14 ± 1	44 ± 2	21 ± 1
B	59	126	36	27	141	533	38	23	31	74	31	171	89
Tyr													
E	26 ± 2	40 ± 0.3	19 ± 0.2	14 ± 0.6	18 ± 1	34 ± 1	9 ± 0.6	9 ± 0.5	5 ± 3	35 ± 1	19 ± 0.7	23 ± 1	16 ± 0.3
B	20	37	19	NR	15	33	NR	NR	NR	31	15	14	NR
Trp													
E	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
B	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Glu													
E	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
B	273	399	163	240	138	338	61	122	76	179	165	306	257
Arg													
E	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
B	111	169	50	124	103	319	20	42	37	57	NR	121	62

[a] NR, not reported.
[b] NA, not analyzed.

8 min of run-time with a good consistency in the retention time (CV $\leqslant$ 0.2%). Relative correction factor (RCF) was calculated for each of the amino acids with reference to the internal standard; the calibration range used was 25–200 nmol/500 μl; standards were run in duplicates (Table 1). The CV for the RCF of the individual amino acids was $\leqslant$12%, except for histidine, which had a higher CV value (ca. 23%), consistent with previous findings (Elmore, Koutsidis, Dodson, Mottram, & Wedzicha, 2005b).

The starting amount of the sample had to be adjusted to the concentration in the products. For whole grain flours and brans, 200 mg were used while, for sifted flour and products, 500 mg were used. The GC chromatograms, for both the raw material and the products, showed good separation and high consistency in the retention time for all the analyzed amino acids (Fig. 1).

Rye bran, sifted rye flour, rye crisp bread and wheat bread were used to test the recovery of amino acids (Table 2). Eight amino acids (Asn, Gly, Val, Ser, Ala, Asp, Trp, and Glu) were chosen for the spiking experiment according to their structure and abundance in cereals. Most of the amino acids had a satisfactory recovery in the order of 100 ± 10%, except for Trp and Glu. To further investigate this problem, a sample of sifted rye flour at different weights (100, 300 and 500 mg) was spiked with Glu and Trp. The recovery of Glu was found to increase with the amount of the sample, while Trp showed a low recovery (in the order of 75%), regardless of the sample amount. The blank sample had a recovery in the order of 100 ± 5% for all amino acids except Trp, which consistently showed about 75% recovery. This method gave satisfactory repeatability within a day and between days. The relative standard deviation was ca. 5% for the major amino acids and ca. 10% for the minor amino acids in cereal fractions.

When comparing the Ez-Faast method with Biochrom, both methods gave comparable results for most of the amino acids. However, some amino acids were better analyzed by one method than the other (Table 3). For example, Pro, Met and Trp are not reported by the Biochrom method used. On the other hand, Arg is not analyzable by the Ez-Faast method as has been reported in other studies as well (Elmore et al., 2005b; Lee & Harnly, 2005) and results for Trp and Glu were unreliable, as discussed earlier. Ez-Faast gives low levels of some amino acids in rye crisp bread, including Asn; however, some of these amino acids were not given by the Biochrom method. On the other hand, His was found at higher concentration using the Biochrom method.

3.2. Amino acid content in cereal fractions and products

The contents of the individual amino acids varied, depending on the type of cereal and its fraction (Fig. 2). In wheat fractions, Ala, Ser, Asn, Asp and Glu were the major amino acids in the samples. There is a difference in

