

Influence and Interactions of Processing Conditions and Starter Culture on Formation of Acids, Volatile Compounds, and Amino Acids in Wheat Sourdoughs

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ABSTRACT

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The aim of this work was to study the influence of process parameters and the starter culture on the characteristics of wheat sourdough by using response surface methodology. Influence of fermentation temperature (16–32°C), ash content of flour (0.6–1.8%), and fermentation time (6–20 hr) were considered as independent factors and their effects were studied in sourdough fermented with *Lactobacillus plantarum*, *L. brevis*, *Saccharomyces cerevisiae*, or with a combination of yeast and lactic acid bacteria. Formation of acidity, free amino acids, and volatile compounds were considered the main responses. A possibility to enhance formation of potential flavor compounds and precursors without excessive acidity formation in wheat sourdoughs was established. The total amount of amino acids increased by 25–50%, depending on the strain and fermenta-

tion conditions. The total amount of volatile compounds increased seven- to 100-fold, depending on the strain and fermentation conditions. Sourdough started with *S. cerevisiae* was an effective way to optimize the amount of volatile compounds without excessive acidity formation in appropriate processing conditions. Ash content of flour and fermentation time were the most significant factors to modify metabolic activity of wheat sourdoughs. Frequent interactions between the studied factors were observed on the formation of acidity, amino acids, and volatile compounds with most of the strains studied. Possibility to improve current industrial fermentation processes and control flavor attributes of breads by using optimized sourdough was established.

Wheat sourdough is an ancient way to improve textural and sensory properties of wheat bread (Brummer et al 1984; Martinez-Anaya 1996; Kulp and Lorenz 2003). The potential of wheat sourdough is based on fermentation processes in which main components of flour (starch and protein) are modified and partly degraded. Starch degraded to monosaccharides and disaccharides is partly used by lactic acid bacteria and yeast present in sourdough to produce acidity (Röcken et al 1992). Proteolysis during sourdough fermentation produces amino acids, which are well-known precursors in bread flavor formation (Schieberle 1990; Gobetti et al 1995; Thiele et al 2002). Lactic acid bacteria and yeasts also produce volatile compounds that may affect bread flavor (Richard-Molard 1979; Lund et al 1989; Hansen and Hansen 1994a,b; Maloney and Foy 2003). Wheat sourdough has a strong acidic flavor which is not appreciated by consumers in most countries. Extensive acidity formation thus limits the amount of sourdough that can be used in the actual bread dough and may cause bitter bread flavor (Salovaara and Valjakka 1987; Meignen et al 2001).

The degree of proteolysis and the amount of acids and volatile compounds formed depend both on what kind of lactic acid bacteria and yeasts are present and on the process parameters of sourdough fermentation such as fermentation time and temperature, dough yield, ash content of flour, and the presence of oxygen. Individual effects of ash content of flour, fermentation temperature and time, and dough yield on the formation of acidity, volatile compounds, and amino acids have been partly elucidated in earlier studies, but the interactions of these factors have not been studied systematically (Hansen et al 1989; Collar 1993; Hansen and Hansen 1994a; Collar and Martinez 1995; Gobetti et al 1995).

One of the major challenges in the use of wheat sourdough is to control excessive acidity of wheat sourdough and still enhance formation of flavor precursors (such as amino acids) and flavor compounds. As sourdough is a very complex system, effects of process factors and especially the interaction of these factors on proteolysis, amylolysis, and formation of acids have to be well clarified to achieve a controlled sourdough process. However, inter-

actions of process factors have been studied only for acidity and growth of microbes (Röcken et al 1992; Rio et al 1996; Simonson 2003). The effectiveness of response surface methodology (RSM) in the development and optimization of cereal products and processes has been well established by various researchers (Röcken et al 1992; Rio et al 1996; Myllymäki et al 1997; Collar et al 1999), so RSM was chosen for this study.

The present study was designed to 1) determine the influence of ash content of flour, fermentation time, and fermentation temperature on the production of acidity, formation of amino acids, and formation of volatile compounds in wheat sourdoughs fermented with single strains of lactobacilli or yeast (*Lactobacillus brevis*, *L. plantarum*, *Saccharomyces cerevisiae*) or with a combination starter (yeast + lactic acid bacteria); 2) highlight interactions of these factors by using different strains and to determine the possibility of enhancing formation of possible flavor compounds (amino acids and volatile compounds) without excessive acidity formation.

MATERIALS AND METHODS

Microbial Strains

Lactic acid bacteria (LAB) and yeast used in these studies were *Lactobacillus plantarum* VTT E 78076, *L. brevis* VTT E 95612, and *Saccharomyces cerevisiae* y VTT B81047. Selected strains originated from Finnish rye sourdoughs. LAB were stored in liquid nitrogen at –196°C and maintained during the experiments on de Man Rogosa Sharpe (MRS) agar slants (Oxoid, Basingstoke, Hampshire). The growth ability of LAB and yeast were checked in preliminary experiments by inoculating a suspension of wheat flour (ash content 0.6, 1.2, or 1.8%) in water (dough yield 250) with LAB or yeast at 10⁶ cfu/g. After 18 hr of fermentation at 30°C, both strains of LAB consistently achieved a level of 10⁸ to 10⁹ cfu/g, and the yeast achieved a level of 10⁷ cfu/g (data not shown). Ability of LAB to compete with natural flour-originating microbes was also checked with AP-PCR. This test confirmed the ability of selected LAB to dominate the growing flora during fermentation (data not shown).

Preparation of Culture Filtrate for Sourdoughs

LAB strains received from the culture collection of VTT were stored in liquid nitrogen at –196°C and were maintained during

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the experiments on MRS agar. The strains were grown in MRS broth overnight at 30°C. The inoculum was further transferred and grown at 30°C for 24 hr. The final LAB starter preparation was prepared using a 1% inoculum and 3 hr of incubation at 30°C without agitation. Cells were harvested by centrifugation at 22°C, 7,000 × g, for 20 min (Sorvall RC-5B refrigerated superspeed centrifuge, DuPont Instruments, Wilmington, DE), washed with sterile distilled water, mixed by vortexing and centrifuged at 22°C, 7,000 × g, for 20 min. The supernatant was discarded and the cells were resuspended in sterile distilled water. The concentration of bacterial cells was estimated by measuring the turbidity of the culture (ABS_{620nm}, turbidimeter Multiscan MCC/340, Labsystems, Helsinki, Finland). Counts of lactic acid bacteria were also determined by plating on MRS agar for three days at 30°C. Fresh cells were added immediately to the sourdough at 1 × 10⁷ cfu/g.

The yeast strain received from the VTT culture collection was stored in liquid nitrogen at -196°C and was maintained during the experiments on Wort Sucrose broth (Difco, Livonia MI). The strains were grown in YM broth (Difco) overnight at 25°C with agitation at 100 rpm. The inoculum was further transferred and grown at 25°C for 24 hr. The final yeast preparation was prepared using a 1% inoculum and 6 hr of incubation at 25°C with agitation at 100 rpm. Cells were harvested by centrifugation at 22°C, 3,000 × g, for 20 min (Sorvall RC-12BP centrifuge, DuPont Instruments), washed with sterile distilled water, mixed by vortexing and centrifuged at 22°C, 7,000 × g for 10 min. The supernatant

was discarded and the cells were resuspended in sterile distilled water. The concentration of yeast cells was estimated by measuring the turbidity of the culture (ABS_{620nm}). Counts of yeast cells were also determined by plating on YM agar for five days at 25°C. Fresh cells were added immediately to the sourdough at 3 × 10⁶ cfu/g.

The sourdough samples were homogenized for 60 sec in a stomacher (400, Seward, Thetford, Norfolk) before dilution in peptone saline and cultivation on MRS agar and YM agar. The MRS agar plates for measuring LAB were incubated under anaerobic conditions for three days at 37°C and the YM plates for measuring yeasts were incubated under aerobic conditions for five days at 25°C.

Preparation of Sourdoughs

Wheat flours (Raisio Factories, Melia, Raisio, Finland) with different ash contents (0.6, 1.2, 1.8% dwb) were used. Sourdough was prepared by mixing 600 g of tap water, 400 g of wheat flour, and the inoculum of LAB or yeasts (10⁶-10⁷ cfu/g) in a large beaker (2,000 mL) covered with aluminum foil. The amount of inoculum for LAB was 10⁷ cfu/g of sourdough and for yeast 10⁶ cfu/g of sourdough. Ash content of flour, fermentation time, and fermentation temperature were in accordance with the experimental design. Samples of sourdoughs were immediately frozen for later measurements of pH, total titratable acidity [TTA], lactic acid, acetic acid, amino acids, and volatile compounds.

Analyses of pH, TTA, Lactic Acid, and Acetic Acid

Frozen sourdough samples were thawed overnight in a refrigerator. The pH value was measured from an aliquot of 10 g of sourdough blended with 100 mL of distilled water (TitroLine Alpha 471217, Schott, Mainz, Germany). TTA was determined by titrating this suspension against 0.1M NaOH to a final value of pH 8.5. TTA was expressed as the amount of NaOH used (mL). Lactic acid and acetic acid were determined enzymatically using Boehringer Mannheim kits. All samples were analyzed in duplicate.

