Reversed-Phase High-Performance Liquid Chromatography Analysis of Changes in Free Amino Acids During Wheat Bread Dough Fermentation[1]

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Cereal Chem. 66(4):283-288.

ABSTRACT

Qualitative and quantitative changes in 22 free amino acids (AA) during wheat bread dough fermentation were determined by reversed-phase high-performance liquid chromatography (RP-HPLC) of their dansyl derivatives in unfermented and fermented straight doughs. With 0–6 hr of fermentation, most major free AA (especially aspartic and glutamic acids, asparagine, and alanine) decreased, although proline did not. As a result, total AA content was depleted by 72%. In addition, free amino acids were metabolized at different rates. Glutamic acid, glycine, γ-aminobutyric acid, and basic AA contents were significantly reduced with 2.5–6 hr of fermentation, whereas asparagine, dicarboxylic acids, hydroxy, aromatic, and monoamino monocarboxylic AA contents decreased first (before 2.5 hr). Between 6 and 24 hr of fermentation, total AA content increased (+105%) because yeast growth stopped and proteolytic activity from lactic acid bacteria increased. Increased alanine, glutamic acid, and proline accounted for 75% of the increase of total AA content during this period.

Nitrogen compounds, especially amino acids, play an important role in the metabolism of dough microorganisms and in production of bread with characteristic flavor.

During bread dough fermentation, amino acids are assimilated by yeast and lactic acid bacteria (Ponomareva et al 1964, Morimoto 1966, El-Dash and Johnson 1970, El-Dash 1971, Faridi and Johnson 1978, Spicher and Nierle 1984a, Benedito de Barber et al 1985). Amino acids are also metabolized as a source of nitrogen for growth (Ng 1976, Spicher and Schröeder 1979, Miyashiro et al 1980) and are released into the medium as *N*-acetyl amino acids (Rothenbuehler et al 1982), α-ketoacids, fusel oils (Woodward and Cirillo 1977), aldehydes, alcohols, or ester derivatives (Lüers 1959, Baca and Golebiewski, 1977) of the amino acids concerned. These compounds have been related with bread flavor (Richard-Molard et al 1979).

The absorption rate of each amino acid depends mainly on the requirements of the microflora. Homofermentative lactic acid bacteria in rye sourdoughs have no requirement for alanine and serine, but certain strains are unable to grow in the absence of glutamic acid and valine (Spicher and Schröeder 1979). In sponge (El-Dash and Johnson 1970) and straight (Morimoto 1966) wheat bread dough systems containing compressed yeasts, most amino acids except arginine (El-Dash and Johnson 1970), glycine, and proline (Morimoto 1966) are rapidly metabolized during fermentation; this is especially true for aromatic amino acids.

Jones and Pierce (1964) were able to classify wort amino acids into four classes depending on rate of assimilation by yeast. Results were later explained by the existence in *Saccharomyces cerevisiae* of two mechanisms for transporting amino acid (Rose and Keenan 1981).

Fermentation conditions (time, temperature, pH, moisture content) and transport competitive inhibition also affect assimilation of nitrogenous compounds by microorganisms (Pekur and Bur'yan 1981).

The rate of amino acid removal from dough will affect their levels before baking, and therefore affect bread flavor and color (Maillard reaction). The addition of proteases induces an important increase of primary amino groups (El-Dash and Johnson 1970); this also occurs in long-fermented doughs (Faridi and Johnson 1978, Morimoto 1966) due to endogenous proteases (Grant and Wang 1972) and lactic acid bacteria (Kosmina 1977, Spicher and Nierle 1984b). The proteolytic activity in wheat flour extracts increases the dialyzable nitrogen fraction, in which leucine and phenylalanine account for 50% or more of free amino acids (Grant and Wang 1972).

Amino acid content is also affected by autolysis. Products released during autolysis of yeast include peptides and amino acids (Thorn 1971). In wine, when fermentation is accomplished, several amino acids increase (Pekur and Bur'yan 1981). In addition, peptides and amino acids (mainly alanine) are released into the medium by brewing yeasts during fermentation from a synthetic medium consisting mostly of glucose and ammonium sulfate (Clapperton 1971).

The qualitative composition of amino acids in dough also influences bread flavor. Arginine, histidine, and leucine produce a characteristic bread flavor (Kiely et al 1960), whereas proline leads to a cracker flavor (Wiseblatt and Zoumut 1963).