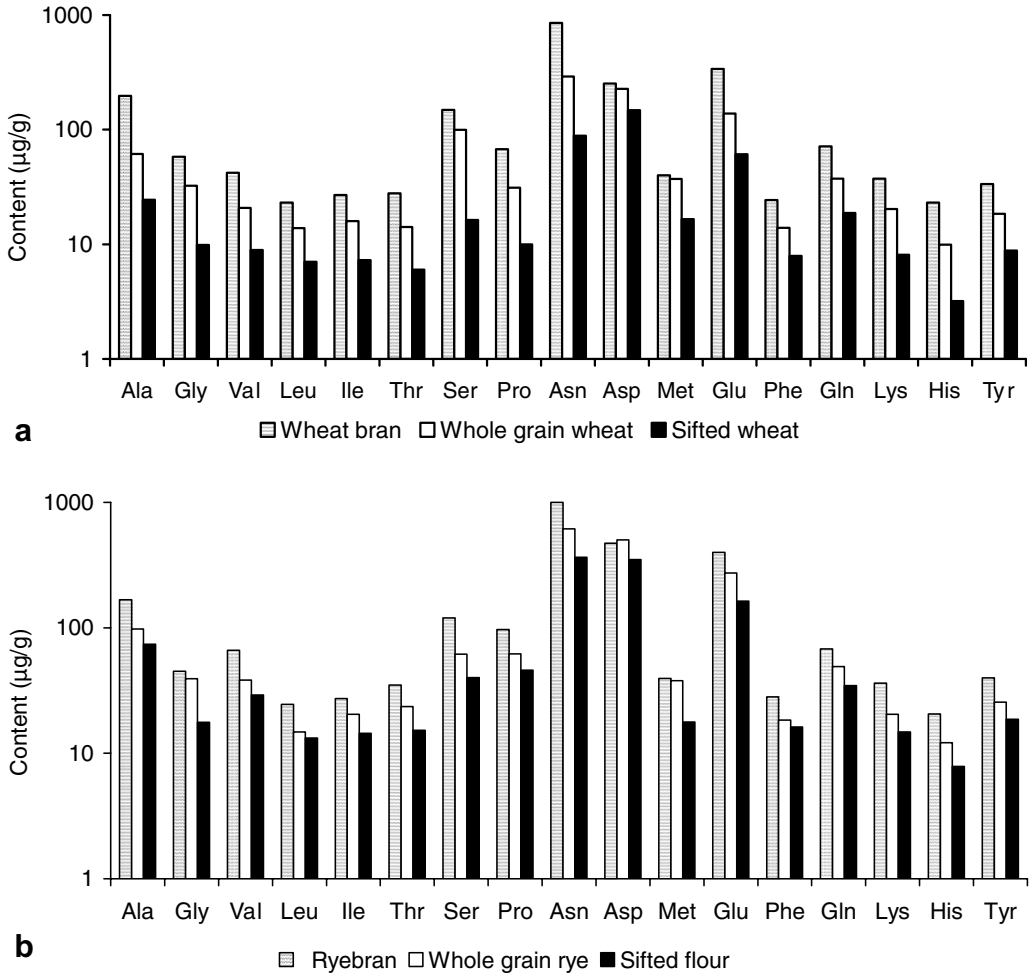

Fig. 2. Amino acid content in different fractions of (a) wheat, and (b) rye. All levels were obtained by the EZ-Faast method except for those of Glu which were obtained by the Biochrom method.

the amino acid contents of cereal fractions, with bran having the highest concentration while the sifted flour showed the lowest concentration, a consistent pattern seen in all amino acids. This difference is very clear in wheat, due to the sharp separation that takes place during the milling of wheat but was less apparent in the rye, due to the fact

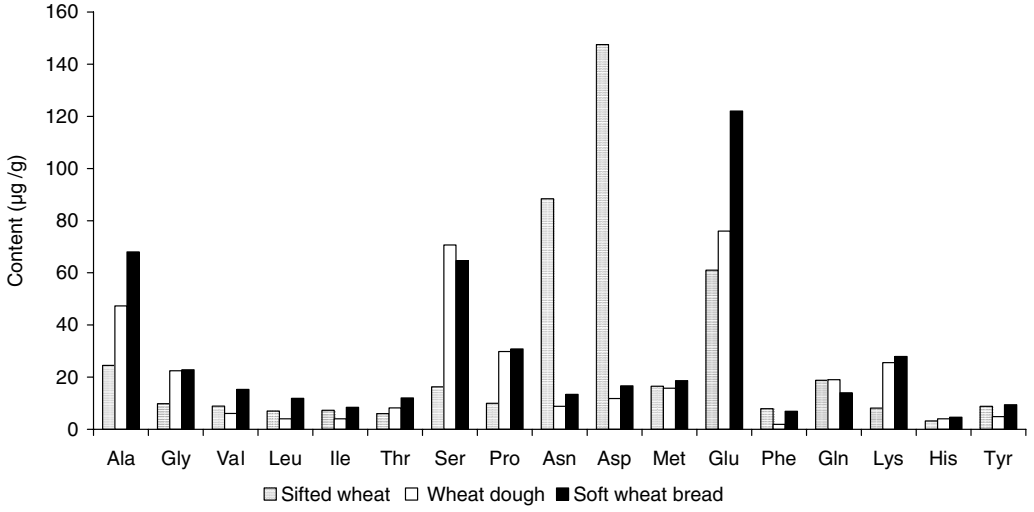

Fig. 3. Effect of fermentation and baking on wheat amino acid content. All levels were obtained by the EZ-Faast method except for those of Glu which were obtained by the Biochrom method.