Volatile Compounds

Volatile compounds were determined by dynamic HS/GC/MS. Sourdough (1 and 20 g in each measuring point, according to the experimental design) were weighed into 120-mL headspace vials and 49 and 30 mL, respectively, of the saturated sodium chloride solution (30%) was added to the vials. The concentrated salt solution facilitates the transfer of volatile compounds from the sourdough samples to the headspace (i.e., salting-out effect) (Hong and Altörfer 1997). Furthermore, concentrated sodium chloride reduces

TABLE I
Composition of Various Runs from the Central Composite Design

Run	Temp. (Te)	Time (Ti)	Ash Content of Flour % (A)
1	16	6	0.6
2	32	6	0.6
3	16	20	0.6
4	32	20	0.6
5	16	6	1.8
6	32	6	1.8
7	16	20	1.8
8	32	20	1.8
9	16	12	1.2
10	32	12	1.2
11	24	6	1.2
12	24	20	1.2
13	24	12	0.6
14	24	12	1.8
15	24	12	1.2
16	24	12	1.2
17	24	12	1.2
18	24	12	1.2

TABLE II
Effects of Factors with Coefficients in Models for Acidity in Sourdough Fermented with *Lactobacillus brevis* and *L. plantarum*^a

Factor ^b	<i>L. plantarum</i>				<i>L. brevis</i>			
	pH*	TTA* ^c	Lactic Acid %*	Acetic Acid %*	pH	TTA*	Lactic Acid %	Acetic Acid %*
Constant	0.807	-0.151	-2.832		9.846	-0.775	-0.363	-3.164
A	ns	1.172	ns	...	ns	1.433	ns	0.005
Te	0.004	-0.025	0.047	...	-0.324	0.024	-0.14	0.002
Ti	0.004	-0.019	0.079	...	0.041	-0.019	-0.037	0.003
A×Te	ns	ns	ns	...	ns	ns	ns	ns
A×Ti	ns	ns	ns	...	ns	ns	ns	ns
Te×Ti	-0.0008	0.003	0.004	...	-0.006	0.002	0.003	ns
A ²	ns	-0.397	ns	...	ns	-0.459	ns	-0.627
Te ²	ns	ns	ns	...	0.007	ns	ns	ns
Ti ²	ns	ns	ns	...	ns	ns	ns	ns
R ²	0.92	0.97	0.95	...	0.92	0.98	0.89	0.89
Q ²	0.67	0.90	0.76	...	0.79	0.94	0.70	0.73

^a Only values of significant coefficients are presented (95% confidence level); ns, no significant effect at the 5% level; *, logarithm of response.

^b A, ash content of flour; Te, temperature; Ti, time; A², quadratic effect of ash content; Te², quadratic effect of temperature; Ti², quadratic effect of time; R², measure of fit of the model; Q², predictive power of the model.

^c TTA, total titratable acidity of sourdough.

TABLE III
Effects of Factors with Coefficients in Models for Acidity in Sourdough Fermented with *Saccharomyces cerevisiae* or with a Combination of *S. cerevisiae*, *Lactobacillus brevis*, and *L. plantarum*^a

Factor ^b	<i>S. cerevisiae</i>				Combination Starter			
	pH	TTA ^{*c}	Lactic Acid %*	Acetic Acid %*	pH*	TTA	Lactic Acid %	Acetic Acid %*
Constant	130.55	-0.210	0.610	-0.774	1.147	-21.290	-1.955	-4.014
A	226.63	0.455	-0.710	-2.033	-0.055	10.380	0.629	0.711
Te	18.96	0.010	-0.133	-0.227	-0.025	1.033	0.095	0.078
Ti	7.44	0.023	-0.105	0.274	-0.024	1.221	0.117	0.078
A×Te	-7.20	0.006	ns	0.045	ns	ns	ns	ns
A×Ti	ns	ns	ns	ns	ns	0.230	ns	0.022
Te×Ti	-1.40	0.0008	0.002	0.003	ns	0.017	0.074	-0.0009
A ²	ns	-0.136	0.260	0.545	0.0410	-3.906	-0.273	-0.261
Te ²	ns	ns	0.002	0.003	0.0004	-0.022	-0.0021	-0.001
Ti ²	ns	ns	0.003	-0.012	0.0006	-0.053	-0.0050	-0.002
R ²	0.89	0.96	0.89	0.95	0.98	0.98	0.98	0.99
Q ²	0.74	0.80	0.70	0.74	0.82	0.81	0.81	0.94

^a Only values of significant coefficients are presented (95% confidence level); ns, no significant effect at the 5% level; *, logarithm of response.

^b A, ash content of flour; Te, temperature; Ti, time; A², quadratic effect of ash content; Te², quadratic effect of temperature; Ti², quadratic effect of time; R², measure of fit of the model; Q², predictive power of the model.

^c TTA, total titratable acidity of sourdough.

TABLE IV
Effects of Factors with Coefficients in Models for Formation of Amino Acids in Sourdough Acidified with *Lactobacillus plantarum* or with *L. brevis*^{a,b}

Factor ^c	<i>L. plantarum</i>					<i>L. brevis</i>				
	Total AA	AliA	ArA	SHCG	BA	Total AA	AliA	ArA	SHCG	BA
Constant	76.17	23.32	0.02	0.47	0.27	499.81	185.85	143.68	33.44	74.63
A	93.03	6.44	1.33	1.24	1.49	-81.66	-47.79	-34.43	33.03	15.27
Te	-8.76	-2.28	-0.04	ns	-0.01	-27.79	-10.19	-6.01	-2.59	ns
Ti	0.27	-0.21	-0.01	ns	0.01	-19.36	-7.55	-7.82	-2.04	-1.90
A×Te	ns	ns	ns	ns	ns	5.51	2.03	2.11	0.76	0.75
A×Ti	3.12	1.14	ns	ns	ns	8.92	3.22	3.34	1.36	1.03
Te×Ti	ns	0.62	ns	ns	ns	0.58	0.22	0.24	0.06	0.05
A ²	-30.22	ns	-0.43	-0.42	-0.47	-18.30	ns	-9.26	-15.75	-10.75
Te ²	ns	ns	ns	ns	ns	0.36	0.13	ns	ns	0.084
Ti ²	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R ²	0.99	0.97	0.99	0.98	0.99	0.99	0.98	0.99	0.99	0.99
Q ²	0.99	0.86	0.93	0.96	0.99	0.98	0.98	0.97	0.94	0.94

^a Only values of significant coefficients are presented (95% confidence level); ns, no significant effect at the 5% level.

^b Total AA, total amino acids; AliA, aliphatic (glycine, alanine, valine, isoleucine, leucine); ArA, aromatic (phenylalanine, tyrosine); SHCG, sulfur-, hydroxy-, cyclic-, and gamma-aminobutyric acid; BA, basic amino acids (arginine, ornithine, lysine, histidine).

^c A, ash content of flour; Te, temperature; Ti, time; A², quadratic effect of ash content; Te², quadratic effect of temperature; Ti², quadratic effect of time; R², measure of fit of the model; Q², predictive power of the model.

TABLE V
Effects of Factors with Coefficients in Models for Formation of Amino Acids in Sourdough Acidified with *Saccharomyces cerevisiae* or with a Combination of *S. cerevisiae*, *Lactobacillus brevis*, and *L. plantarum*^{a,b}

Factor ^c	<i>S. cerevisiae</i>					Combination Starter				
	Total AA	AliA	ArA	SHCG	BA	Total AA	AliA	ArA	SHCG	BA
Constant	-93.743	-42.65	-22.25	17.98	-21.67	104.84	0.658	0.062	5.24	-1.63
A	102.86	8.00	2.60	4.92	5.38	-43.21	0.498	0.46	18.08	14.61
Te	ns	ns	ns	ns	ns	-1.81	-0.014	-0.01	ns	-0.18
Ti	ns	ns	ns	ns	ns	-13.34	-0.04	-0.03	ns	-0.44
A×Te	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
A×Ti	ns	ns	ns	1.21	ns	ns	ns	ns	ns	ns
Te×Ti	ns	ns	ns	ns	ns	0.318	0.003	0.002	ns	0.04
A ²	ns	ns	ns	ns	ns	50.45	ns	ns	ns	ns
Te ²	ns	ns	-0.04	ns	ns	ns	ns	ns	ns	ns
Ti ²	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
R ²	0.87	0.87	0.86	0.96	0.92	0.93	0.90	0.85	0.89	0.95
Q ²	0.71	0.62	0.63	0.75	0.63	0.76	0.81	0.73	0.81	0.87

^a Only values of significant coefficients are presented (95% confidence level); ns, no significant effect at the 5% level.

^b Total AA, total amino acids; AliA, aliphatic (glycine, alanine, valine, isoleucine, leucine); ArA, aromatic (phenylalanine, tyrosine); SHCG, sulfur-, hydroxy-, cyclic-, and gamma-aminobutyric acid; BA, basic amino acids (arginine, ornithine, lysine, histidine).