Despite interest in amino acids in fermented bread dough and the variability of changes expected, available information is constrained by the low resolution of methods used for their determination, usually ion-exchange chromatography. Thus, quantitative changes during fermentation are reported for fewer than 18 amino acids, mainly for sponge doughs. Chromatography may also fail to quantify some pairs of amino acids, such as aspartic acid and asparagine, glutamic acid and glutamine (Morimoto 1966), and histidine and ammonia (El-Dash and Johnson 1970). Tryptophan and γ-aminobutyric acid (El-Dash and Johnson 1970) and cysteine and γ-aminobutyric acid (Morimoto 1966) also may not be determined.

In this study, changes in free amino acid levels during bread dough fermentation were investigated in straight doughs by reversed-phase high-performance liquid chromatography (RP-HPLC) of their dansyl derivatives. In addition, we describe a rapid, sensitive, and reproducible method for separation and quantification of dansyl-amino acids, suitable for determination of free amino acids in doughs.

MATERIALS AND METHODS

Reagents and Solvents

Dowex 50W-X2 cation exchange resin was obtained from Fluka AG (Buchs, Switzerland). Amino acids (Sigma grade), dansyl amino acids, and dansyl chloride (dansyl-Cl) were purchased from Sigma (St. Louis, MO). Acetonitrile (HPLC grade) and acetone (ultraviolet grade) were obtained from Panreac (Barcelona, Spain). All buffers were prepared from analytical grade chemicals and ultrapure water obtained from a Milli Q system (Millipore, Bedford, MA). A standard mixture of the amino acids to be analyzed was prepared by dissolving in 0.01*N* HCl the following amounts (mmol): aspartic acid, 1.00; glutamic acid, 0.91; asparagine, 0.36; glutamine, 0.30; serine, 0.24; threonine, 0.33; tyrosine, 0.17; glycine, 0.21; alanine, 0.19; valine, 0.28; isoleucine, 0.24; leucine, 0.24; tryptophan, 0.11; phenylalanine, 0.17; proline, 0.13; ornithine, 0.17; lysine, 0.11; arginine, 0.20; histidine, 0.19;

[1]Presented at the 8th International Cereal & Bread Congress, May 28–June 3, 1988, Lausanne, Switzerland. This paper is part of the doctoral thesis of J. A. Prieto.

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γ-aminobutyric acid, 0.24; methionine, 0.25; and cystine, 0.11. The solution was made up to 200 ml with 0.01*N* HCl.

Apparatus

A Waters Associates (Milford, MA) HPLC system was used. It consisted of two 510 solvent delivery units, a 721 programmer, and a U6K universal liquid chromatography injector coupled to a 490 variable wavelength ultraviolet detector (Waters). An ODS HS/3 column (3 μm × 8.3 cm × 4.6 mm i.d.) and a precolumn (3.6 cm × 4.6 mm i.d.) packed with pellicular C_{18} both from Perkin-Elmer (Norwalk, CT) were used for analyses. The detector response was displayed and peak areas were calculated by a Waters 730 printerplotter automation system. Samples were injected with a microliter 802 syringe (Hamilton, Reno, NV). Solvents were filtered using an Afora filter holder (Afora, Barcelona, Spain), and samples were filtered using a Swinney filter (Millipore). Millipore HA grade 0.45-μm filters and a Millipore FH grade filter were used for aqueous solvent and sample preparation, and for acetonitrile filtration. Solvents were degassed in an ultrasonic bath (Selecta, Barcelona, Spain) before use.

Bread Dough Preparation

A commercial wheat bread flour (energy of deformation [*W*] = 208 × 10^3 ergs, curve configuration ratio [*P*/*L*] = 0.93, and 11.9% protein) was used. Unfermented bread dough was prepared by mixing 5 kg of flour, 125 g of salt, 94 g of commercial compressed yeast, and 2,800 ml of water for 15 min in an arm mixer of 10 kg flour capacity. Unfermented dough was divided into 500-g portions and fermented at 28°C and 80% rh for 2.5 (F2.5), 6 (F6), 12 (F12), and 24 hr (F24). Doughs were frozen, freeze-dried, and then ground in a mortar to a fine powder.