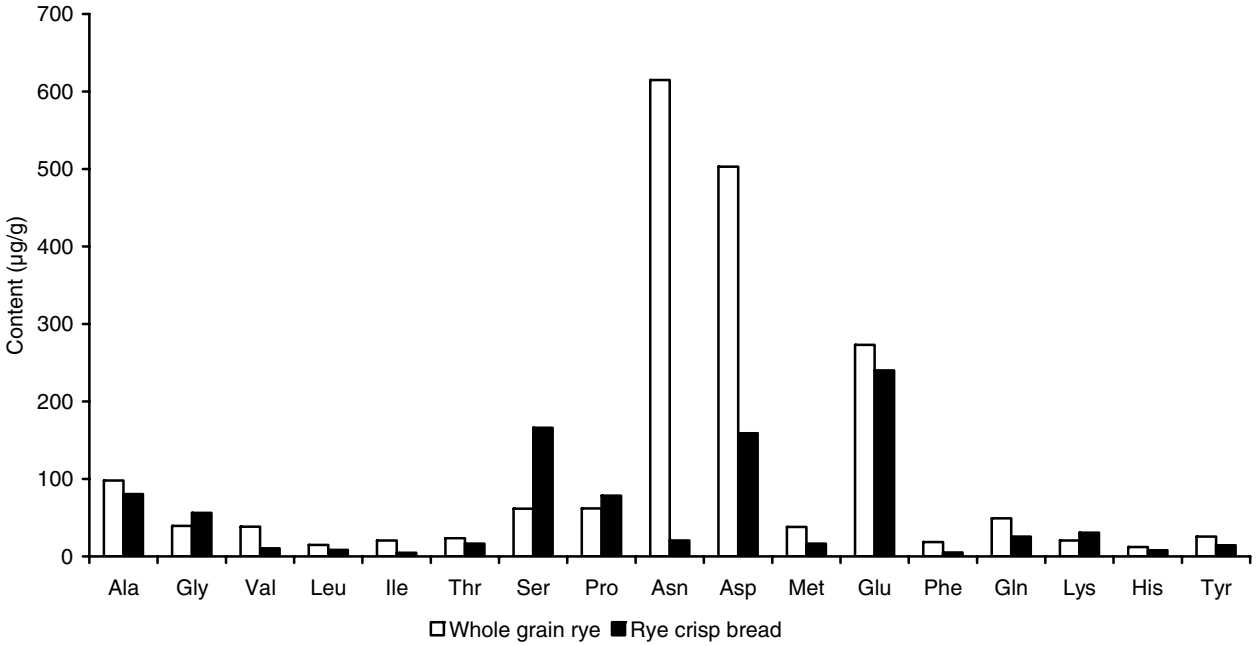

Fig. 4. Effect of baking on rye amino acid content. All levels were obtained by the EZ-Faast method except for those of Glu which were obtained by the Biochrom method.

that there is more of the outer parts of the kernel in the sifted fractions. Samples of rye were richer in individual amino acids than were those of wheat, although varietal differences may exist (Abdel-Aal & Hucl, 2002). These results indicate that the rye samples have higher content of free Asn than have wheat samples and that free Asn is concentrated in the outer parts of the kernel. Oats were rich mainly in Ala, Asn, Asp and Glu. The bran was richer in free amino acids than were oat groats and the low fibre oat flour (Table 3).

3.3. Change of amino acid content during fermentation and baking

Fermented dough from sifted wheat flour showed a major decrease in Asn (Fig. 3), in agreement with Fredriksson et al. (2004). A similar decrease was also observed in Asp. The variation in the amino acid content after fermentation could be explained by yeast metabolism (Benedito De Barber et al., 1989) and their release by proteases (Collar & Matinez, 1993). For example, there was increase mainly in Ala and Ser during wheat bread-making that could partially be due to the fermentation process, a metabolic state that consumes, as well as releases, different amino acids. Rye crisp bread fermentation and baking showed that Asn, and Asp are decreased during bread-making as well, while Gly, Ser, Pro and Lys showed a smaller increase (Fig. 4).

