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Flavour of sourdough wheat bread crumb

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Abstract This study investigates the effect of adding sourdough to wheat bread dough on the production of flavour compounds in wheat bread crumb. The sourdoughs were fermented with starter cultures of lactic acid bacteria alone and in combination with sourdough yeasts. The volatile compounds in the bread crumb were isolated by a dynamic headspace technique and extraction analysis, analysed by gas chromatography (GC), and identified on the basis of GC retention times for reference compounds and mass spectrometry (MS). The chemical analyses were combined with sensory evaluation. The volume of the loaves increased significantly when the doughs had 5–20% sourdough added compared with the control bread (bread without sourdough). In the sourdough bread, the content of acetic acid, 2-methylpropanoic acid, and 3-methylbutanoic acid was generally higher, and loaves made with the addition of sourdoughs fermented with *Lactobacillus plantarum*, *L. delbrueckii*, or *L. sanfrancisco* had a higher content of 2- or 3-methyl-1-butanol than control bread. Interactions were seen between the starter cultures and the sourdough yeasts, and the production of the following compounds was increased depending on the starter culture used and on the sourdough yeast: ethanol, 2-methylpropanol, 2/3-methyl-1-butanol, 2-phenylethanol, benzyl alcohol, acetic acid, 2-methylpropanoic acid, and 3-methylpropanoic acid. Bread made with an addition of 5% to 15% sourdough fermented with *L. sanfrancisco* had a pleasant, mild and sour odour and taste. *L. plantarum* bread had a strong, sour and unpleasant odour and a metallic sour taste with a sour aftertaste, but when the sourdough was also supplemented with the sourdough yeast *Saccharomyces cerevisiae*, the bread attained a more aromatic wheat bread flavour, which may be caused, in part, by a higher content of 2/3-methyl-1-butanol, 2-methylpropanoic acid, 3-methylbutanoic acid and 2-phenylethanol.

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Introduction

The flavour of bread depends upon the ingredients, the dough fermentation, and the baking process. The flavour of bread crumb is influenced mainly by the enzymatic reactions during the dough fermentation, whereas the flavour of bread crust is more influenced by the thermal reactions during the baking process [1].

Many studies have been concerned with the identification of bread flavour compounds, and today about 300 volatile compounds have been identified, with most work having been done on bread crust [2]. The influence of volatile compounds on bread flavour has been studied [1–6]. Chemical analyses of the flavour compounds have been combined with sensory analysis of the bread or a synthetic mixture of volatile compounds in bread to find out what influence the compounds have on the flavour sensed. Compounds that have been positively correlated with the flavour of wheat crumb are: acetaldehyde, 2-methyl-propanal, 3-methyl-butanal, isopentanal, 2-nonenal, benzylethanol, 2-phenylethanol, dimethyl sulphide and 2-furfural [1–3, 6, 7]. The flavour compounds with buttery flavour, i.e. 2,3-butandione (diacetyl) and 3-hydroxy-2-butanone (acetoin), have been related to both a positive influence [1] and no influence [3] on bread flavour. Acetic acid, at levels of 100–200 ppm, was found to act as a flavour enhancer in the bread crumb [8].

Few investigations have focused on the influence of the bread-making processes on the formation of bread flavour. Richard-Molard and co-workers [8] studied the influence of different dough-making techniques on wheat bread flavour and they found that the content of the volatile acids (acetic, isobutyric and isovaleric acids) varied considerably according to the mixing techniques and fermentation method used. The iso-acids had a negative influence on bread flavour and the levels of these acids were generally proportional to the amounts of baker's yeast added to the dough, which were greater in the short bread-making processes than in bread made with the sourdough process. On the other hand, the concentration of acetic acid was higher in bread made with a prolonged dough fermentation compared with the straight dough process. Schieberle and Grosch [5] found that prolonged dough fermentation increased the concentration of 3-methyl-butanol and 2-phenylethanol, and Frasse et al. [9] found that 2-phenylethanol, 3-methyl-1-butanol and 2-hydroxy-3-butanone were directly linked to the fermentative activity of the yeast in the dough fermentation step.

In sourdough fermentation, lactic acid, acetic acid and volatile flavour compounds such as alcohols, esters and carbonyls are produced, depending on the microorganisms in the dough [10]. As well as flavour compounds, precursors of flavour compounds can also be formed, such as free amino acids produced by the proteolytic activity in sourdough [11], which are later involved in the Maillard reactions during baking.