Amino Acid Extraction

Samples (100 g) of freeze-dried doughs were suspended in 300 ml of 0.01*N* acetic acid and vigorously mixed for 5 min in a Virtis homogeneizer. The homogenate was centrifuged at 23,000 × *g* for 20 min at 1–3°C, and the sediment extracted again with 250 ml of solvent. The clear supernatant fractions were pooled, made up to 500 ml, and used for further purification (Benedito de Barber et al 1988).

Amino Acid Purification

The purification sequence was carried out as described by Benedito de Barber et al (1989). Extracts were deproteinized by ultrafiltration using an Amicon cartridge (cut-off 10,000 daltons) against 0.25*N* acetic acid, in an extract-to-acid ratio of 1:4 at 4°C and a flow of 200–250 ml/hr. Aliquots (40 ml) of each protein-free extract were brought up to pH 2.0 and applied to a column packed with 5 ml of Dowex 50W-X2 resin (50–100 mesh, H^+ form) previously equilibrated with 0.01*N* HCl. The resin was washed with 15 ml of water and then eluted with 30 ml of 4*N* ammonium hydroxide. The ammonia eluates were evaporated to dryness in a rotary evaporator (<40°C). Residues were redissolved in 5 ml of water and used for amino acid analyses.

Amino Acid Derivatization

Purified dough extracts and amino acid standard mixtures were derivatized with dansyl-Cl (Navarro et al 1984). Norvaline was used as the internal standard (69 mg/200 ml of 0.01*N* HCl). Aliquots (0.2 ml) of the amino acid standard mixture and 2.25–5.0-ml aliquots of purified dough extracts, containing approximately 50–75 μg of amine nitrogen, were placed in small vials, and the internal standard (0.2 ml for dough samples, and 0.1, 0.15, or 0.2 ml for standard mixture) was added. Samples were evaporated to dryness in a heating-stirring module coupled to an evaporating unit (Pierce, Rockford, IL) and redissolved in 0.5 ml of 0.1*N* K_2CO_3 buffer, pH 10.5, followed by addition of 0.2 ml of dansyl-Cl solution (0.5 g/25 ml of dry acetone) and 1.0 ml of dry acetone (final pH 9.3–9.6). The mixture was heated at 100°C for 2 min, then evaporated to dryness and redissolved in 1.7 ml of potassium phosphate buffer (pH 7.0)/acetonitrile (90:10, v/v). An aliquot (10 μl) of each sample and standard was injected into the HPLC system.

TABLE I
Solvent Program for Amino Acid Separation[a]

Time (min)	% Solvent A	% Solvent B
0	90	10
42	56.4	43.6
45	30	70
55	30	70
60	90	10

[a]Solvent A was 12 m*M* K_2HPO_4, pH 7.00, and solvent B was acetonitrile. The gradient was initiated upon injection at a column temperature of 30°C and a pressure of approximately 2,500 psi. Flow rate was 1.50 ml/min. Gradient steps in the program were linear.

Chromatography

Different ionic strength buffers, flow rates, and solvent gradient programming were tested. Solvent A was 12 m*M* K_2HPO_4 (adjusted to pH 7.00 with orthophosphoric acid), and solvent B was acetonitrile. The solvent program is summarized in Table I. Succesive samples could be injected every 65 min. The dansyl derivatives were detected at 250 nm and 0.15 absorbance units full scale (AUFS).

Quantification

Quantification was accomplished using the norvaline internal standard addition method. Response factors were calculated for each amino acid in the standard mixture as means of 10 determinations, varying the relative proportion of added internal standard.

Statistical Treatment

One-way analysis of variance (ANOVA) was performed with an AT-IBM computer (BMDP statistical package, 7D program). Least significant differences among means were obtained after applying the Q test.

