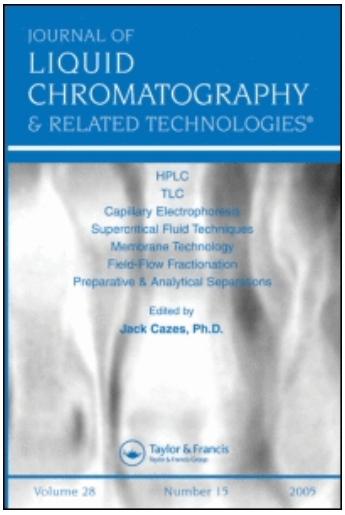

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Influence of the Microbial Starter and the Breadmaking Step on the Free Amino Acid Profiles of Wheat Sours, Doughs, and Breads by Reversed-Phase High-Performance Liquid Chromatography

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INFLUENCE OF THE MICROBIAL STARTER AND THE BREADMAKING STEP ON THE FREE AMINO ACID PROFILES OF WHEAT SOURS, DOUGHS, AND BREADS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

Reversed-Phase High Performance Liquid Chromatography profiles of twenty-two free amino acids from sour doughs (240 dough yield, 35 °C, 20 h, 1.1% ash content), bread doughs (17.5% sour dough addition, 0.5-0.6% ash content) and breads respectively inoculated with *Lactobacillus brevis*, 25a (with and without yeast) and *Lactobacillus plantarum*, L-73 and B-39 (without yeast) are investigated. Interactive influence of both the microbial composition of the starter and the breadmaking steps are observed on the individual amino acid pattern. After proofing, a significant decline is noticed in dicarboxylic acids and amides, Ser, and hydrophobic amino acids as well as in total amino acid content in all samples. The extent in the amino acid assimilation by lactobacilli and mainly by yeast is closely concerned with the strain of lactobacilli. In general, homofermentative lactobacilli induce bigger decreases. In addition, aromatic amino acids noticeably deplete during fermentation of both unsoured doughs and doughs started with *L. plantarum* strains. Predominant amino acids such as Ala and GABA are sharply promoted as a consequence of enzymatic reactions. Baking generally induces a significant increase in main amino acids except in unsoured doughs and doughs started with L-73. The presence of yeast in the microbial composition of the heterofermentative bacterial starters (25a) leads to greater increases in amino acid levels during baking.

Introduction

The positive effects of sour dough addition in breadmaking performance and in extension of bread microbial shelf-life have been extensively supported in the literature for rye (1-2) and wheat (3-7) systems, and related to the production of suitable metabolites by the starting microflora. Sour dough improving action is associated with the equilibrated balance between ethanolic and lactic fermentation (1) and depends on many interactive factors -microbial starter composition (4, 6, 8), sour dough process (9), processing conditions (3, 10-12) and ingredients (8)- that regulate the metabolism of starting yeast and lactic acid bacteria.

In wheat products, sour dough plays a dual role as rising and acidifying agent to promote both aroma and volume of bread (5). Consequently, in addition to acidification degree, active metabolites such as amino acids are of interest since they serve as an important source of bread flavor precursors (13), are involved in metabolism of dough microorganisms (14-17), and improve sour dough functionality (18) and bread dough machinability (19).

During sour dough fermentation of both rye and wheat systems, amino acid metabolism regarding nutritional requirements and involved enzymatic activities, depends mainly on the microbial starter composition (20-23) and on some processing conditions (13, 17, 24). Concerning wheat sours, the extraction rate of flour and the fermentation temperature are reported as the main factors with positive influence on level of total free amino acids (24) and on extent of accumulation of hydrophobic and basic amino acids during fermentation (17). Incorporation of sour doughs to bread doughs affects initial levels of amino acids in doughs depending on the sour dough starter composition (25) since microbial mass of microorganisms constitutes a source of amino acids (23). As well, qualitative and quantitative composition of amino acids of fermented doughs affects intensity and typical bread flavor mainly through the formation of specific Maillard compounds during baking.

The influence of the microbial starter on the individual amino acid pattern has been investigated in rye (13, 26) and wheat (17, 24) sour doughs so as in wheat straight doughs and breads (15, 16, 19, 27, 28); but no systematic research has been carried out on free amino acid profiles of started wheat sours, doughs and breads despite the interest of the dynamics of these metabolites along breadmaking.

In this paper, a previously optimized methodology for the determination of 22 individual amino acids is successfully applied to wheat sours, doughs and breads started with homo and heterofermentative bacterial starters.

Experimental

Reagents and chemicals. Amino acids (AA, Sigma grade), dansylaminoacids, and dansyl chloride were purchased from Sigma. Acetonitrile (ACN, HPLC grade) and water (chromatography-grade) were furnished by Merck; acetone (ultraviolet grade), was from Panreac. Buffers were prepared from analytical-grade chemicals. Solvents were filtered with an Afora filter holder, and samples, with a Swinney filter (Millipore). Millipore HA-grade 0.45-μm filters and Millipore FH-grade 0.22-μm filters were used for aqueous solvent and ACN filtration, respectively.