In the current study we investigated what effect the addition of sourdoughs to wheat bread dough had on the production of volatile compounds in wheat bread crumb. The sourdoughs were fermented with starter cultures of lactic acid bacteria alone and in combination with sourdough yeasts, as a mixed culture fermentation might result in a more fully developed bread flavour. The chemical analyses were combined with sensory evaluation.

Materials and methods

Flour. Danish commercial wheat flour (protein content 14.3% of dry matter, d.m.) for breadmaking with the addition of 70 mg/kg ascorbic acid (flour weight basis) was used according to [10].

Preparation of sourdoughs. Starter cultures of the genus *Lactobacillus* originating from rye sourdoughs: *L. plantarum*, *L. delbrueckii*, *L. sanfrancisco*, and *L. brevis* and four different strains of sourdough yeasts: *Saccharomyces cerevisiae*, *Candida milleri*, strain A and strain B were used for the fermentations [10]. The sourdough sponge and the sourdough were prepared according to [10] and fermented for 20 h at 30 °C. Yeast sponges were prepared and combined with the sourdough sponges [10].

Baking procedure. The loaves were made using Funai Baking Machines [12] and the control bread formulation was as follow: 550 g wheat flour, 385 ml tap water (30 °C), 7.5 g salt and 6.0 g dried yeast (The Danish Destilleries A/S, Danisco). The baking tests were carried out on loaves with the addition of 0%, 5%, 10%, 15% and 20% sourdough (% fermented flour of total flour amount). The total flour and water amounts were the same in control bread and in sourdough fermented bread.

Analytical assays. The loaves were analysed for pH, total titratable acidity (TTA) and the bread volumes were determined as the specific loaf volume (ml/g) according to [12]. The crust of the loaves was cut away to a depth of 1 cm and the crumb was gently comminuted in a Waring blender 20 h after baking. TTA was determined by suspending 10 g comminuted crumbs in 100 ml distilled water and titrated to pH 8.5 with 0.1 M sodium hydroxide.

The flavour analysis was conducted on the crumb of the wheat bread made with the addition of 15% sourdough. The flavour compounds were collected by a headspace technique and extraction analysis and analysed by gas chromatography (GC) with identification based on the GC retention time for reference compounds and mass spectrometry [13, 14]. For the headspace analysis, 1 ml of 50 ppm 4-methyl-1-pentanol was added to 50 g comminuted crumb as an internal standard. The volatile compounds were collected by a tube containing Poropak Q as an absorbent polymer. For the extraction analysis, 4 ml of 50 ppm 4-methyl-1-pentanol and 200 ml water were added to 50 g comminuted crumb. The method is described in detail by Hansen et al. [14], but for this work the method was modified by concentration of the ether phase to 0.20 ml. The data were analysed by an analysis of variance (ANOVA) procedure available in SAS/STAT software [10, 15]. The baking tests were carried out three times and analyses were made in duplicate.

Sensory evaluation. The test panel consisted of eight well trained and experienced students who have been selected for a professional sensory panel. The panellists were trained prior to the evaluations. A ranking method was used to test the level of sour odour and sour taste in sourdough fermented bread with the addition of various amounts of sourdough: 0%, 5%, 10%, 15% and 20% (fermented with *L. plantarum* and *L. sanfrancisco*). The judges used a qualitative scale for sour odour and sour taste from "not very sour" to "very sour" which was transformed to a scale from 0 to 10. The descriptive sensory evaluations were made on bread without the addition of sourdough (control bread) and on loaves with the addition of 15% sourdough fermented with *L. sanfrancisco* or *L. plantarum*. *L. plantarum* was also tested in combination with the sourdough yeast *S. cerevisiae*. The crumb of the loaves was cut into pieces (about 1.5 cm) 30 min before testing and presented in dark bottles covered with foil and served at room temperature. The samples were coded with a two-digit random number and presented in following combinations: control bread against *L. plantarum* or *L. sanfrancisco*, *L. plantarum* against *L. sanfrancisco*, and *L. plantarum* against *L. plantarum* plus *S. cerevisiae*. The judges were instructed to describe the odour and taste of the breads. The samples were tested three times.