RESULTS AND DISCUSSION

Chromatographic Separation and Quantification

Figures 1 and 2 show RP-HPLC gradient separations of a dansyl-amino acid standard mixture and of free amino acids in dough samples, respectively. Separations are remarkably good, especially for critical regions such as dansyl-glutamine and dansyl-serine (Wilkinson 1978); dansyl-threonine and dansyl-glycine (Hsu and Currie 1978); dansyl-alanine, dansyl-arginine, and dansyl-proline (Navarro et al 1984); dansyl-isoleucine and dansyl-leucine (Bayer et al 1976, Khayat et al 1982); and dansyl-phenylalanine and dansyl-amide (Takeuchi et al 1984). The conditions we adopted for separation of dansyl-amino acids were based on those of Navarro et al (1984) but differ in concentration of the eluting buffer (0.012*M* vs. 0.025*M* K_2HPO_4). Under these conditions, retention times of all derivatives except dansyl-arginine and dansyl-amide decreased. It is thus possible to mantain the resolution of dansyl-arginine, now eluting between dansyl-γ-aminobutyric acid and dansyl-proline, while also notably improving resolution of dansyl-phenylalanine and dansyl-amide (*unpublished data*). Chromatograms of samples (Fig. 2) show larger amounts of dansyl-Cl by-products than in the standard mixture, probably because of the complex composition of doughs. Other unidentified peaks in the chromatograms (Fig. 2) may correspond to amino acids not considered here, or to small peptides. The peak eluted just before dansyl-isoleucine disappeared after hydrolysis (*unpublished data*). All these unidentified products were well separated from dansyl-amino acids.

Initially, assays were made to choose an internal standard for quantification. Four nonprotein amino acids (cysteic acid, norvaline, norleucine, and ornithine) were screened. Cysteic acid and norleucine coeluted with aspartic acid and tryptophan, respectively, and ornithine was present in bread doughs (Fig. 2).

Fortunately, norvaline was satisfactorily resolved and near the midpoint of the run.

Reproducibilities of the derivatization method and chromatography were established by succesive injections (10 determinations) of the derivatived amino acid standard mixture including different proportions of internal standard. Results (Table II) show good reproducibility. The coefficient of variation for relative retention time was less than 1% and for response factors, less than 6% for most amino acids. Variability in amino acid quantification (Table III) was similar in all doughs to that for response factor in the standard mixture. In a few cases, the coefficient of variation was greater than 10%, owing to the low concentration of amino acids in the reaction mixture. Short analysis time, high resolution capacity and good reproducibility of results make this method suitable for determination of the free amino acid content in doughs. Accurate control of operating conditions and adequate reconditioning of the column after each run are essential to achieve maximum resolution and reproducibility.

Changes in Total Amino Acid Content

Total amino acid content, determined by the sum of the individual content of amino acids (Table III) in each dough, changed during fermentation. Total amino acids decreased markedly (72%) during the first 6 hr of fermentation, especially from 0 to 2.5 hr (55%); total amino acids increased between 6 and 24 hr (105%). Similar trends are reported by others for bread doughs and cracker sponges, but the data compared differ in absolute values (Ponomareva et al 1964, Morimoto 1966, El-Dash 1971, Faridi and Johnson 1978).

The variation in total amino acids can be explained as a balance between their absorption by yeast (MacWilliam and Clapperton 1969, Krauze et al 1965, Spicher and Nierle 1984a) and their release by endogenous proteases from flour (Grant and Wang 1972) and/or lactic acid bacteria (Spicher and Nierle 1984b, Kosmina 1977).

At the beginning of fermentation, yeast rapidly reaches the log phase, producing an intensified demand for nitrogen as cells multiply (Baca and Golebiewski 1977). Thorn and Ross (1960) found in straight doughs that compressed yeast multiplies 37% in 3–3.5 hr. Flour protease activity is low in this period because the dough pH (5.67–5.05) is not optimum (McDonald and Chen 1964). No significant change was found in the amine nitrogen content of doughs made without compressed yeast and allowed to stand for 3 hr (Benedito de Barber et al 1985). In addition, lactic acid bacteria have a long lag phase (Morimoto 1966). All these factors decrease the total amino acid content. The variation of this trend after 6 hr of fermentation apparently results because

