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Analysis of free amino acids in cereal products

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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).

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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).