Results and discussion

Analytical data

The analytical data of the sourdoughs are given in [10]. The effects of various amounts of sourdough added to the wheat bread are shown in Table 1. The pH of the loaves varied from 5.9 (control bread) to 4.5 in bread with the addition of 20% sourdough, and the corresponding loaves had TTA values of between 1.6 and 4.1. The optimum quantity of sourdough to be added to wheat bread was dependent on the TTA of the sourdoughs. According to the ranking tests, TTA values in wheat bread between 1.9 and 4.1 are to be recommended, which corresponds to the addition of 10–35 acid Eq/100 g unfermented flour. These loaves had a regular crumb porosity, good crumb elasticity and good flavour. This is in accordance with Brümmer [16], who recommended TTA values between 3.5 and 4.0 to obtain a good taste in wheat bread. Wheat bread with the addition of 15% sourdough was used for all the following analyses and measurements. The analytical data of wheat bread with the addition of sourdough and sourdough yeasts are shown in Table 2. The pH of the sourdough breads varied from 5.1 to 4.5, and the loaves had TTA values between 3.3 and 4.6. The specific volumes of the sourdough breads varied between 3.8 and 4.3 ml/g, and it is noteworthy that the volume of the loaves increased significantly with the

Table 1 Characteristics of wheat bread with the addition of sourdough fermented with *L. plantarum* and *L. sanfrancisco*

Conditions	Sourdough (%) added:				
	0	5	10	15	20
L. plantarum:					
pH	5.9[a]	5.2[b]	4.9[c]	4.6[d]	4.5[d]
TTA	1.6[a]	2.2[b]	2.9[c]	3.8[d]	4.1[d]
Spec. volume (ml/g)	3.5[a]	3.7[a]	3.8[ab]	4.1[b]	3.8[ab]
Acid odour	0[a]	1.7[b]	3.8[c]	7.3[d]	8.5[e]
Acid taste	0[a]	1.6[b]	3.2[c]	7.0[d]	8.1[e]
L. sanfrancisco:					
pH	5.9[a]	5.4[b]	5.1[c]	4.8[d]	4.6[e]
TTA	1.6[a]	1.9[a]	2.7[b]	4.0[c]	4.1[c]
Spec. volume (ml/g)	3.5[a]	3.8[ab]	3.8[ab]	3.8[ab]	4.0[b]
Acid odour	0[a]	1.7[b]	3.6[c]	6.5[d]	8.7[e]
Acid taste	0[a]	1.3[b]	2.9[c]	6.2[d]	7.5[e]

Means in each row followed by the same letter are not significantly different ($P < 0.05$)
Sourdough (%), Fermented flour of total flour amount; TTA, total titratable acidity, expressed in ml of 0.1 mol/ml NaOH; acid odour, ranking test for sour odour with a quantitative scale from 0 to 10; acid taste, ranking test for sour taste with a quantitative scale from 0 to 10

Table 2 Analytical data of bread crumb prepared with 15% sourdough fermented with starter cultures of *Lactobacillus* and sourdough yeasts

Starter culture + sourdough yeast	pH	TTA	Specific loaf vol (ml/g)
L. plantarum	4.5[a]	3.7[abc]	4.1[bcd]
L. plantarum + strain A	4.5[a]	4.6[e]	4.3[d]
L. plantarum + strain B	4.8[abc]	3.3[a]	4.3[d]
L. plantarum + *S. cerevisiae*	4.8[bcd]	3.8[bc]	4.1[bcd]
L. plantarum + *C. milleri*	4.7[abc]	3.7[abc]	4.2[cd]
L. delbrueckii	4.8[bcd]	4.3[de]	3.9[ab]
L. delbrueckii + strain A	4.7[abc]	4.3[de]	4.2[cd]
L. delbrueckii + strain B	4.8[bcd]	3.8[b]	4.2[cd]
L. delbrueckii + *S. cerevisiae*	4.9[cde]	3.9[bcd]	4.2[cd]
L. delbrueckii + *C milleri*	4.7[abc]	4.3[de]	4.2[cd]
L. sanfrancisco	4.7[abc]	4.0[bcd]	4.0[abc]
L. sanfrancisco + strain A	4.8[bcd]	4.1[cd]	4.0[abc]
L. sanfrancisco + strain B	4.8[bcd]	3.8[bc]	4.3[d]
L. sanfrancisco + *S. cerevisiae*	4.9[cde]	3.3[a]	3.9[ab]
L. sanfrancisco + *C. milleri*	5.1[e]	3.6[ab]	4.2[cd]
L. brevis	4.5[a]	3.8[bc]	4.0[abc]
L. brevis + strain A	4.6[ab]	3.7[abc]	4.2[cd]
L. brevis + strain B	4.6[ab]	3.3[ab]	4.3[d]
L. brevis + *S. cerevisiae*	4.7[abc]	3.7[abc]	3.8[a]
L. brevis + *C. milleri*	4.7[abc]	3.7[abc]	4.1[bcd]