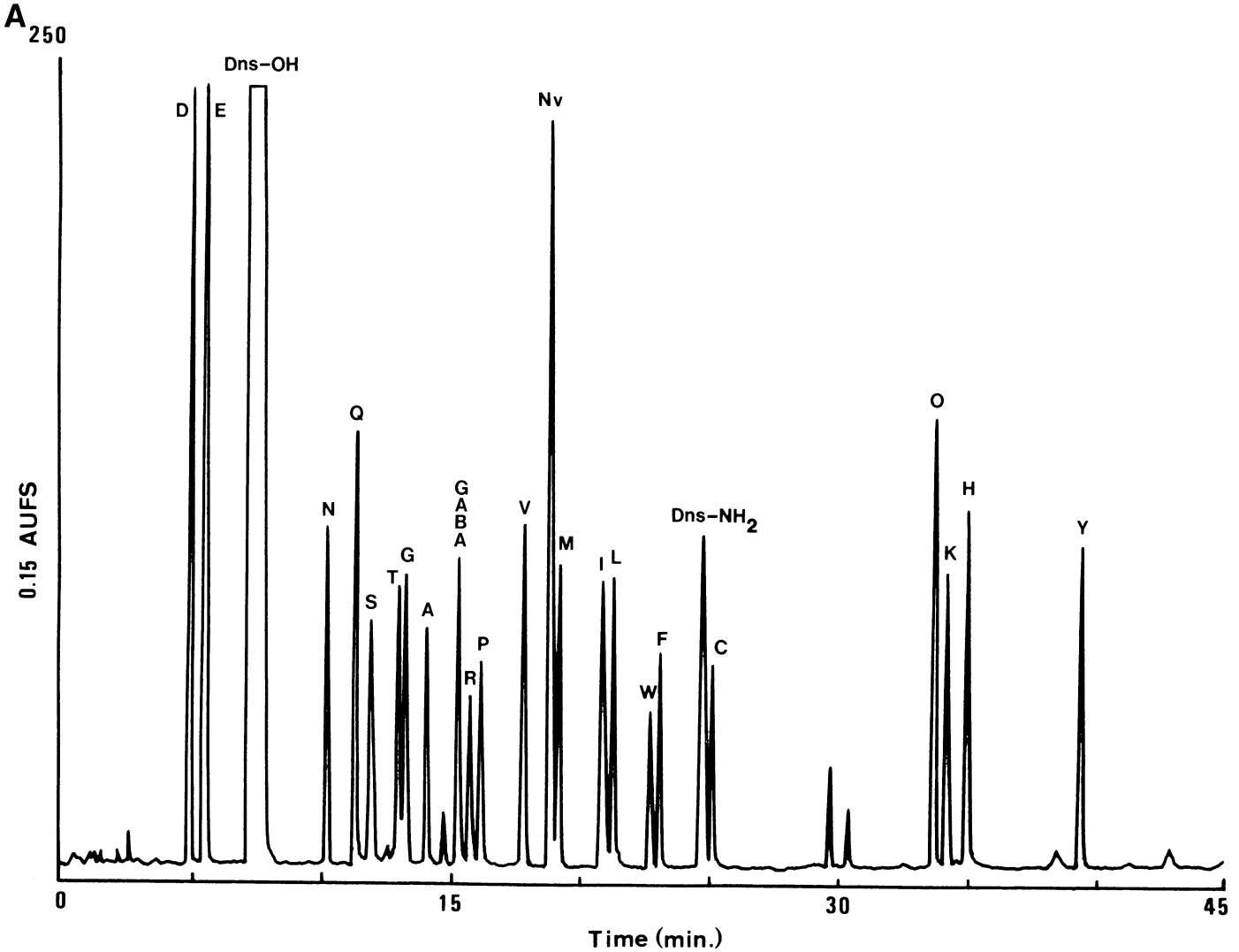

Fig. 1. Separation of dansyl amino acids standards by high-performance liquid chromatography. Column: ODS (3 μm, 83 × 4.6 mm i.d.). Solvents: A = 12 m*M* K_2HPO_4, pH = 7.0; B = CH_3CN. The linear gradient was 10 to 43.6% B during 42 min. Flow rate: 1.5 ml/min. Injection volume: 10 μl. Column temperature: 30°C. Dansyl (**Dns**)-amino acids are symbolized by one letter abbreviations: aspartic acid = **D**, glutamic acid = **E**, asparagine = **N**, glutamine = **Q**, serine = **S**, threonine = **T**, glycine = **G**, alanine = **A**, arginine =**R**, proline = **P**, valine = **V**, methionine = **M**, isoleucine = **I**, leucine = **L**, tryptophan = **W**, phenylalanine = **F**, cystine = **C**, ornithine = **O**, lysine = **K**, histidine = **H**, and tyrosine = **Y**. **AUFS** is absorbance units full scale, **GABA** is γ-aminobutyric acid, **Nv** is norvaline, **Dns-OH** is dansylic acid, and **Dns-NH_2** is dansyl amide.

yeast growth stops and proteases and lactic acid bacteria activity increase at the lower dough pH (Faridi and Johnson 1978).