HPLC equipment. A Hewlett-Packard (Palo Alto, CA) HPLC system, composed of a 1050 pumping system, a rheodyne injector, a 1040 A diode array detector set at 254 nm, a 9000 Pascal Chem Station and an oven set at 30 °C, was used.

Chromatographic conditions. An ODS HS/3 column (3 μm particle size, 8.3 cm length, 4.6 mm i. d.) and a precolumn (3 μm, 3 cm, 4.6 mm) both from Perkin-Elmer (Norwalk, CT) were used as stationary phase. Mobile phase consisted of buffer 12 mM K_2HPO_4, pH 7 (A) and ACN (B). Gradient program was from 10% to 43.6% B in 42 min,

up to 70% B in 3 min, and isocratic elution during 10 min for washing purposes. Flow rate was 1.50 mL/min.

Sample preparation. Three lactobacilli strains (*Lactobacillus brevis*, 25a with (+) and without (-) yeast, and *Lactobacillus plantarum*, L-73 and B-39 without yeast) were used as starters. Composition of microbial starters (propagated cultures: CHR. Hansen's Laboratorium, Denmark A/S), analytical data on flours used (supplier: Federal Center for cereal, potato and lipid research, Detmold, Germany), and conditions for sour dough (FSD) preparation and dough formula are summarized in Table I. Control doughs (without starter) were also prepared for comparative purposes. The German Standard method "Kastengebächversuch" was used for baking test (5). Briefly, doughs were mixed at 26-27 °C (UBD); after dough resting (20 min), scaling (700 g), hand rolling and intermediate proofing (10 min), doughs were panned and proofed (FBD) (60 min, 32°C, 75-80% RH) before baking (B) (240°C, 40 min).

Extraction, purification and chromatographic determination of free AA. Freeze-dried FSD, UBD, FBD (10 g) and B (5 g) were each extracted twice with 20 mL 40% ethanol (v:v) (25). After centrifugation at 23 000 g for 20 min at 1-3°C, supernatants were made up to 50 mL. Aliquots of extracts were deproteinized by ultrafiltration (cut-off 10 kDa, Millipore cartridges). Protein-free extracts were brought to pH 2.0 and applied onto a column packed with 5 mL Dowex 50W-X2 resin (50-100 mesh, H^+ form), previously equilibrated with 0.01 M HCl (16). The ammonia eluates were evaporated to dryness in a rotary evaporator, Dried extracts were dansylated (28) and used for AA determination (15, 27) using the internal standard addition method for AA quantification. A standard mixture of 20 protein AA, γ-aminobutyric acid and ornithine were dissolved in 0.01 N HCl and made up to 200 mL. Norvaline was used as the internal standard (69 mg/200 mL 0.01 HCl). Aliquots of the AA standard mixture and purified sample extracts, containing approximately 50 μg of amino nitrogen were placed in small vials, and the internal stardard (0.1 mL) was

Table I.- Starters and flours used for sour and bread dough preparation.

STARTER COMPOSITION AND FLOUR CHARACTERISTICS		
lactobacilli	**strain**	**cfu/g**
Lactobacillus plantarum	L-73	2.0×10^{10}
Lactobacillus plantarum	B-39	5.0×10^{11}
Lactobacillus brevis	25-a	6.0×10^{11}
yeast	**cells/g**	
Instant Active Dried Yeast (IADY)	2.7×10^{10}	
flour	**sour dough**	**bread dough**
energy of deformation ($\times 10^3$ ergs)	-	233
curve configuration ratio	-	0.92
ash (%, mb)	1.11	0.54
moisture (%)	12.22	14.79
Hagberg Index	-	360
SOUR DOUGH CONDITIONS		
dough yield	240	
fermentation time	20 h	
fermentation temperature	35 °C	
bacterial inoculum	10^7 bacteria/g flour	
yeast inoculum (25-a+)	10^6cells/g flour	
DOUGH FORMULA		
sour dough (%, flour basis)	17.5	
flour (g)	1000	
ascorbic acid (mg)	50	
malt flour (g)	1.8	
water (ml)	620	
vegetable fat (g)	10	
sugar (g)	10	
salt (g)	15	
IADY (g)	20	

respectively 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 chloride (Dns-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 initial mobile phase. Aliquots (20μ L) of each sample and standard were injected into the HPLC system.

Statistical evaluation. Two paralell experiments and three replicates per experiment were made. Data of individual amino acid levels in FSD, UBD, FBD and B were statistically analyzed in a Microvax computer (Digital, Northboro, MA) using programs of the BioMeDical statistical Package (BMDP). Univariate analysis (two-ways analysis of variance) was performed to calculate significant interactions (starter x breadmaking step) by applying 7D program (Tukey test). Multivariate data handling procedures (factor and K-means clustering analysis) were conducted in order to classify samples on the basis of the significant grouping individual amino acids, by applying respectively 4M and KM programs.

Results and Discussion

Chromatographic separation and quantification of amino acids

Previously optimized RP-HPLC experimental conditions (28) lead to the complete separation of 23 amino acids (20 protein AAs, GABA, Orn and Nval as internal standard) in a standard mixture and in FSD, UBD, FBD and B samples, in less than 37 min (Figure 1).