^c A, ash content of flour; Te, temperature; Ti, time; A², quadratic effect of ash content; Te², quadratic effect of temperature; Ti², quadratic effect of time; R², measure of fit of the model; Q², predictive power of the model.

fermentation as determined in preliminary experiments (data not shown). The vials were closed with a Teflon-faced butyl rubber septum and an aluminum crimp cap. Samples were frozen immediately and analyzed later. Frozen samples were thawed and allowed to stabilize for 2 hr at room temperature (25°C) before analysis. The volatile compounds were purged from the headspace vial for 4 min (40 mL/min) into a Tekmar 3000 dynamic headspace sampler that was interfaced to the GC/MS system (Carlo Erba 8000^{top}/Automass II, Finnigan Mat, Bremen, Germany). The temperature of Tenax absorbent during the purge was 35°C, the dry purge time was 8 min, the desorption temperature was 220°C. The transfer line temperature was 200°C. Liquid nitrogen that cooled the cryo trap temperature was held at -120°C during the desorption. The cryo inject time was 0.75 min and the temperature was 220°C. The GC/MS interface temperature was 250°C and the ion source temperature was 200°C, and the ionization energy was 70 eV in EI mode. The filament current was 500 µA, and the photomultiplier voltage was 750V. The scan rate of the mass spectrometer was 0.7 sec/scan over the mass range 35–500 amu. The column was a 50-m HP-PONA (0.20 mm, i.d.; 0.5 m film thickness). The carrier gas was helium, and the inlet pressure was 200 kPa. The oven temperature program started at 0°C, with a hold time of 3 min, and then the temperature was raised to 50°C at a rate of 5°C/min, and then continued directly at 25°C/min to

300°C. The final temperature was maintained for 5 min. The compounds were identified on the basis of mass spectra, and amounts were quantified using selective ions for each compound from total ion chromatograms against the standard solution mixture series that were prepared in 30% sodium chloride solution. Methyl cyclohexane was used as an internal standard. The samples and standards were analyzed in duplicate.

Reference Compounds

Dimethyl sulfide (DMS), isobutanol, hexyl acetate, diacetyl, pentyl acetate, 3-methyl butanal, 2-methyl butanal, 2-methyl butanol and hexanal were obtained from Fluka Chemie Ag (Buchs, Switzerland). 1-Penten-3-ol, ethyl propionate, 3-methyl butanol, and 1-hexanol were obtained from Sigma-Aldrich (St. Louis, MO). Ethyl acetate, propanol, butanol, and isobutanol were obtained from Merck KGaA (Darmstadt, Germany). The 2-methyl butyl acetate, and 2-pentylfuran were obtained from Toyo Kasei Kogyo (Osaka, Japan). Ethanol was obtained from Altia Oyj (Helsinki, Finland).

Amino Acids

Free amino acids were extracted with water and proteins were precipitated with sulfosalicylic acid. Amino acids were quantified by liquid chromatography according to Dong and Gant (1985).

TABLE VI
Effects of Factors with Coefficients in Models for Formation of Volatile Compounds in Sourdough Acidified with *Lactobacillus plantarum* or with *L. brevis*^a

Factor ^b	<i>L. plantarum</i>					<i>L. brevis</i>				
	Total AV	Ethanol	Diacetyl*	Ethyl Acetate	3-Me Butanol	Total AV*	Ethanol	Diacetyl	Ethyl Acetate*	3-Me Butanol*
Constant	1.49	0.78	-0.71			1.95	18,200		-5.30	-6.033
A	0.34	0.50	ns	...	nf	1.45	ns	...	ns	3.39
Te	ns	ns	ns	...	nf	0.097	-7,006	...	0.170	ns
Ti	0.0012	0.026	0.046	...	nf	0.060	-29,975	...	0.026	ns
A×Te	ns	ns	ns	...	nf	ns	ns	...	ns	ns
A×Ti	ns	ns	ns	...	nf	ns	ns	...	ns	ns
Te×Ti	ns	ns	ns	...	nf	ns	2,152	...	0.011	ns
A ²	ns	ns	ns	...	nf	-0.57	ns	...	ns	-0.96
Te ²	ns	ns	ns	...	nf	ns	ns	...	ns	ns
Ti ²	ns	ns	ns	...	nf	ns	ns	...	ns	ns
R ²	0.76	0.89	0.70	...	nf	0.96	0.98	...	0.84	0.95
Q ²	0.56	0.73	0.54	...	nf	0.84	0.82	...	0.63	0.80

^a Only values of significant coefficients are presented (95% confidence level); ns, no significant effect at the 5% level; *, logarithm of response; nf, no reliable model found.

^b Total AV, total amount of volatile compounds formed (without ethanol); A, ash content of flour; Te, temperature; Ti, time; A², quadratic effect of ash content; Te², quadratic effect of temperature; Ti², quadratic effect of time; R², measure of fit of the model; Q², predictive power of model.

TABLE VII
Effects of Factors with Coefficients in Models for Formation of Volatile Compounds in Sourdough Acidified with *Saccharomyces cerevisiae* or with a Combination of *S. cerevisiae*, *Lactobacillus plantarum*, and *L. brevis*^a

Factor ^b	<i>S. cerevisiae</i>					Combination Starter				
	Total AV*	Ethanol*	Diacetyl*	Ethyl Acetate*	3-Me butanol*	Total AV*	Ethanol*	Diacetyl*	Ethyl Acetate*	3-Me Butanol*
Constant	-0.983	-0.489	-1.693	1.278	-7.491	-2.356	-4.772	-2.262	-6.069	-3.352
A	ns	ns	ns	-0.844	ns	3.334	0.608	3.830	-0.160	3.70
Te	0.155	0.202	0.138	-0.074	0.426	ns	ns	ns	ns	0.021
Ti	0.214	0.321	ns	-0.015	0.539	0.384	0.345	ns	0.407	0.417
A×Te	ns	ns	ns	0.025	ns	ns	ns	ns	ns	ns
A×Ti	ns	ns	ns	0.036	ns	0.049	ns	0.041	0.056	0.059
Te×Ti	0.005	ns	ns	0.008	ns	ns	ns	ns	ns	ns
A ²	ns	ns	ns	ns	ns	-1.459	ns	-1.564	ns	-1.629
Te ²	-0.004	ns	ns	ns	-0.008	ns	-0.007	ns	0.007	ns
Ti ²	-0.010	-0.009	ns	ns	-0.016	-0.014	-0.012	ns	-0.014	-0.015
R ²	0.95	0.96	0.70	0.96	0.92	0.94	0.82	0.95	0.92	0.98
Q ²	0.80	0.85	0.45	0.72	0.78	0.82	0.73	0.81	0.80	0.88

^a Only values of significant coefficients are presented (95% confidence level); ns, no significant effect at the 5% level; *, logarithm of response.

^b Total AV, total amount of volatile compounds formed (without ethanol); A, ash content of flour; Te, temperature; Ti, time; A², quadratic effect of ash content; Te², quadratic effect of temperature; Ti², quadratic effect of time; R², measure of fit of the model; Q², predictive power of the model.

The column was an amino acid analyzer (30 cm, Waters Corp., Milford, MA) with a column oven temperature of 63°C, flow rate was 0.4 mL/min, detection was made using a fluorescence detector (Waters 474), and data was processed by Millennium 3.2 software program. Postcolumn reagents were 0.01% hypochlorite and 0.07% *o*-phthalaldehyde solutions. The first eluent was 88 mM citrate buffer, pH 2.86, which was gradually changed over 60 min, to 65 mM borate buffer, pH 9.75, containing 31M sodium nitrate. The samples were analyzed in duplicate.

Experimental Design and Statistical Methods

To study the effects of the main factors on sourdough fermentation, the parameters selected as independent variables were temperature (Te, 16–32°C), ash content of flour (A, 0.6–1.8%), and fermentation time (Ti, 6–20 hr). A central composite design was used to arrange experiments, and four replicates were made at the center point of the design to allow estimation of the pure error at the sum of the square. The experimental design is presented in Table I. Levels of variables were selected on the basis of those commonly used in real sourdoughs and sponge fermentation processes applied in Finnish bakeries.

In the mathematical models, selected points in the experimental area were measured (three points for each variable in this study) and the rest of the points are predicted according to the created equations and can be presented also as graphical presentations such as response surfaces. This approach decreased the amount of experiments. Testing three variables at three levels would involve 27 experiments, but by using a CCF experimental design, the amount of experiments could be reduced to 18, four of which consist of all three variables at their central levels. The results were analyzed by a multiple regression method (MLR or PLS), which describes the effects of variables in second-order polynomial models. For each response (acidity, amino acids, and volatile compounds), the quadratic model used was

$$Y = \beta_0 + \beta_1Te + \beta_2Ti + \beta_3A + \beta_{11}Te^2 + \beta_{22}Ti^2 + \beta_{33}A^2 + \beta_{12}Te \times Ti + \beta_{13}Te \times A + \beta_{23}Ti \times A + \epsilon$$

This model took into account the effects of the variable alone (Ti), the effects of the interactions between two variables (Ti ×

Te), and quadratic effects of the variables alone (Ti²). Regression analysis was calculated and the response surfaces were graphed with Modde 4.0 software (Umametri AB, Umeå, Sweden). The fit of the model to the experimental data was given as the coefficient of determination (*R*²), which explains the extent of the variance in a modeled variable that can be explained with the model. Each model was validated by calculating the predictive power of the model (*Q*²), which is a measure of how well the model will predict the responses for new experimental conditions. *Q*² is based on the prediction of the residual sum of squares (PRESS). For determining *Q*², the computations are repeated several times, each time omitting different objects from the calculation of the model. PRESS is then computed as the squared difference between observed *Y* and predicted values (cross validation of *R*²). Large *Q*² (>0.7) values indicate that the model has good predictive ability and will have small prediction errors.