Changes in the Individual Amino Acid Content

Table III shows individual amino acid contents of unfermented and fermented doughs. Twenty-two amino acids were detected in every dough. Cystine, tryptophan, and threonine could not always be quantified because they were present in trace amounts. Aspartic and glutamic acids, asparagine, and alanine predominated in unfermented doughs and together accounted for 43% of free amino acids. Two nonprotein amino acids, ornithine and γ-aminobutyric acid, were also detected. Ornithine has a microbial origin; it is involved in the biosynthetic pathway of arginine (Wiame 1971), and it is known that yeast releases amino acids during mixing (El-Dash and Johnson 1970). γ-Aminobutyric acid is formed from glutamic acid in flour by glutamate carboxylase (Ponomareva et al 1964).

With the exception of proline, all free amino acids decreased considerably at the beginning of fermentation (UF to F6). In addition, amino acids were metabolized from dough at different times. From UF to F2.5, asparagine, dicarboxylic acids, hydroxy, aromatic, and monoamino monocarboxylic amino acid contents, except glycine, underwent considerable depletion, mainly aspartic acid (−88%), asparagine (−92%), and tryptophan (−91%), whereas arginine increased by 31%. From F2.5 to F6, glutamine, glycine, γ-aminobutyric acid, and basic amino acid contents were reduced sharply, especially glutamine, γ-aminobutyric acid, and histidine (−80%). Similar behavior was reported in bread doughs, and in sponge (El-Dash and Johnson 1970) and straight dough (Morimoto 1966) systems.

The constant proline content suggests not only that it is not consumed by yeast (yeast assimilates proline only under aerobic conditions [Pekur and Bur'yan 1981]), but its production by proteolytic activity appears nonsignificant. Proline is prominent in the amino acid composition of wheat flour proteins (Kasarda et al 1971). Therefore, differences in the amino acid elimination