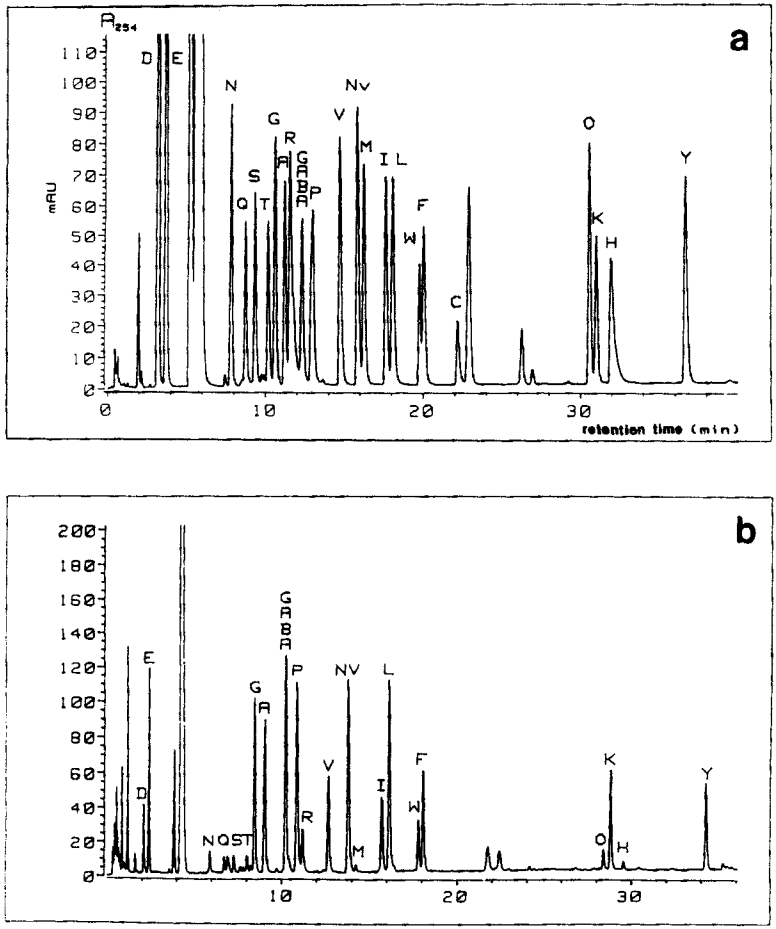


Figure 1.- Separation of dansylamino acids (Dns-AA) of a standard mixture (a) and of fermented sour dough samples started with *Lactobacillus brevis*, 25a+ (b), by reversed-phase high-performance liquid chromatography. Concentration of Dns-Cl [Dns-Cl] and molar ratio [Dns-Cl]/[AA]= 8.5 mM, 4.6. Column: ODS (3 μm particle size, 8.3 cm length, 4.6 mm i.d.). Mobiles phases: A= 12mM K_2HPO_4, pH 7.0; B= ACN. Linear gradient, 10 to 43.6% B over 42 min; flow rate: 1.5 mL/min; injection volume: 20 μL; column temperature: 30 °C. Dns-AAs: (D) Asp, (E) Glu, (N) Asn, (Q) Gln, (S) Ser, (T) Thr, (G) Gly, (A) Ala, (GABA) γ-amino butyric acid, (P) Pro, (R) Arg, (V) Val, (M) Met, (I) Ile, (L) Leu, (W) Trp, (F) Phe, (C) Cys, (O) Orn, (K) Lys, (H) His and (Y) Tyr.

Good resolution and reproducibility are achieved in the separation of individual AAs. Coefficients of variation (CV) for relative retention times in standards and samples range from 0.10 and 1.30%, being less than 1% for most AA. Variability in response factors (f) relative to Nval and in AA quantification are less than 5% except for Thr, Tyr and Arg (<9%). Maxima diode array detector (DAD) responses correspond to Gly (f= 0.5713), Orn (f= 0.6033) and Lys (f=0.6347); minima are for Cys (f= 3.2076), Arg (f=2.6836) and Thr (f= 2.3913). Peak purity checked through specific DAD software and by comparison of UV spectra at different wavelength is higher than 99.5% (match> 995) for all AA except for Cys and Met (match= 900) when present in trace amounts.

Interactive effects of microbial starter and breadmaking step on the free amino acid profile of samples

Data of individual and total amino acid content of sours, doughs and breads are summarized in Table II. Interactive effects between type of starter (B-39, 25a-/+, L-73) and type of sample (FSD, UBD, FBD, B) are observed on most AA. Among the pool of samples, total amino acid content (mg/ 100 g sample, dry basis) ranges from 167.25 mg (B-39-) to 216.19 mg (L-73-) within FSD, from 86.25 mg (control, 25a+) to 125.99 mg (L-73-) within UBD, from 58.93 mg (control) to 107.59 mg (25a-) within FBD, and from 66.26 mg (control) to 123.03 mg (25a+) within B. Prominent AA include Glu, Ala, GABA, and Pro in all samples; and Arg, Ile, Leu, Trp, Phe and Lys in FSD. In an opposite way, Cys and Met are minor AA, sometimes present in trace amounts.