RESULTS

The 18 experiments were performed according to the experimental design. Formation of acidity, amino acids, and selected volatile compounds for the yeast and LAB starters and for the combination starter were determined in each experimental point. For each response group, a quadratic equation was formed with relevant terms (*P* < 0.05) to obtain as high *R*² and *Q*² values as possible. Based on these equations, behavior of response can be predicted in the experimental area and presented as a response surface. The coefficients of a particular model and the *R*² and *Q*² values obtained are presented in Tables II–VII. Using these tables, the pH level of sourdough, with *L. brevis* for example, can be predicted by the equation for the experimental area under consideration

$$pH_{\text{pred}} = 9.846 - 0.324Te + 0.041Ti - 0.006Te \times Ti + 0.007Te^2$$

If logarithm transformation of responses is used (to normalize standard distribution of data), logarithm of the particular response value is predicted from the equation. Selected response surface figures are also presented to illustrate these equations. Figures are presented by choosing two main variables (time and temperature)

TABLE VIII
Correlation Matrix Between Acidity, Volatile Compounds, and Amino Acids in Sourdoughs

	Starter	pH	TTA ^a	Lactic Acid	Acetic Acid
<i>Lactobacillus brevis</i>	Total amount of volatile compounds	-0.65	0.86	0.75	0.85
	Total amount of amino acids	-0.65	0.86	0.75	0.85
	Aliphatic amino acids	-0.61	0.78	0.60	0.74
	Aromatic amino acids	-0.61	0.81	0.71	0.74
	SHCG ^b	-0.21	0.85	0.85	0.77
	Basic amino acids	-0.22	0.74	0.74	0.66
<i>L. plantarum</i>	Total amount of volatile compounds	-0.74	0.90	0.83	...
	Total amount of amino acids	-0.24	0.71	0.59	...
	Aliphatic amino acids	-0.24	0.71	0.60	...
	Aromatic amino acids	-0.20	0.67	0.51	...
	SHCG	-0.03	0.50	0.31	...
	Basic amino acids	-0.24	0.66	0.50	...
<i>Saccharomyces cerevisiae</i>	Total amount of volatile compounds	-0.83	0.59	0.45	0.65
	Total amount of amino acids	0.19	0.64	0.24	0.34
	Aliphatic amino acids	0.11	0.69	0.25	0.38
	Aromatic amino acids	0.24	0.56	0.08	0.20
	SHCG	0.13	0.69	0.28	0.40
	Basic amino acids	0.12	0.62	0.10	0.25
Combination starter	Total amount of volatile compounds	-0.61	0.85	0.82	0.63
	Total amount of amino acids	0.03	0.51	0.39	0.73
	Aliphatic amino acids	-0.16	0.63	0.51	0.70
	Aromatic amino acids	-0.24	0.64	0.56	0.70
	SHCG	0.14	0.43	0.24	0.66
	Basic amino acids	-0.13	0.65	0.50	0.81

^a Total titratable acidity

^b Sulphur-, hydroxy-, cyclic- and gamma amino acids.

most influencing a particular response (such as lactic acid formation) for x - and y -axes. The correlation matrix of the measured responses is also presented (Table VIII).

Validity of models (R^2 and Q^2 values), linear interactions, and quadratic effects of variables (time, temperature, and ash content of flour) are presented for each response type (acidity, amino acids, and volatile compounds) separately. For each response, effects are presented with four different types of starters (*L. plantarum*, *L. brevis*, *S. cerevisiae*, and combination starter).

Acidity Development in Sourdoughs

Relatively high R^2 (goodness of fit) values and Q^2 (predictive power of the model) values were obtained in the acidity models (Tables II and III); typical R^2 values were >0.9 and typical Q^2 values were 0.7–0.9.

In general, increased fermentation time, temperature, and ash content of flour resulted in increased acidity (measured either as pH, TTA, or acid content) in the sourdoughs. The most influential parameter on acidity was fermentation time in all sourdough types. Ash content of flour and fermentation time had an equal effect on acidity formation in general. The level of acidity obtained and the role of the different parameters creating acidity were, however, partly strain-dependent.

The acidity level of the sourdoughs varied at pH 3.9–6.4 and TTA 1.4–19.1 in the experimental region. The amount of lactic acid varied at 0.03–1.3% and amount of acetic acid varied at 0.003–0.25%. Acidity development was dependent on both the strain and the fermentation conditions. The most acidic sourdough was obtained with *L. brevis* as the starter and the least acidic sourdough was obtained with *S. cerevisiae* as the starter.

The lowest pH value of sourdoughs was obtained with all strains when fermentation time was 18–20 hr and fermentation temperature was 32°C. As expected, the pH level of the sourdoughs was linearly dependent on both time and temperature. There was also a strong interaction between time and temperature in all sourdough types, indicating that effective lowering of pH required both long fermentation time and high temperature (Table II).

The highest TTA value was obtained with a fermentation time of 18 hr, fermentation temperature of 32°C, and ash content of flour of $>1.2\%$ in sourdoughs fermented with single strains of lactobacilli or with yeast. Increasing ash content of flour greatly increased TTA values in most types of sourdough. Time and temperature also had a significant linear effect on TTA. Significant TTA increase required both long fermentation time and high temperature as a strong interaction between time and temperature was

observed with all types of sourdough. An example of this interaction is shown in Fig. 1A for the sourdough fermented with *L. brevis*. Interaction of fermentation time and ash content of flour was also noticed in the sourdough acidified with the combination starter (Fig. 1B). Ash content of flour also had a quadratic effect on TTA values in all sourdoughs, indicating that the highest value of TTA was obtained with ash content of flour of 1.2%, and it did not increase beyond this level, even if ash content of flour was increased (Fig. 1B). With the combination starter, a similar quadratic relationship was observed between time and temperature; maximum values for TTA were obtained with fermentation times of 12–14 hr and fermentation temperatures of 24–26°C.

The role of fermentation parameters varied with different types of sourdough in the formation of lactic acid and acetic acid. With sourdoughs fermented with single strains of lactobacilli, the highest amount of lactic acid was obtained with a fermentation time of 20 hr and a fermentation temperature of 32°C, as longer time and higher temperature increased formation of lactic acid (Tables II and III). Also, the ash content of flour had a linear effect on formation of lactic acid, but the effect was smaller and statistically insignificant in most types of sourdough. In pure yeast fermentation, the ash content of flour, however, was the most significant factor influencing lactic acid formation (Table III). Interaction of temperature and time was also significant in all sourdoughs. Thus, for the formation of lactic acid, the influence of time was dependent on the temperature. In sourdough fermented with yeast or with the combination starter, maximum formation of lactic acid was obtained at 26–32°C and in 14–20 hr due to significant quadratic effects of time and temperature. This quadratic relationship, as well as the interaction of fermentation time and temperature, is shown in Fig. 2 for sourdough fermented with the combination starter.

The highest amount of acetic acid was obtained in the sourdough fermented with *L. brevis* when ash content of flour was 1.8%, fermentation temperature was 32°C, and fermentation time was 20 hr. Ash content of flour, time, and temperature had a linear effect on acetic acid formation in the model (Table II). With *S. cerevisiae* and with the combination starter, maximum concentration of acetic acid was obtained at 24°C in 14 hr with 1.2% ash content of flour (Table III); interaction between time and temperature was observed in the model. Also, fermentation temperature and fermentation time had a strong quadratic effect on acetic acid formation in the sourdough fermented with *S. cerevisiae*. Similar quadratic effects of time, temperature, and ash content of flour were observed with the combination starter (Table III).

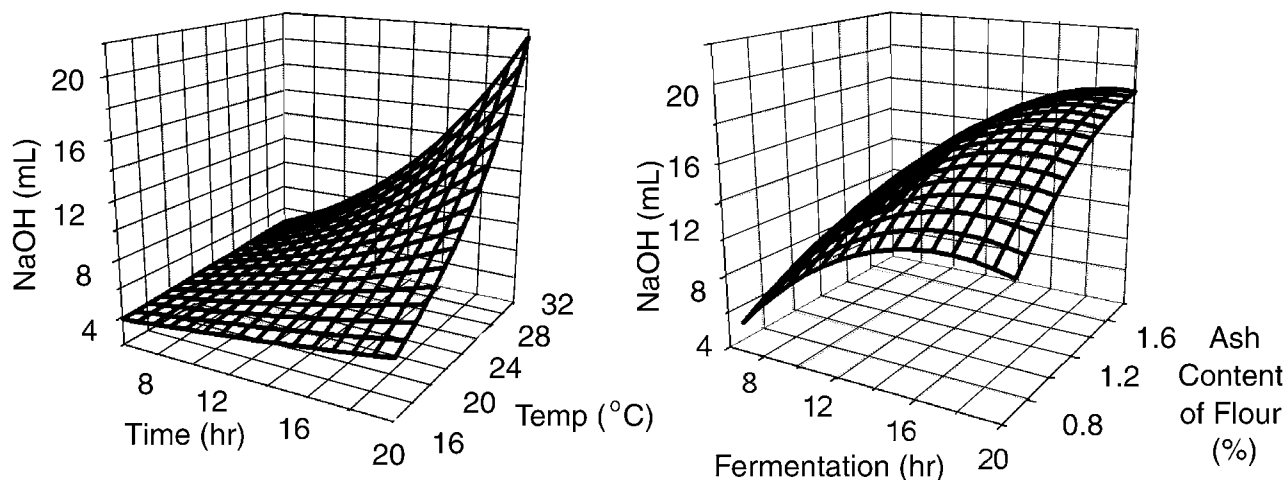


Fig. 1. A, Formation of acidity (TTA value) in sourdough fermented with *L. brevis* (ash content of flour 1.2%). B, Formation of acidity (TTA value) in sourdough fermented with *S. cerevisiae*, *L. plantarum*, and *L. brevis* (fermentation at 24°C).