The alcohol extraction, coupled with the EZ-Faast amino acid analysis kit, was able to extract and determine free amino acids that are found in low concentrations. It gave reproducible results for most of the amino acids (except Arg, Trp, and Glu) in all the different fractions of wheat, rye, oats and barely, and products from wheat and rye. The chromatograms show about 20 free amino acids appearing in around 8 min, with good separation, as well as consistency, in the retention times. Total analysis time is 1 h including the GC run. Spiking to test the effect of matrix gave satisfactory results for most of the amino acids. The most common free amino acids were Ala, Ser, Asn and Asp. Milling fractions that contain the outer part of the kernel are richer in free amino acids than are the sifted fractions. The rye samples showed higher contents of free amino acids than did the wheat samples. Fermentation and baking seem to use-up, as well as release, certain amino acids.

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References

Abdel-Aal, E.-S. M., & Hucl, P. (2002). Amino acid composition and in vitro protein digestibility of selected ancient wheats and their end products. *Journal of Food Composition and Analysis, 15*, 737–747.

Benedito De Barber, C., Prieto, J. A., & Collar, A. (1989). Reversed-phase high-performance liquid chromatography analysis of changes in free amino acids during wheat dough fermentation. *Cereal Chemistry, 66*(4), 283–288.

Collar, C., & Matinez, C. S. (1993). Amino acid profile of fermenting wheat sour doughs. *Journal of Food Science, 58*(6), 1324–1328.

Collar, C., Mascarods, A. F., & Benedito De Barber, C. (1992). Amino acid metabolism by yeast and lactic acid bacteria during bread dough fermentation. *Journal of Food Science, 57*(6), 1423–4126.

Davies, M. (2002). *The biochrom hand book of amino acid analysis*. Cambridge: Biochrom Ltd.

Elmore, J. S., Koutsidis, G., Dodson, A. T., Mottram, D. S., & Wedzicha, B. L. (2005a). The effect of cooking on acrylamide and its precursors in potato, wheat and rye. In M. Friedman & D. Mottram (Eds.), *Chemistry and Safety of Acrylamide in Food* (pp. 255–269). Heidelberg, Germany: Springer-Verlag GmbH.

Elmore, J. S., Koutsidis, G., Dodson, A. T., Mottram, D. S., & Wedzicha, B. L. (2005b). Measurement of acrylamide and its precursors in potato, wheat, and rye model systems. *Journal of Agricultural and Food Chemistry, 53*(4), 1286–1293.

Farkas, T., & Toulouee, J. (2003). Asparagine analysis in food products. *LC GC Europe*, 14–16.

Fredriksson, H., Tallving, J., Rosen, J., & Åman, P. (2004). Fermentation reduces free asparagine in dough and acrylamide content in bread. *Cereal Chemistry, 81*(5), 650–653.

Husêk, P. (1998). Chloroformates in gas chromatography as general purpose derivatizing agents. *Journal of Chromatography B, 717*(1–2), 57–91.

Husêk, P., & Sweeley, C. C. (1991). Gas-chromatographic separation of protein amino-acids in 4 min. *HRC-Journal of High Resolution Chromatography, 14*(11), 751–753.

Lee, J., & Harnly, J. M. (2005). Free amino acid and cysteine sulfoxide composition of 11 garlic (*Allium sativum* L.) cultivars by gas chromatography with flame ionization and mass selective detection. *Journal of Agricultural and Food Chemistry, 53*(23), 9100–9104.

Molnar-Perl, I. (2000). Role of chromatography in the analysis of sugars, carboxylic acids and amino acids in food. *Journal of Chromatography A, 891*(1), 1–32.

Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Acrylamide is formed in the Maillard reaction. *Nature, 419*(6906), 448–449.

Mustafa, A., Andersson, R., Rosen, J., Kamal-Eldin, A., & Åman, P. (2005). Factors influencing acrylamide content and color in rye crisp bread. *Journal of Agricultural and Food Chemistry, 53*(15), 5985–5989.

Phenomenex (2001). *Ez-Faast easy-fast amino acid sample testing kit manual*. Torrance, CA USA: Phenomenex USA.

Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., et al. (2002). Acrylamide from Maillard reaction products. *Nature, 419*(6906), 449–450.

Surdyk, N., Rosén, J., Andersson, R., & Åman, P. (2004). Effects of asparagine, fructose, and baking conditions on acrylamide content in yeast-leavened wheat bread. *Journal of Agricultural and Food Chemistry, 52*, 2047–2051.

Taeymans, D., Wood, J., Ashby, P., Blank, I., Studer, A., Stadler, R. H., et al. (2004). A review of acrylamide: An industry perspective on research, analysis, formation and control. *Critical Reviews in Food Science and Nutrition, 44*(5), 323–347.