Fermentation and baking steps influence the individual and total AA levels depending on the microbial composition of started sour doughs (Table II). After prooving, in general, a significant ($P<0.05$) decline is observed in dicarboxylic acids and amides, Ser and hydrophobic AA as well as in total amino acid content in all doughs (Figure 2). As it was

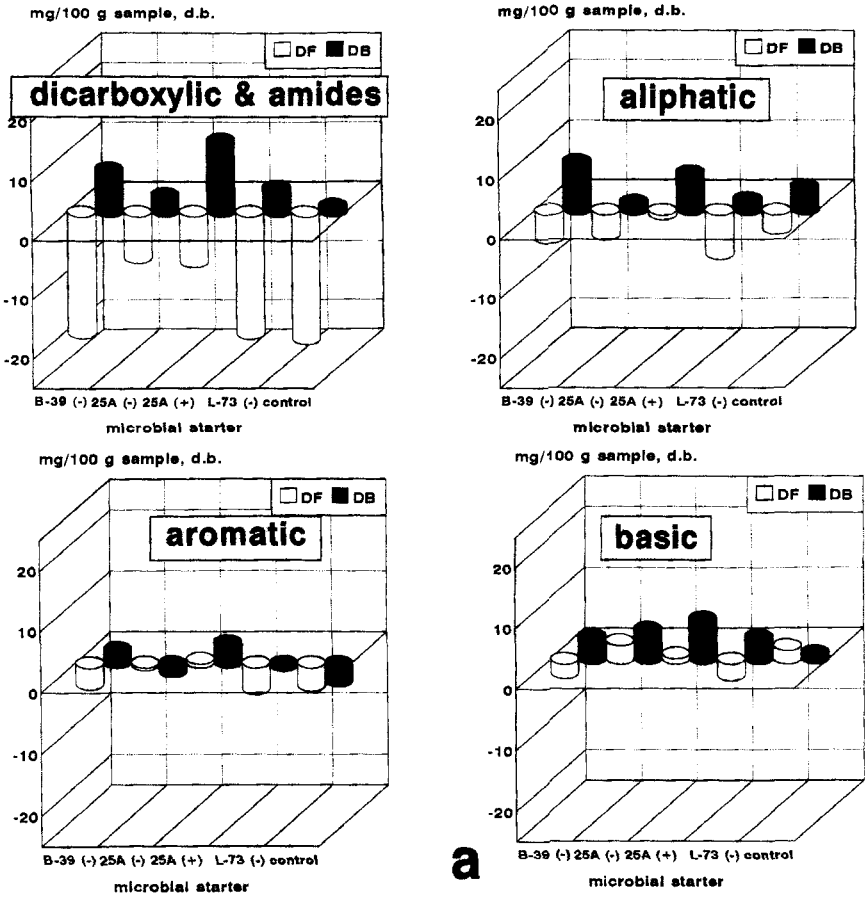


Figure 2.- Changes in main (a), minor (b) and total (c) free amino acid groups during fermentation (DF) and baking (DB).

(continued)

previously reported (16), the extent in the AA assimilation by lactic acid bacteria and mainly by yeast is closely concerned with the strain of lactobacilli in good accordance with their nutritional requirements on AA (14). In general, homofermentative lactobacilli (B-39, L-73) induce bigger decreases during proofing than the heterofermentative lactobacillus 25a (+/-) (Table II). In addition, aromatic AA noticeably decrease during fermentation of control