Amino Acids

Relatively high R^2 values (goodness of fit) and Q^2 (predictive power of the model) values were obtained in the amino acid models (Tables IV and V); typical R^2 values were >0.9 and typical Q^2 values were 0.6–0.9.

The amount of 19 amino acids were determined in unfermented and fermented doughs acidified with *L. plantarum*, *L. brevis*, *S. cerevisiae*, or with the combination starter. For easier discussion, individual amino acids were grouped as aliphatic (glycine, alanine, valine, isoleucine, leucine), basic (arginine, ornithine, lysine, histidine), aromatic (phenylalanine, tyrosine), sulfur-containing, hydroxy, cyclic, and gamma-aminobutyric acid (serine, threonine,

proline, gamma-aminobutyric) as reported in the literature (Spicher and Nierle 1984a,b; Collar et al 1991)

In unfermented doughs, the total amount of amino acids (TA) varied at 40–81 mg/100 g of dough depending on the ash content of flour (0.6–1.8%); the highest values were obtained with whole meal flour. In fermented doughs, the highest accumulation of amino acids was obtained in the sourdough fermented with *L. brevis* (367 mg/100 g of dough) and the lowest in the sourdough fermented with *S. cerevisiae* (190 mg/100 g of dough). Thus, depending on the strain in question and the fermentation conditions, amounts of amino acids increased by 55–90% during the sourdough fermentation.

The most effective accumulation of amino acids occurred when ash content of flour was 1.8%, fermentation time was 20 hr, and fermentation temperature was 32°C, as increased ash content of flour, fermentation time, and fermentation temperature increased the TA and amino acid groups in all types of sourdough (Tables IV and V). With all of the strains, the most important parameter influencing proteolysis was clearly ash content of flour.

The influence of interaction of ash content of flour and fermentation time on TA and on aliphatic amino acids was significant in sourdoughs fermented with the starter containing *L. brevis* or *L. plantarum*. Interaction between the time and temperature on the TA and on aliphatic amino acids was also observed in the sourdoughs acidified with *L. brevis*. This indicates that effective accumulation of these amino acids required both a long fermentation time and the use of a flour with a high ash content. The influence of the fermentation time and ash content of flour is presented in Fig. 3 for sourdough fermented with *L. brevis*.

Ash content of flour had a significant quadratic effect on most amino acid groups (but not for aliphatic amino acids) in sourdough fermented with *L. brevis* or *L. plantarum*. This indicates that the optimum ash content of flour was 1.4–1.8% to maximize formation of amino acids (Table IV). When *S. cerevisiae* was used to start sourdough fermentation, almost the only significant factor

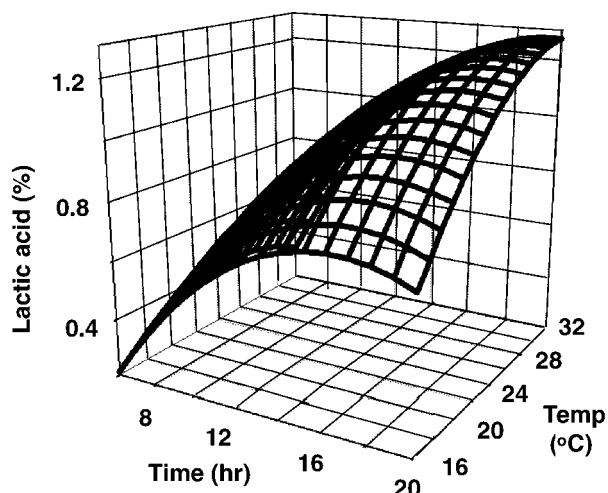


Fig. 2. Formation of lactic acid in sourdough fermented with *S. cerevisiae* + *L. plantarum* + *L. brevis* (ash content of flour 1.2%)

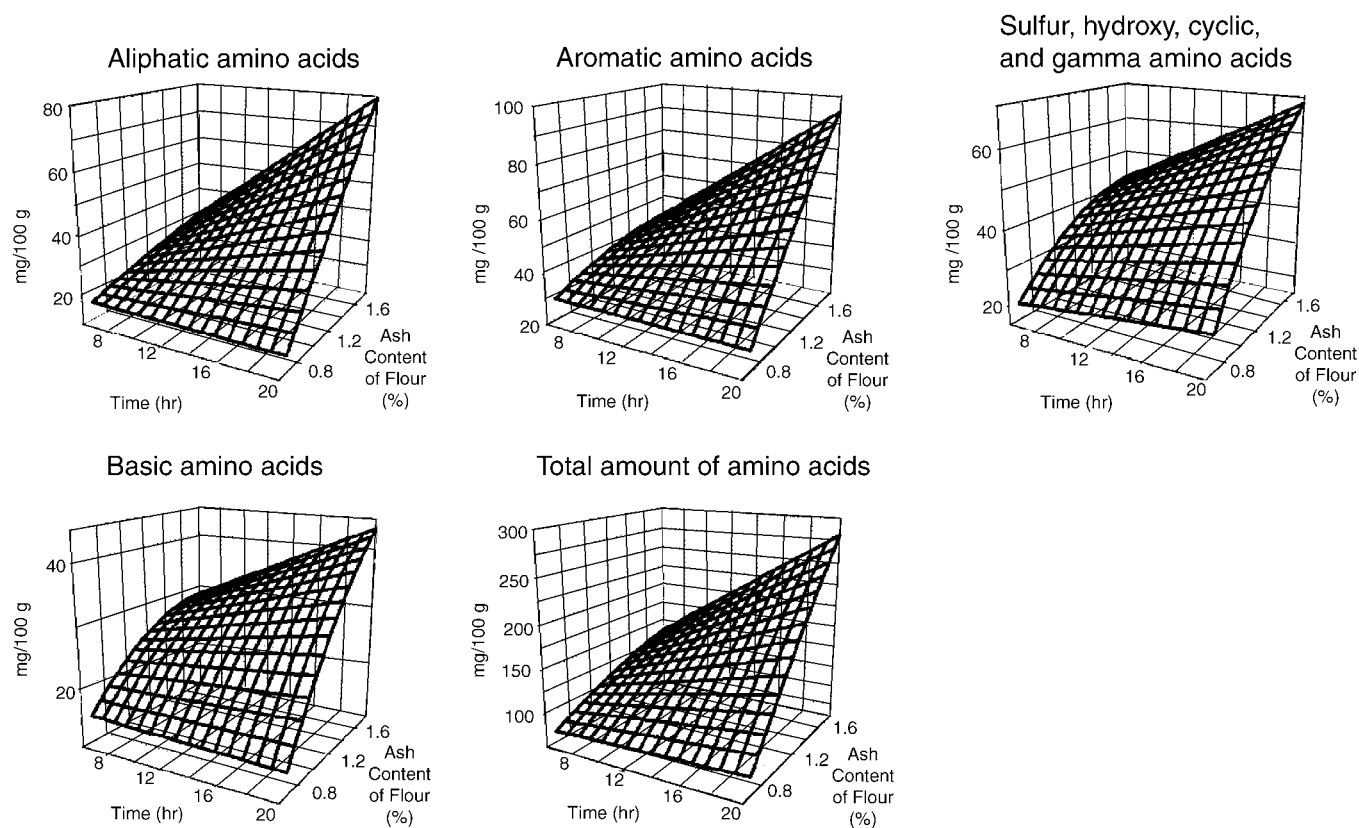


Fig. 3. Formation of amino acids in wheat sourdough fermented with *L. brevis* (fermentation at 24°C).

influencing formation of amino acids was the ash content of flour (Table V, Fig. 4). Thus, the amount of amino acids in the sourdough fermented with *S. cerevisiae* was mainly dependent on properties of flour in the yeast preferment. According to the model, the amount of some amino acids decreased slightly during yeast fermentation if ash content of flour was 0.6% and increased slightly when ash content of flour was 1.8%.