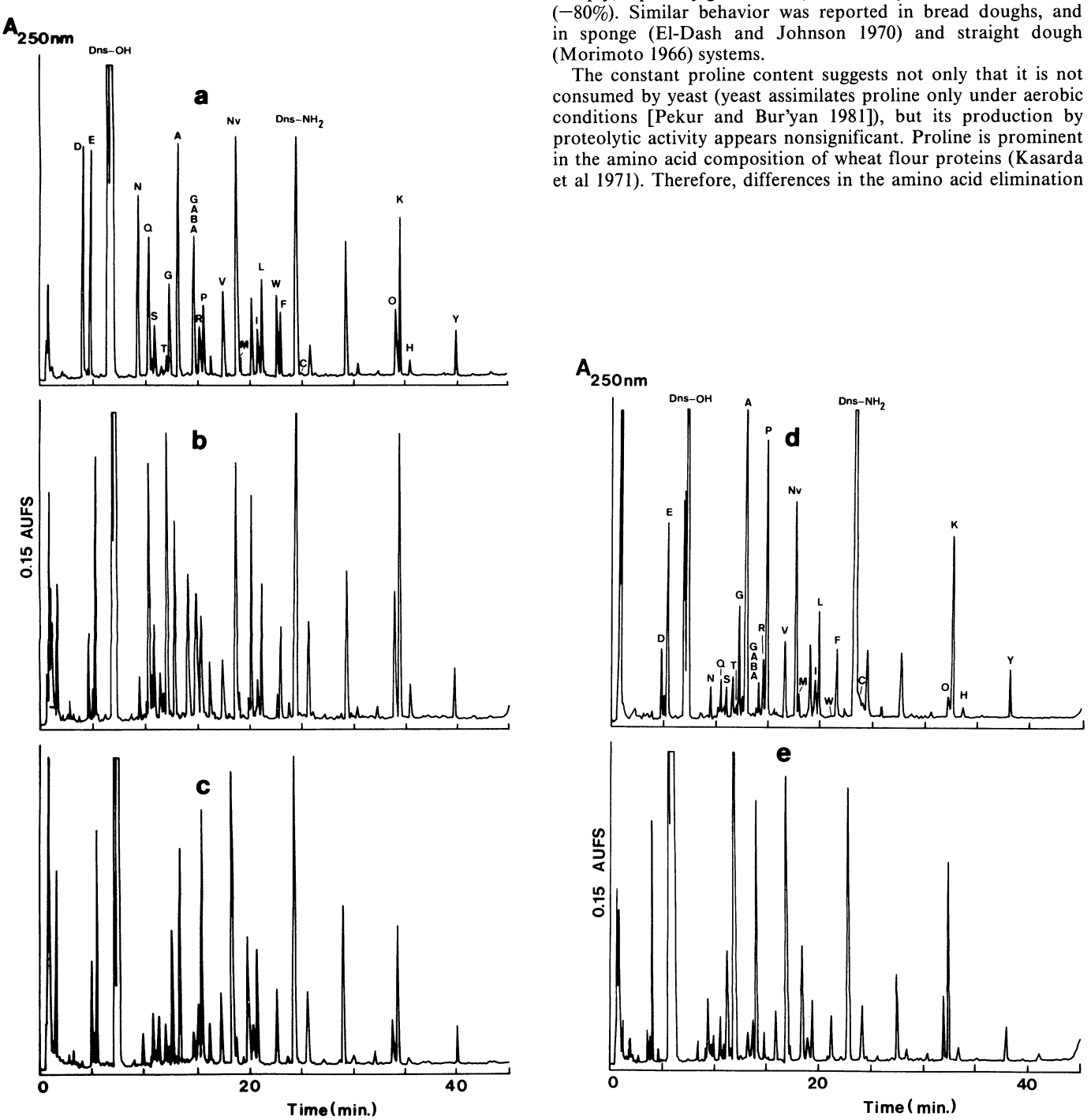

Fig. 2. Reversed-phase high-performance liquid chromatograms of dansyl amino acids from unfermented (**a**) and fermented (**b–e**) bread doughs. Fermentation time: 2.5 (**b**), 6 (**c**), 12 (**d**), and 24 hr (**e**). Separation procedure as in Fig. 1. Aspartic acid = **D**, glutamic acid = **E**, asparagine = **N**, glutamine = **Q**, serine = **S**, threonine = **T**, glycine = **G**, alanine = **A**, arginine =**R**, proline = **P**, valine = **V**, methionine = **M**, isoleucine = **I**, leucine = **L**, tryptophan = **W**, phenylalanine = **F**, cystine = **C**, ornithine = **O**, lysine = **K**, histidine = **H**, and tyrosine = Y. **AUFS** is absorbance units full scale, **GABA** is γ-aminobutyric acid, **Nv** is norvaline, **Dns-OH** is dansylic acid, and **Dns-NH_2** is dansyl amide.

rates may relate to differences in their absorption rates by yeast (Jones and Pierce 1964).

The sequence of amino acid elimination from dough can be explained by the existence of at least 11 transport systems, specific for one or only a few amino acids, and by a general amino acid permease (Rose and Keenan 1981). Thus uptake of amino acids reflects affinity for the transport system they share.

TABLE II
Retention Times and Response Factors of Amino Acids Relative to Norvaline (internal standard)

	Retention Times		Response Factors	
Amino Acid	Mean[a]	CV (%)	Mean[b]	CV (%)
Aspartic acid	0.2305	1.52	1.3612	5.20
Glutamic acid	0.2643	1.26	1.5508	6.04
Asparagine	0.4999	0.85	1.4198	6.24
Glutamine	0.5542	0.51	1.2414	2.97
Serine	0.5888	0.40	1.0538	2.36
Threonine	0.6461	0.25	1.6599	1.89
Glycine	0.6650	0.19	0.5808	1.61
Alanine	0.7093	0.20	0.7373	1.31
γ-Aminobutyric acid	0.7762	0.42	0.8350	1.64
Arginine	0.8078	0.46	2.0604	4.59
Proline	0.8323	0.21	0.8760	1.71
Valine	0.9363	0.14	1.0254	1.31
Methionine	1.0193	0.09	1.4146	2.62
Isoleucine	1.1160	0.15	1.2135	1.57
Leucine	1.1411	0.12	1.1660	1.31
Tryptophan	1.2261	0.16	1.7318	1.29
Phenylalanine	1.2484	0.21	1.5098	1.35
Cysteine	1.3937	0.40	1.5079	13.61
Ornithine	1.8730	0.40	0.6470	3.38
Lysine	1.9001	0.42	0.7351	3.19
Histidine	1.9526	0.41	0.7299	3.32
Tyrosine	2.2289	0.41	1.0815	4.47

[a]Not corrected with respect to holdup time. Mean of 10 determinations.
[b]Response factor = (weight of amino acid/weight of internal standard) × (peak area of internal standard/peak area of amino acid). Mean of 10 determinations.