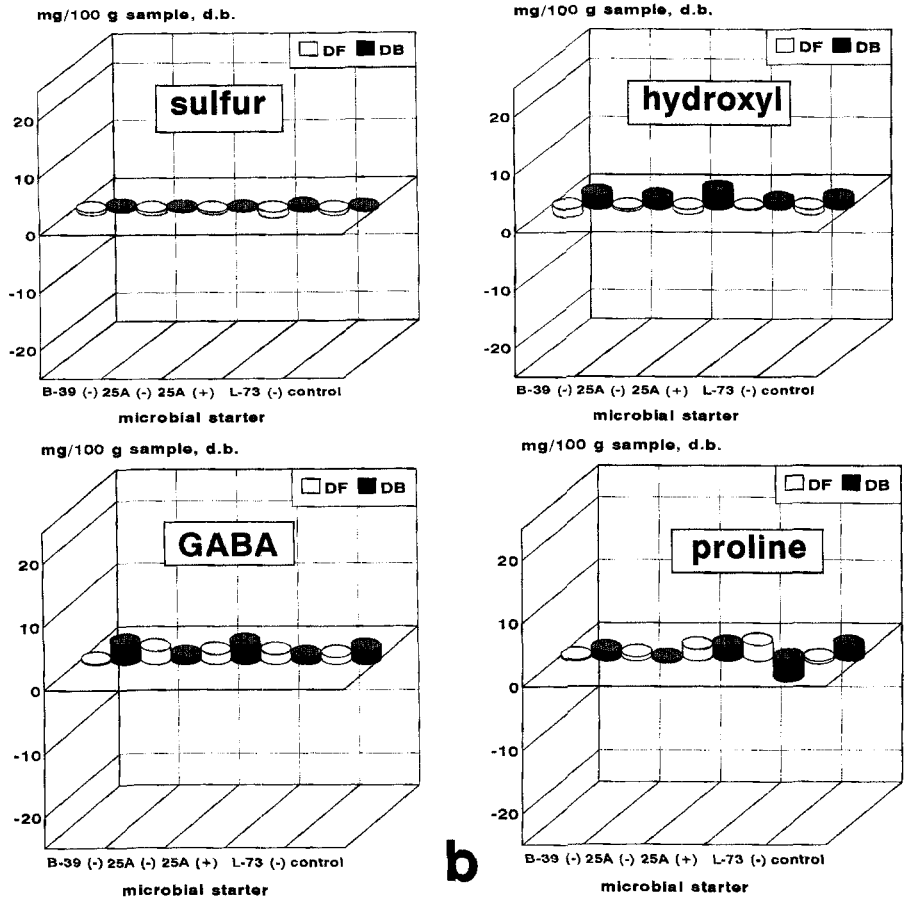
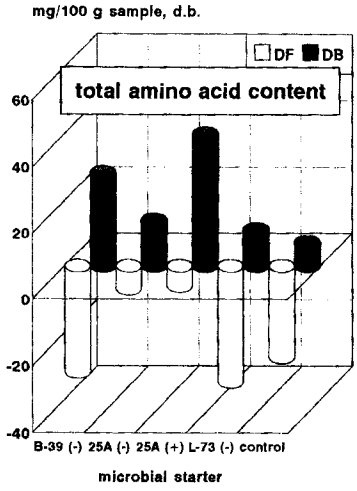


Figure 2 (Continued)

doughs and of doughs started with *L. plantarum* strains (Figure 2). On the other hand, predominant AAs such as Ala and GABA are sharply promoted as a consequence of enzymatic reactions. Protein AAs accumulate by exoproteolysis (13) because lactic acid bacteria no longer metabolize AAs so rapidly. GABA (25a, L-73) increases as a consequence of the effect of glutamate decarboxylase (29) as there is a simultaneous reduction in Glu content. Orn, which is involved in the biosynthetic pathway of Arg (30), is released particularly in 25a started doughs, probably from the microbial mass (25).



C

Figure 2 (Continued)

Baking generally induces a significant ($P<0.05$) increase in main AAs except in control doughs and in doughs started with L-73 (Figure 2). This observation should be attributed to an hydrolytic thermal degradation and to an intense proteolysis of proteins favoured by the acidic pH (4.43-4.56) of soured fermented doughs during oven spring. The net AA increase masks their utilization in non-enzymatic Maillard reactions. The protein degradation is not so intense in control doughs (pH 5.57) in which the reaction between AAs and sugars during baking to form melanoidins becomes more evidenced. Concerning heterofermentative 25a as starter, the presence of yeast in its microbial composition (25a-, 25a+), leads to the biggest increases in amino acid levels during baking (Figure 2).

Starter composition does not significantly influence the levels of Ser, Thr and Cys in all samples, leading to similar amounts in both soured and control samples (<4 mg). Within FSD, homofermentative B-39 started samples show the highest amounts of Ala (9.58 mg) and

Table II.- Individual and total amino acid content of unstarted and started sours, doughs and breads.

starter	sample	MEAN CONTENT OF INDIVIDUAL AND TOTAL FREE AMINO ACIDS*																					
		Asp	Glu	Asn	Gln	Ser	Thr	Gly	Ala	GABA	Pro	Arg	Val	Met	Ile	Leu	Trp	Phe	Cys	Orn	Lys	His	Tyr	TAA
Lactobacillus plantarum B-39 (-)	fermented sour dough	5.22	8.22	8.27	4.01	3.73	2.70	4.71	9.58	16.04	6.50	21.68	8.87	1.79	7.03	18.28	8.70	10.84	0.54	0.24	8.71	3.04	7.75	167.25
	unfermented bread dough	6.59	19.81	5.33	4.93	2.32	2.12	2.07	6.37	5.79	11.05	9.35	4.36	0.70	2.80	4.29	7.18	3.07	0.37	0.80	4.11	0.87	3.04	106.26
	fermented[a] bread dough	1.99 **	10.03 **	1.46 **	2.56 **	1.06 **	1.99	2.45	7.89 **	5.95	11.27	8.00	3.20	0.30	0.66 **	0.94 **	6.49	1.15 **	0.23	0.77	3.26 **	0.74	2.16 **	74.50 **
	bread[b]	4.20 **	14.36 **	2.77 **	2.03 **	2.12 **	3.17	3.18	10.62 **	8.86 **	12.59 **	11.06	4.23	0.42	2.18 **	3.21 **	6.40	2.58 **	0.53 **	1.43 **	3.10	1.05	3.18 **	102.93 **
Lactobacillus brevis 25a (-)	fermented sour dough	6.53	9.60	8.52	3.77	5.71	2.82	5.89	6.83	18.99	8.49	5.58	11.04	2.58	10.61	23.08	9.76	14.40	0.67	9.06	4.37	3.71	3.12	174.59
	unfermented bread dough	7.08	17.30	5.52	6.17	2.45	1.73	2.20	7.06	6.06	12.33	1.93	4.92	1.01	4.07	7.62	7.91	4.86	0.24	2.34	2.69	0.66	2.35	107.59
	fermented[a] bread dough	5.66	15.36 **	3.16 **	4.05 **	1.80 **	2.00	2.68	9.28 **	8.16 **	12.97	2.50	4.72	0.54 **	1.98 **	3.04 **	7.99	3.96	0.12	4.01 **	3.42 **	0.75	2.75 **	100.80
	bread[b]	6.57	17.38 **	4.37 **	2.88 **	2.09 **	3.39	2.90	10.44 **	9.21 **	12.70	8.49 **	4.75	0.38	3.51 **	1.59 **	6.60 **	3.65	0.49 **	3.43	2.76	1.04	2.93 **	114.95 **
Lactobacillus brevis 25a (+)	fermented sour dough	5.43	10.45	5.54	4.18	4.08	2.70	6.45	8.12	20.88	10.42	3.25	13.43	2.93	12.34	26.52	11.19	17.02	0.40	9.81	4.89	2.47	3.27	186.08
	unfermented bread dough	6.14	14.42	4.54	6.64	2.24	1.97	1.83	5.47	4.87	8.11	1.98	4.10	0.82	2.75	5.29	5.65	3.56	0.28	2.70	2.48	0.98	1.55	88.81