Volatile Compounds

Varying values of R^2 (goodness of fit) and Q^2 (predictive power of the model) were obtained in the volatile models (Tables VI and VII); R^2 values of 0.6–0.9 and Q^2 values of 0.5–0.9 were typical. The amount of 23 volatile compounds was determined in unfermented doughs and doughs fermented with *L. plantarum*, *L. brevis*, *S. cerevisiae*, or with the combination starter. The volatile compounds selected based on earlier studies of their potential to influence flavor were: ethanol, 2-methyl butanol, 3-methyl butanol, propanol, butanol, isobutanol, 1-penten-3-ol, hexanol, heptanol, hexyl acetate, ethyl acetate, pentyl acetate, ethyl propionate, heptanal, isobutanol, 2-methyl butanol, 3-methyl butanol, hexanal, diacetyl, 2-pentylfuran, 2-methyl butyl acetate, acetone, and dimethylsulfide (Schieberle and Grosch 1985, 1987, 1989, 1991; Baltes and Song 1994; Gassenmeier and Schieberle 1995; Hansen and Hansen 1996; Zehentbauer and Grosch 1998a,b; Czerny and Schieberle 2002). The chosen method, headspace analysis with GC-MS, was limited to the most volatile compounds evolved in sourdough fermentation. Influence of process parameters on the total amount of volatile compounds (without ethanol), as well as the amounts of ethanol, 3-methyl butanol, diacetyl, and ethyl acetate were measured.

In unfermented doughs, the total amount of volatile compounds (TV) varied at 42–83 $\mu\text{g}/100\text{ g}$ of dough, depending on the ash content of flour (0.6–1.8%); the highest values were obtained with

whole meal flour. Among fermented doughs, the highest value for TV was obtained in the sourdough fermented with *S. cerevisiae* (3,696 $\mu\text{g}/100\text{ g}$) and the lowest TV value was obtained in the sourdough fermented with *L. plantarum* (300 $\mu\text{g}/100\text{ g}$). Thus, depending on the strain used and the fermentation conditions, the TV amount increased sevenfold to 100-fold during the sourdough process.

In sourdoughs fermented with *L. brevis* or *L. plantarum*, the highest TV value was obtained when fermentation time was 20 hr, fermentation temperature was 32°C, and ash content of flour was 1.8%. According to the model, the formation of volatile compounds was linearly dependent on fermentation time, fermentation temperature, and ash content of flour (Tables VI and VII). This means that in a short fermentation time, volatile compounds are formed in minor amounts only. This relationship is shown in Fig. 5 for *L. brevis*. However, with individual compounds, the role of fermentation parameters varied, that is, formation of ethyl acetate was mainly dependent on time and temperature, whereas formation of 3-methyl butanol was mainly dependent on the ash content of flour (Table VI). In pure lactic acid fermentations (either homofermentative or heterofermentative), interactions of time and temperature were observed only for formation of ethanol and ethyl acetate with *L. brevis* as the starter. Thus, increased concentrations of these compounds required a combination of long fermentation time and high fermentation temperature.

In yeast fermentation (sourdough started with *S. cerevisiae*) or with the combination starter, the effects of time, temperature, and ash content of flour were more complicated. Frequent interactions and quadratic effects of factors were obtained with both types of fermentation (Table VII).

The highest TV value and maximum amount of 3-methyl butanol and ethyl acetate (combination starter) were reached when fermentation time was 14–16 hr because fermentation time exhib-

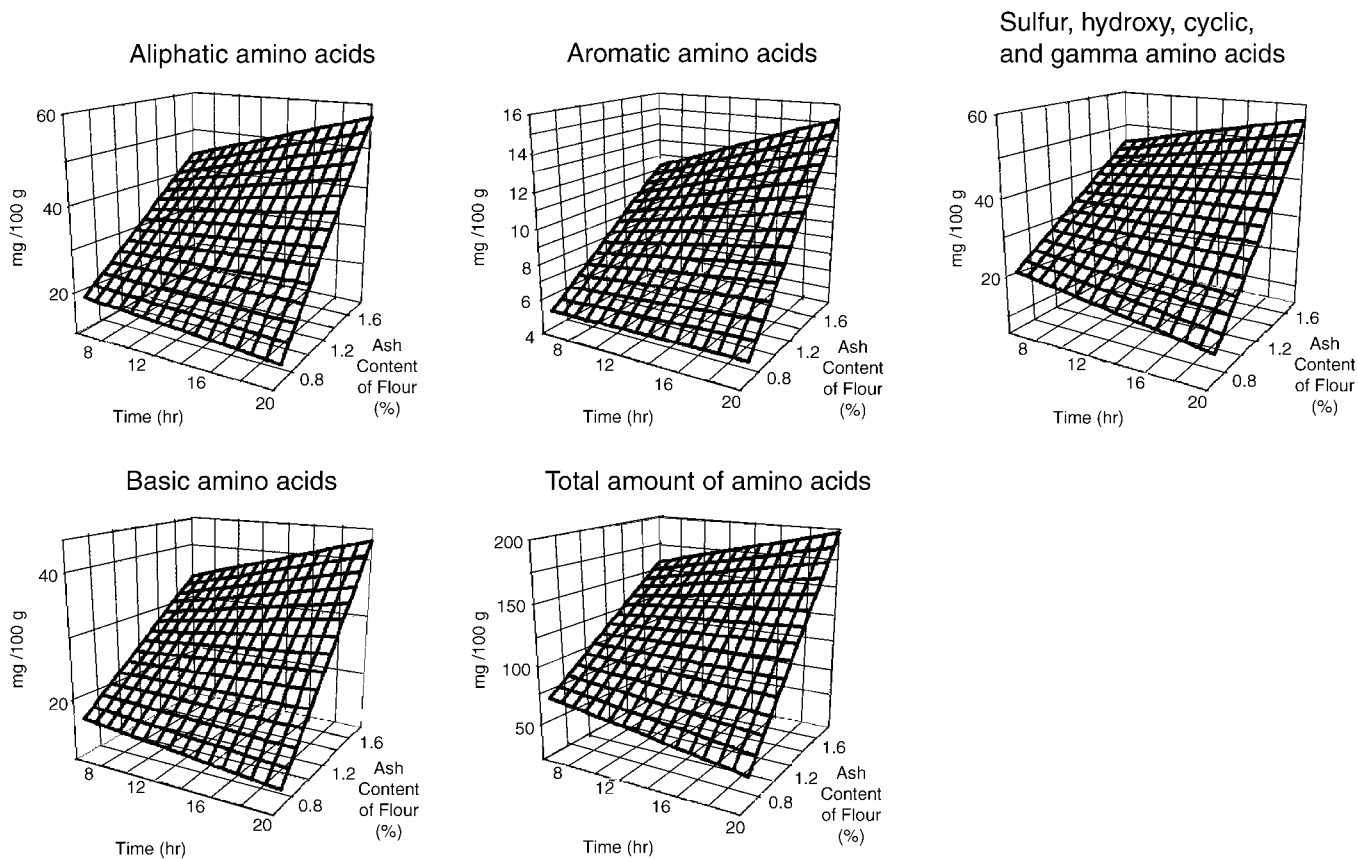


Fig. 4. Formation of amino acids in sourdough fermented with *S. cerevisiae* (fermentation at 24°C).

ited a significant quadratic effect on the formation of these compounds. This is exemplified by the sourdough fermented with the combination starter (Fig. 6). In pure yeast fermentation, maximum TV and ethanol amounts were reached with 18–20 hr of fermentation, and the maximum amount of 3-methyl butanol was obtained with 14–16 hr of fermentation.

The maximum TV, 3-methyl butanol and ethyl acetate (combination starter) were obtained when the ash content of flour was 1.2–1.6% because the ash content of flour also had a significant quadratic effect on the formation of volatile compounds. With the combination starter, temperature also exhibited a quadratic effect on the production of ethyl acetate and 3-methyl butanol. Optimum temperatures for production of 3-methyl butanol and ethyl acetate were 24–32°C and 24–28°C, respectively.

According to models with yeast fermentation or the combination starter, frequent interaction and quadratic effects of studied factors were observed; such effects were not observed with sourdoughs containing pure LAB. These effects also varied with different volatile compounds and with different types of fermentation. The main factors influencing flavor formation in pure yeast fermentation were time and temperature, whereas in the combination starter, fermentation time and ash content of flour had major roles in determining the activity of sourdough. Optimization of TV compounds or that of a particular compound in these sourdoughs requires a strict control of ash content of flour, fermentation time and sometimes fermentation temperature.

Correlation Matrix of Different Responses

With sourdoughs fermented with *L. brevis* or *L. plantarum*, the overall acidity, TV, and TA were highly correlated ($r = 0.6–0.9$)

(Table VIII). Strain-specific differences were observed as lower correlations (0.5–0.66) of acidity with formation of certain amino acid groups (basic aromatic amino acids) were observed with *L. plantarum*. In the sourdough started with yeast, the correlation of acidity, TV, and TA was much lower in comparison with sourdoughs started with pure LAB strains ($r = 0.56–0.69$) (Table VIII). A high correlation ($r = 0.85$) between acidity (TTA or lactic acid) and formation of volatile compounds was observed in the sourdough with the combination starter. A moderate correlation was observed between TTA or lactic acid and formation of amino acids ($r = 0.43–0.65$) but correlation with amino acids was significantly higher with acetic acid ($r = 0.66–0.81$) in the sourdough fermented with combination starter.