TABLE III
Free Amino Acid Content of Unfermented (UF) and Fermented (F) Wheat Bread Dough Samples

	Mean Content (mg/100 g of dough, db)				
Amino Acid	UF ($n = 6$)	F2.5[a] ($n = 3$)	F6[a] ($n = 5$)	F12[a] ($n = 4$)	F24[a] ($n = 4$)
Aspartic acid	12.15	1.50*[b]	1.39	1.31	0.73
Glutamic acid	8.98	3.61*	4.00	5.70*	6.07
Asparagine	8.21	0.64	0.50	0.67	0.58
Glutamine	4.72	3.52	0.64*	0.68	1.51
Serine	1.80	0.99*	0.60	0.58	0.53
Threonine	1.77	0.53*	tr	0.96*	0.78
Glycine	2.18	2.12	0.87*	1.14	1.25
Alanine	8.05	1.91*	2.05	8.30*	15.65*
γ-Aminobutyric acid	4.96	2.14*	0.30*	0.68	0.57
Arginine	4.26	5.71*	1.76*	2.55	2.28
Proline	2.27	2.35	3.18	5.04*	6.60*
Valine	3.35	1.02*	0.95	1.39	1.35
Methionine	0.91	0.57	0.53	0.73	0.59
Isoleucine	1.93	0.64*	0.74	0.88	0.60
Leucine	4.04	2.09*	1.89	2.38	1.69
Tryptophan	4.68	0.42*	tr	tr	0.16
Phenylalanine	3.33	1.68*	1.42	1.78	1.48
Cystine	tr	tr	tr	tr	tr
Ornithine	1.45	1.09	0.35*	0.22	0.86*
Lysine	3.73	2.83	1.29*	2.38*	3.15
Histidine	0.49	0.50	0.10*	0.21	0.35
Tyrosine	1.82	0.87*	0.53	1.00	0.88
TAA[c]	84.84	37.00*	23.37*	38.76*	47.95*

[a]Fermentation time (hours).
[b]An asterisk indicates a statistically meaningful change between mean values ($P < 0.05\%$) for the longer fermentation time and the shorter time to its left.
[c]Total amino acids.

The slow removal of basic amino acids suggests that the general amino acid permease, which shows a high capacity for the transport of basic amino acids (Crabeel and Grenson 1970, Woodward and Cirillo 1977) and their specific transport systems (Grenson 1966, Grenson et al 1966) is repressed or is not synthetized at the beginning of fermentation. This fact would be explained by the existence of a high NH_3 level in unfermented dough (El-Dash and Johnson 1970), the best source for nitrogen. In addition, NH_3 can inhibit the catabolic pathway of arginine (Wiame 1971) and promote its release during the first 2.5 hr of fermentation.

During longer fermentation periods (6–24 hr), most individual amino acids varied little (Table III). From 6 to 12 hr, only glutamic acid, threonine, alanine, proline, and lysine increased significantly (mainly threonine [200%] and alanine [300%]). From 12 to 24 hr, alanine and proline again increased by 90 and 30%, respectively. The largest increase was shown by ornithine (285%). These results explain the changes in total amino acid contents (Table III). Thus, the increases in alanine, glutamic acid, and proline contents from 6 to 24 hr together account for 75% of the increase in total free amino acids.

During fermentation, yeast is less metabolically active (stationary phase); consequently, the absorption rate of amino acids decreases. This metabolic state promotes the release of amino acids, especially alanine and lysine (Clapperton 1971). In parallel, proteolytic activity increases as dough pH decreases (Faridi and Johnson 1978). As a result, glutamic acid and proline contents increase. This activity is not very specific and should be attributed principally to lactic acid bacteria (Spicher and Nierle 1984b). The endogenous proteases of wheat flour release mainly leucine and phenylalanine (Grant and Wang 1972). At the end of fermentation (between 12 and 24 hr), increased amino acid absorption rate by lactic acid bacteria and their specific nutritional requirements balance the content of some amino acids. Thus, lactic acid bacteria in sourdough have no requirement for alanine, whereas they are unable to grow without glutamic acid (Spicher and Schröeder 1979).

Our results showed dynamic changes in amino acids during fermentation, mainly of microbial origin. The slow removal of basic amino acids and the release of arginine by yeasts can be important for selection of microbial starters, because basic amino acids are most reactive during baking (El-Dash and Johnson) and enhance bread flavor (Kiely et al 1960).

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[Received September 22, 1988. Revision received February 22, 1989. Accepted February 26, 1989.]