	fermented[a] bread dough	4.21	11.80 **	2.77 **	4.38 **	1.49	1.82	2.00	7.97 **	6.43 **	9.94 **	2.64	4.12	0.45 **	1.72 **	2.89 **	5.94	3.38	0.25	3.20	2.88	0.32 **	2.07 **	82.82 **
	bread[b]	8.03 **	18.69 **	5.25 **	3.36 **	2.87 **	3.49 **	2.95 **	10.71 **	9.31 **	12.02 **	7.34 **	5.04	0.43	3.10 **	3.37	7.17 **	4.69 **	0.41	4.22 **	2.94	1.25 **	2.93 **	123.03 **
Lactobacillus plantarum L-73 (-)	fermented sour dough	5.72	7.85	9.11	4.92	1.40	2.67	5.31	8.80	20.62	10.72	25.98	12.15	2.95	9.87	30.33	13.83	17.98	0.53	0.37	9.86	2.85	12.41	216.19
	unfermented bread dough	7.04	18.36	6.76	6.91	1.77	2.08	2.24	7.49	6.76	13.00	10.35	5.48	1.15	3.61	8.13	8.45	5.15	0.34	1.09	4.72	1.36	3.84	125.99
	fermented[a] bread dough	2.17 **	11.52 **	1.80 **	2.81 **	1.33	2.38	3.49 **	10.82 **	8.31 **	15.51 **	8.18 **	3.51 **	0.18 **	0.75 **	0.98 **	8.01	1.96 **	0.27	1.11	4.61	0.90 **	3.14	91.31 **
	bread[b]	3.86	14.57 **	2.28	1.81	1.61	3.26	3.37 **	10.62	9.29 **	12.26 **	13.02 **	4.80	0.51	0.98	1.75	6.44 **	2.59 **	0.47	1.18	3.21	1.16	3.57	102.56 **
CONTROL	unfermented bread dough	7.88	20.25	6.31	5.82	2.13	1.86	1.55	5.74	4.31	9.74	2.68	2.18	0.32	1.37	1.43	6.16	1.04	0.63	0.92	1.81	0.58	1.53	86.25
	fermented[a] bread dough	4.98 **	9.46 **	0.66 **	3.53 **	1.24 **	1.80	2.03 **	3.96 **	5.39	9.31	4.78	2.10	0.23	0.32 **	0.65	4.28 **	0.28 **	0.11 **	0.68	2.34	0.39	0.38 **	58.93 **
	bread[b]	3.49	13.65 **	1.14	1.47 **	1.75	2.88	2.32	5.95 **	7.48 **	11.42 **	5.62	3.37	0.27	0.55	0.94	0.59 **	0.45	0.32	0.82	2.12	0.73	0.85	66.26

(*) mg amino acid/100 g sample, d.b.

TAA= total amino acid content

(**) Indicates a statistically significant change between mean values ($P < 0.05$) for fermentation (a) and baking (b) steps when 2nd order interactions (starter / breadmaking step) are significant.