DISCUSSION

High R^2 (goodness of fit) and Q^2 (predictive power of model) values obtained for most responses indicate that the quadratic polynomial models were adequate in most cases to illustrate the effects of fermentation time, fermentation temperature, and ash content of flour on the formation of acidity, amino acids, and volatile compounds. However, for formation of some compounds such as 3-methyl butanol in the sourdough fermented with *L. plantarum*, the quadratic polynomial model could only partly illustrate the effects of the studied factors, as indicated by the lower values of R^2 and Q^2 obtained with the model. This is most likely due to the fact that formation of a particular volatile compound is not dependent on measured variables.

Increasing the length of fermentation time, fermentation temperature, and ash content of flour enhanced acidity formation in

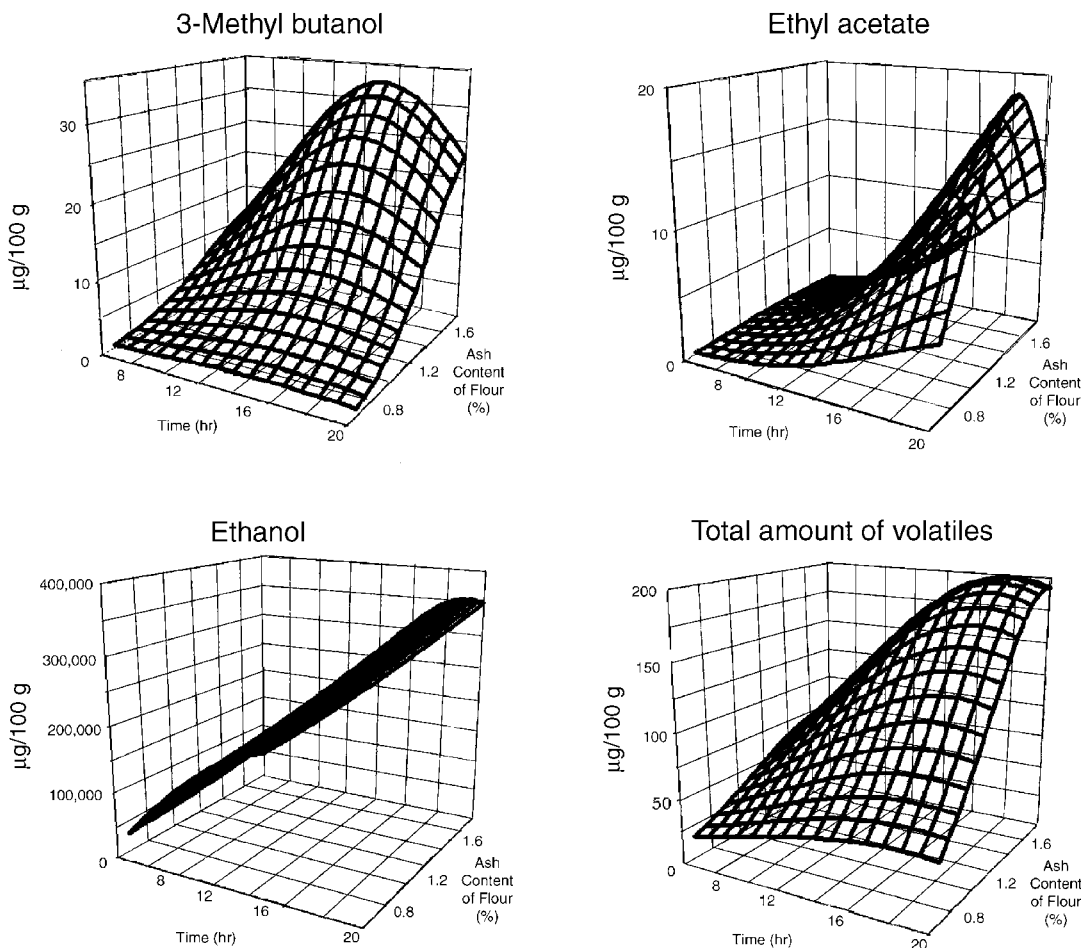


Fig. 5. Formation of volatile compounds in the sourdough fermented with *L. brevis* (fermentation at 24°C).

all sourdoughs. Such an influence from long fermentation time is logical because lactic acid fermentation allows more time to produce acids. However, it is interesting that according to the obtained models, maximum acidity is obtained in the experimental region with the combination starter as the formation of acids starts to slow down (quadratic terms significant in the model) but with pure lactic acid fermentation (sourdoughs with *L. plantarum* or *L. brevis*), this is not the case. This might be due to competition of nutrients and the inhibitory effect of yeast on lactic acid fermentation. Also, according to the results of Thiele (2002), acidity formation slows down when the stationary phase of microbial growth is reached. With the combination starter, microbial growth may slow down sooner in comparison with a single LAB.

The positive effect of temperature on acidity development has also been reported by other workers (Spicher and Nierle 1984a; Salovaara and Valjakka 1987; Rio et al 1996). The observed significance of ash content of flour on production of acids is in accordance with observations of Martinez-Anaya (1994) and Rio et al (1996). The positive effect of ash content of flour on acidity may be explained by a nutritional enrichment of the dough (more vitamins and minerals are present in whole meal flour) and by an increase in buffering capacity. In the experimental region studied, a strong interaction between fermentation time and temperature was observed for most types of sourdough as reported in earlier studies for rye and wheat sourdoughs (Hansen et al 1989; Lorenz and Brummer 2003). This means that significant formation of acids occurred only with a combination of long fermentation time (>16 hr) and high temperature (>30°C). In practice, control of sourdough acidity levels requires reduced fermentation time and fermentation temperature, and reduced ash content of flour in all sourdough types.

This study confirmed the importance of ash content of flour on the formation of amino acids during sourdough fermentation as reported in earlier studies (Spicher and Nierle 1988; Collar et al

1993; Collar and Martinez 1995). The important role of flours with a higher extraction rate is based on the presence of endogenous endo- and exoproteolytic activity in bran-rich flour (Loponen et al 2004). Darker flour also promotes development of acidity, and lower pH values promote activity of both microbe- and flour-originated proteases that usually have optimum pH 3–4.

Effective amino acid formation was most prominent with sourdoughs fermented with LAB. Accumulation of amino acids in sourdoughs is only possible when degradation of amino acids for microbial growth is less than production of amino acids due to proteolysis or autolysis of yeast. Higher proteolytic activity of LAB sourdoughs as compared with yeast preferments has also been reported in several studies (El Dash et al 1970; Spicher and Nierle 1984a; De Barber et al 1989). This is most likely due to a higher demand of yeast for amino acids as compared with most *Lactobacillus* species. Most available amino acids are degraded during yeast fermentation because at the beginning of fermentation, yeast follows a log phase of growth that induces a strong demand for nitrogen (El Dash et al 1970). Furthermore, higher pH values in yeast preferments do not favor action of proteases, especially at the beginning of fermentation. Most homo- and heterofermentative LAB during their active growth period exhibit a demand for certain specific amino acids such as glycine, glutamic acid, asparagine, and tryptophan.

Process conditions favoring intensive acidification also enhance accumulation of amino acids. This was confirmed by the high correlation of acidity parameters and amino acids in these sourdoughs. Positive effects of acidity on protein degradation have been reported by several workers (Loponen et al 2004; Thiele et al 2002, 2003, 2004). Recent studies of sourdough proteolysis show that degradation of cereals proteins during sourdough fermentation is not dependent on proteolytic activity of LAB but is mainly due to pH-dependent activation of cereal enzymes, especially aspartic proteinase (Loponen et al 2004; Thiele et al 2004). In