the lowest in Glu (8.22 mg), Gly (4.71 mg), GABA (16.04 mg), Pro (6.50 mg), Val 8.87 mg), Ile (7.03 mg), Leu (18.28 mg), Trp (8.70 mg) and Phe (10.84 mg). Heterofermentative 25a accounts for greater amounts of Glu (9.60 mg) and Ser (5.71 mg), being hydrophobic AAs promoted in presence of yeast (25a+). Within UBD, inoculated doughs show higher levels of individual and total AAs except in Asp, Glu and Asn, when data are compared with control doughs. In general, homofermentative lactobacilli (L-73, B-39) lead to higher levels of AAs than heterofermentative (25a+). The presence of yeast significantly decreases the amount of AAs. FBD including uninoculated samples do not statistically differ in addition in Gly (2.0-3.5 mg), Met (0.2-0.5 mg) and His (0.3-0.9 mg) contents, but soured samples show higher levels of free AAs than control doughs with the exception of Asp content (4.98 mg). *L. plantarum* strains (L-73, B-39) leads to similar AA levels, and lower than *L. brevis* starters (25a-, 25a+). Differences in AA pattern are minimized in B samples. Inoculated samples account for greater amounts of AAs than control breads, but similar levels of Ser (1.4-2.9 mg), Thr (2.9-3.5 mg), Gly (2.4-3.4 mg), Pro (11.4-12.7 mg), Met (0.3-0.5 mg) and Cys (0.3-0.5 mg) are observed. In addition, soured samples follow the same profile of Ala (10.4-10.7 mg), GABA (8.9-9.3 mg), Val (4.2-5.0 mg), Trp 6.4-7.2 mg), Lys (2.8-3.2 mg), His (1.0-1.3 mg) and Tyr (3.0-3.6 mg). Within homofermentative lactobacilli, B-39 induces higher hydrophobic AA content; and with heterofermentative lactobacillus 25-a, the presence of yeast increases dicarboxylic acids and amides (+13%) as well as Orn contents (+23%). Levels of Asp, Glu, Asn, Gln, Orn, Ile and total AAs are more prominent in breads started with hetero than with homofermentative lactobacilli (Table II).

Classification of samples according to the amino acid pattern

Multivariate data handling procedures (factor and K-means clustering analysis) are applied in order to classify samples according to the effects of microbial starter and type of

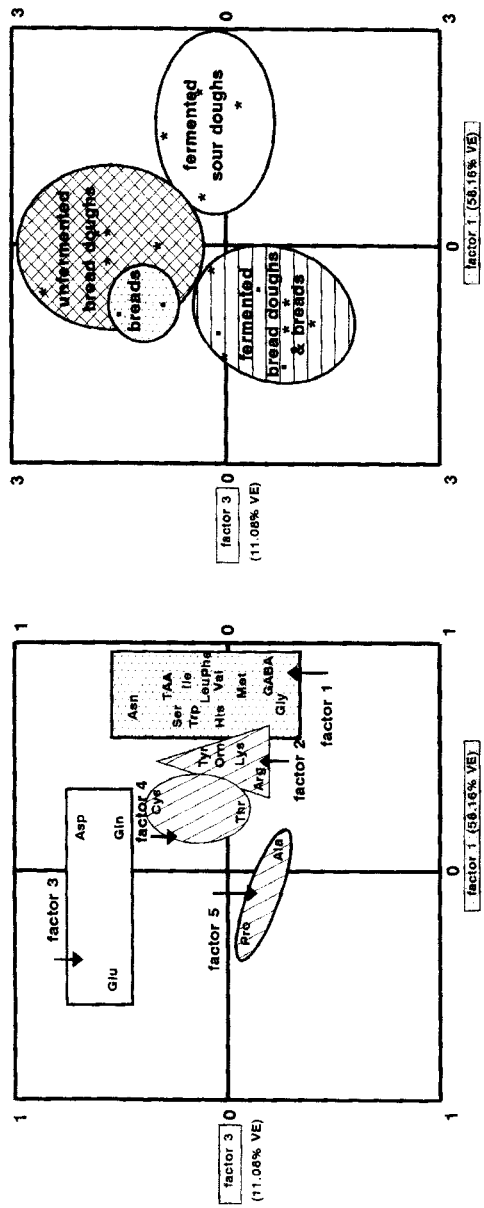


Figure 3.- Distribution of samples (N = 19) in the plane defined by factor 1 vs. factor 3 (factor analysis) on the basis of their individual amino acid pattern (N = 23).

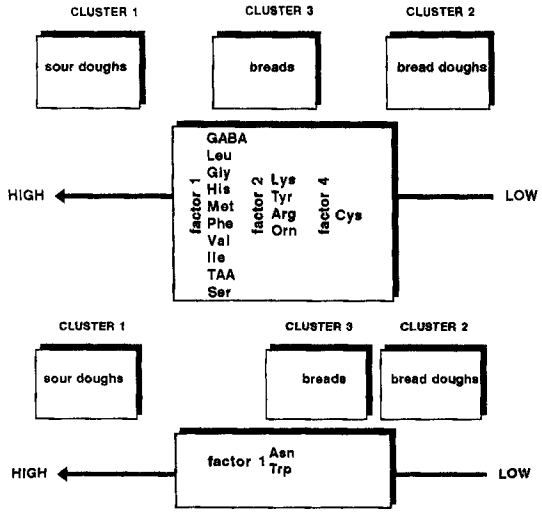


Figure 4.- Trends of individual amino acids of samples distributed in three prefixed clusters (K-means clustering).

sample on the significant grouping individual AAs. When absolute values for analytical variables (Nr= 23) are considered for FSD, UBD, FBD and B samples (Nr= 19), three factors account for 84% of the total cumulative variance (VE) (Figure 3a). Factor 1 (58.16% VE) positively correlates with Val, Phe, Ile, Met, Leu, GABA, TAA, Gly, His, Trp, Asn and Ser (loadings over 0.600); factor 2 (15.16% VE) is closely connected with Arg, Tyr, Lys (positive correlation) and Orn (negative correlation), and factor 3 (11.08%) relates with Asp, Glu and Gln contents. The most suitable classification of samples was obtained after plot of scores of factor 1 vs factor 3 (Figure 3b). Three groups are outlined: (a) FSD (4 cases) showing the higher levels of AAs of factor 1 and medium content of AAs of factor 3, (b) UBD and B (25a) (7 cases) characterized by high levels of AAs of factor 3 and medium contents of AAs of factor 1, and (c) FBD and B (8 cases) defined by low levels of AAs of factor 1 and factor 3. Maximum and minimum values of AAs of factor 1/factor 3

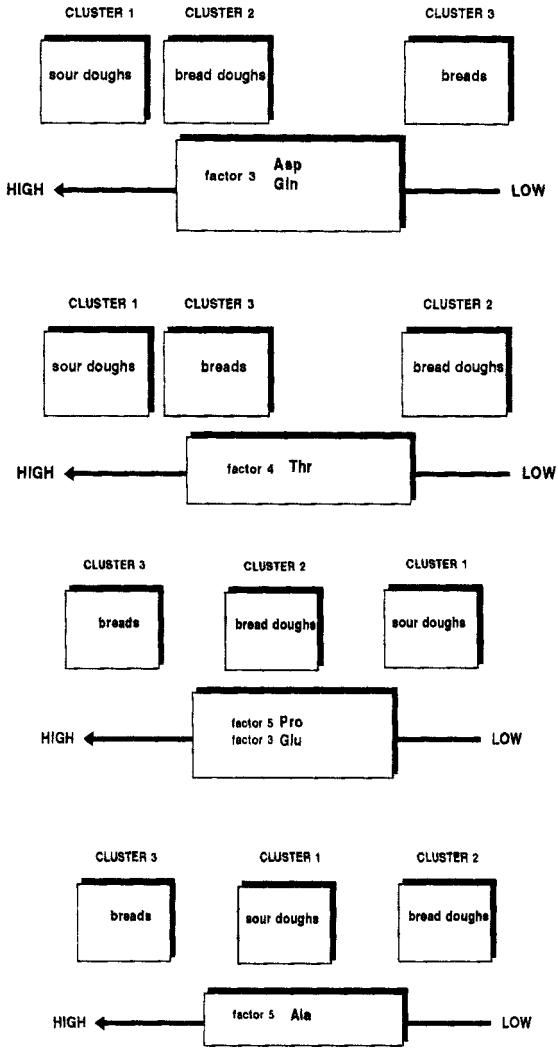


Figure 4 (continued)

correspond respectively to FSD (25a+)/ UBD (control), and B (control)/ FBD (B-39) (Table II).

Sample distribution from factor analysis is in good accordance with AA profiles of samples after K-means clustering analysis of data (Figure 4). In three predefined clusters, the pool of samples is grouped according to samples included in each breadmaking step: FSD (cluster 1), UBD and FBD (cluster 2) and B (cluster 3). FSD account for maximum levels of main AA (factors 1-4), minimum amounts of Pro (factor 5) and Glu (factor 3) and intermediate values for Ala (factor 5). UBD and FBD reach the lower values of main AAs (included in factors 1, 2, 4 and Ala) and intermediate contents of Pro and Glu. B samples characterize by higher amounts of Pro, Glu and Ala, lower in Asp and Gln and intermediate values for main AA. Similarities among samples are also observed: BD and B (Asn, Trp), SD and B (Thr), and SD and BD (Asp, Gln).

Results ponted out that almost all individual AAs play a significant role in characterizing breadmaking samples. Breadmaking step is a separative factor more relevant than the type of starter in the classification of samples.

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Appendix

Key to Abbreviations

RP-HPLC	reversed-phase high performance liquid chromatography
AA	amino acid

FSD	fermented sour dough
UBD	unfermented bread dough
FBD	fermented bread dough
B	bread
Dns-Cl	dansyl chloride
Dns-AA	dansyl amino acid
Asp	aspartic acid
Glu	glutamic acid
Asn	asparagine
Gln	glutamine
Ser	serine
Thr	threonine
Gly	glycine
Ala	alanine
GABA	γ -aminobutyric acid
Pro	proline
Arg	arginine
Val	valine
Nval	norvaline
Met	methionine
Ile	isoleucine
Leu	leucine
Trp	tryptophan
Phe	phenylalanine
Cys	cystine
Orn	ornithine
Lys	lysine
His	histidine
Tyr	tyrosine
A_{254}	absorbance at 254 nm
mAU	absorbance milliunits
Rt	retention time
CV	coefficient of variation
f	response factor
DAD	Diode Array Detector
UV	ultraviolet
TAA	total amino acids
ACN	acetonitrile
VE	variance explained

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