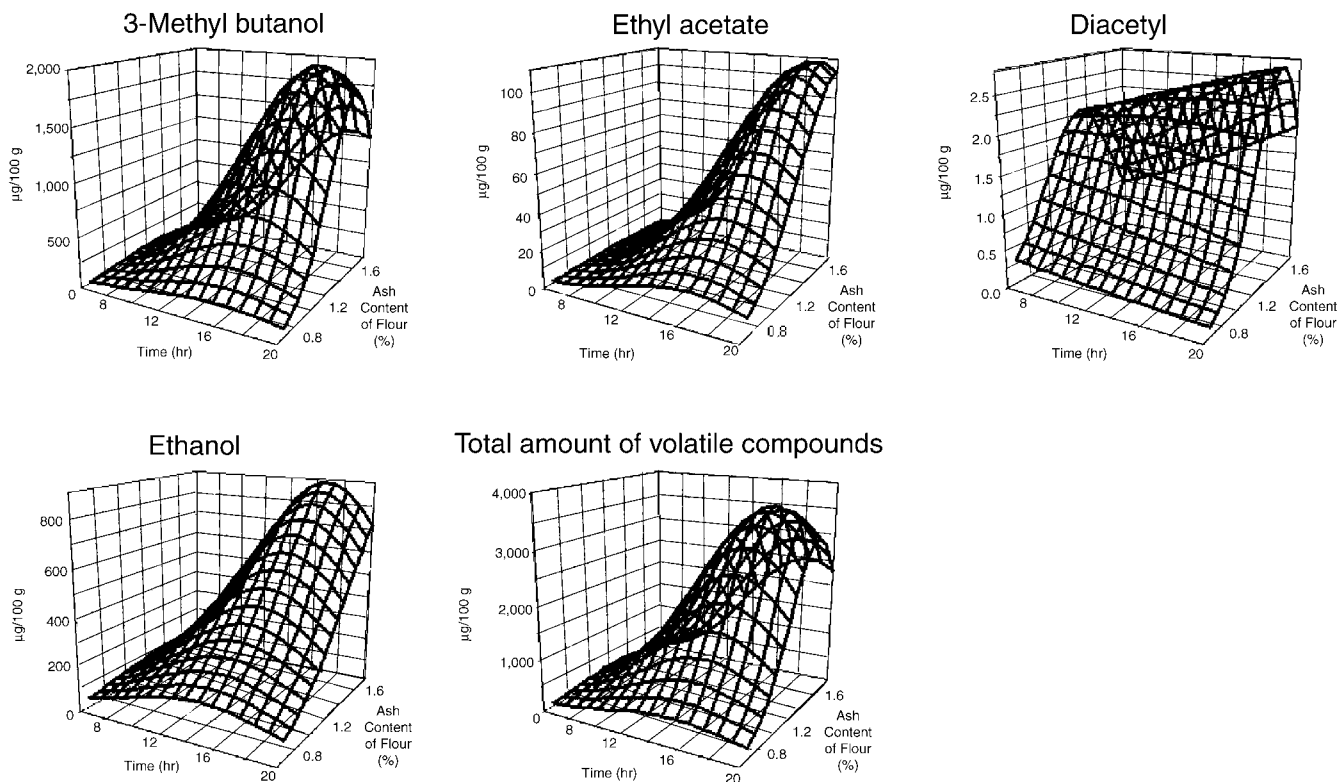


Fig. 6. Formation of volatile compounds when sourdough was fermented with *S. cerevisiae*, *L. plantarum*, and *L. brevis* (fermentation at 24°C).

practice, this means that effective accumulation of amino acids would not be obtained with flours with low ash content (0.6%), despite long fermentation times and high temperatures. Also, development of acidity would be very modest under these conditions and it could be hypothesized that pH-dependent activation of cereal proteases would not happen. This mutual dependence of the ash content of flour, fermentation time, and fermentation temperature on each other and the effect on proteolysis has not been reported.

The observed difference between *L. brevis* and *L. plantarum* in their proteolytic activity in relation to acidifying potential could be due to different proteolytic enzymes, as proteolytic activities vary greatly among different LAB strains (Gobbetti et al 1996; Di Gagno et al 2002). Also, acidification patterns with the LAB are different: *L. plantarum* produces only lactic acid and *L. brevis* produces both lactic acid and acetic acid, which might have a different influence in activation of cereal proteases. However, by choosing appropriate processing conditions for sourdough started with *L. plantarum*, formation of certain amino acids such as basic amino acids, which are important with respect to flavor, could be considerably increased without producing excessive acidity. The most well-known flavor precursor of basic amino acids is ornithine, which is converted to diacetyl 1-pyrroline in the Maillard reaction, creating roasty flavor of bread crust (Schieberle 1990).

In yeast fermentation, the observed depletion of amino acid concentration during fermentation with white wheat flour (ash content 0.6%) has been confirmed in other studies (El Dash and Johnson 1970; Thiele 2002). However, when flour with higher extraction rate was utilized, the amount of amino acids slightly increased during fermentation, as did the acidity level of sourdough, which tallies well with the theory of pH-dependent activation of endogenous proteases (Loponen et al 2004; Thiele et al 2004).

Significant enhancing effect of whole meal flour on formation of volatile compounds in sourdough started with LAB or with the combination starter has been also confirmed in other studies (Hansen and Hansen 1994a; Gobbetti et al 1995; Czerny and Schieberle 2002). A higher amount of endogenous precursors and higher proteolytic activity of whole meal flour could be responsible for this higher amount of volatile compounds (Loponen et al 2004). The initially higher number and versatility of volatile compounds in whole meal flour is one possible explanation (Czerny and Schieberle 2002). Proteolysis is of great importance in flavor formation in two ways: 1) amino acids can form volatile compounds in yeast metabolism during dough fermentation by the Erlich pathway (Gassenmeier and Schieberle 1995), and 2) they can react with sugars in the Maillard reaction during baking to produce crust flavor compounds (Grosch and Schieberle 1991). However, in pure yeast fermentation, use of whole meal did not have any effect on formation of volatile compounds. This interesting difference between LAB and yeast fermentation in response to ash content of flour is probably due to different metabolic routes to produce secondary metabolites in yeast cells as compared with bacteria cells.

The quadratic relationship between volatile compounds and fermentation time (also in some cases with fermentation temperature) observed in our study has not been reported before. This kind of trend might be due to stationary phase of yeast growth after 14–18 hr (Gobbetti et al 1994), which will lead to a gradual slow down in the metabolic activity of yeast. Also, volatilization of compounds is expected to some extent during long fermentation periods. As yeast sourdoughs or sourdoughs fermented with the combination of yeast and *L. plantarum* and *L. brevis* are frequently utilized in industrial wheat sourdoughs, this information suggests a possibility to control flavor attributes of various bread types by choosing appropriate process conditions. Furthermore, according to the general bakery practice in Finland, yeast sourdoughs are fermented for 4–6 hr at room temperature with ash content of flour 0.6–1.1%, but according to our results these are

not the optimum conditions for the production of flavor compounds.

The highest production of volatile compounds during sourdough fermentation was obtained when *S. cerevisiae* was used as a starter, fermentation time was 18–20 hr, and temperature was 32°C. Acidity development under these conditions was relatively low; predicted values according to the model were only pH 5.2 and TTA 3.1 (ash content 0.6%). With short fermentation time, no significant accumulation of volatile compounds occurred, despite utilizing high temperatures or high ash content of flour. Sourdough started with *S. cerevisiae* is thus an efficient way to optimize the amount of volatile compounds without excessive acidity formation, provided that appropriate conditions are utilized.

Maximum accumulation of amino acids without extensive acidification is difficult to obtain by adjusting process parameters in sourdough fermentation because proteolysis is so closely related to the acidity of the sourdough. However, fermentation conditions could be adjusted to obtain moderate increase in amino acids without excessive acidification. For example, this could be obtained by using *L. brevis* as starter, a fermentation time of 14 hr, a fermentation temperature of 26°C, and ash content of flour of 1.8%. Under those conditions, predicted values for acidity are pH 4.5, TTA 11.5, and TA 240 mg/100 g. However, such sourdough would be at the linear phase of acidification and proteolysis and would need an instant method (such as addition of salt or cooling) to stop the metabolic activities to preserve the required level of metabolites.

In principle, only the combination starter could produce the maximum amount of both volatile compounds and amino acids without excessive acidity in the same sourdough. However, as proteolysis and formation of volatile compounds are so closely related to the acidity of the dough with this starter, fermentation conditions could be adjusted to obtain only a moderate increase in amino acids and volatile compounds without excessive acidification. This could be obtained by using fermentation time of 9 hr, fermentation temperature of 22°C, and ash content of 1.8%. Under those conditions, predicted values for acidity are pH 4.6, TTA 11.1, TA 111 mg/100 g, and TV 1,520 µg/100 g. It is noteworthy that accumulation of amino acids would be rather modest in this type of sourdough (25% increase in comparison to unfermented sourdough), probably due to high demand of yeast for liberated amino acids. Thus, combination starter (resembling industrial sourdoughs) could not be considered a potential choice to increase the amount of free amino acids without excessive acidity formation.

In general, wheat sourdoughs are utilized in bread dough at the level of 5–30% (of dough weight), most likely due to the fact the higher amounts of sourdough would increase dough acidity and have a detrimental effect on subsequent bread flavor. Optimization of the sourdough process to produce the maximum amount of volatile compounds or amino acids without excessive acid formation by adjusting process parameters according to the starter would make it possible to increase the level of sourdough in bread dough, and thus enhance subsequent bread flavor. However, as formation of amino acids is greatly dependent on acidity level of the sourdough, new methods for improving amino acids liberation in sourdoughs would be useful. Use of exogenous proteases or even peptidases during sourdough fermentation could enhance proteolysis and produce flavor precursors for subsequent baking. Effectiveness of this approach was confirmed in a recent study by Di Cagno et al (2003): combined use of proteases with *L. hildgardii* increased the amount of free amino acids 15% but acidity level of sourdough remained nearly unaltered.

CONCLUSIONS

The significant role of process parameters and their partly strain-dependent influence on metabolic activity of wheat sourdough during fermentation were confirmed in this study. This study also

shows significance of frequent interactions between process factors, and the importance of taking these interactions into account for processing. Response surface studies of this kind are a pertinent choice for predicting effects of various fermentation conditions on sourdough properties and give valuable information on fermentation conditions to achieve a desired product.

The necessity to monitor processing parameters and the possibility, through the choice of these conditions and by choosing appropriate starter to influence acidity, formation of potential flavor compounds, or flavor precursors were confirmed herein. Accordingly, a controlled sourdough process would make it possible to modify final product characteristics. Further studies are needed to determine the properties of the final bread resulting from variations of the factors investigated in this study.

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