Means in each row followed by the same letter are not significantly different ($P < 0.05$)
TTA is expressed in ml of 0.1 mol/l NaOH

addition of sourdough compared with control bread (Table 1). The addition of sourdough yeasts also had a positive effect on volumes compared with those loaves which had no added sourdough, except for bread fermented with heterofermentative cultures with the addition of *S. cerevisiae*, which seems to have a negative effect on specific loaf volume compared with the other sourdough bread. The positive effect on bread volume of both starter culture and sourdough yeast can reduce the demand for addition of baker's yeast to the dough.

Content of flavour compounds

The flavour compounds detected in the sourdough wheat bread are shown in Table 3. The control bread and the sourdough breads showed nearly the same qualitative composition of flavour compounds, but the amount of flavour compounds was increased in the sourdough breads compared with the control bread. The identified compounds in the bread were primarily alcohols and acids. The alcohols were dominated by ethanol, 2/3-methyl-1-butanol, 2-methyl-1-propanol, and *n*-propanol, and the production of acids was dominated by 2-methylpropanoic acid, 3-methylbutanoic acid and acetic acid.

The production of ethanol was higher in sourdough breads than in control bread, and the production varied according to the starter culture and the yeast strain used. Generally, the content of ethanol was higher in those sourdough breads which also had sourdough yeasts, except for sourdough bread fermented with *L. sanfrancisco*. The production of 2-methyl-1-propanol was not higher in sourdough bread as in control bread except for sourdough bread fermented with *L. delbrueckii* plus *C. milleri* and *L. brevis* plus yeast strain A. In contrast, the content of 2/3-methyl-1-butanol was higher in sourdough breads than in control bread except for sourdough bread fermented with *L brevis*. Schieberle and Grosch [5] found also higher content of 2/3-methyl-1-butanol in sourdough bread compared to bread made without the addition of sourdough.

Generally, the loaves had a low content of esters and the following esters were identified: ethyl acetate, ethyl *n*-hexanoate and ethyl lactate. A higher content of ethyl acetate was seen in sourdough bread fermented with *L. delbrueckii* with the addition of *C. milleri*. In particular, the content of ethyl acetate was low in the breads compared with a much higher content in the respective sourdoughs [10].

The production of 2-phenylethanol was dependent on both starter culture and yeast strain. Sourdough bread fermented with *L. plantarum* had a higher content of 2-phenylethanol than sourdough bread fermented with the other starter cultures except for *L. sanfrancisco* combined with yeast strain A. The content of 2-phenylethanol in sourdough bread fermented with *L. delbrueckii* was the same as in the control bread. The content of benzyl alcohol was generally low, but high amounts were seen in sourdough breads fermented with *L. plantarum* and *L. brevis* with the addition of *C. milleri*.

The production of the volatile acids, 2-methylpropanoic acid, 3-methylbutanoic acid, and acetic acid, was increased in sourdough breads compared with control bread, and it varied according to the starter culture used. The greatest amounts of the acids were seen in sourdough bread fermented with *L. sanfrancisco*, and the addition of sourdough yeast increased the production of 2-methylpropanoic acid and 3-methylbutanoic acids. A high content of 2-methyl-

Table 3 Volatile compounds in sourdough bread crumb. The bread was prepared with 15% sourdough fermented with starter cultures of *Lactobacillus* and sourdough yeast

Starter culture	Control	*L. plantarum*					*L. delbrueckii*					*L. sanfrancisco*					*L. brevis*				
Sourdough yeast		0[a]	A	B	*S. cer*	*C. mill*	0[a]	A	B	*S. cer*	*C. mill*	0[a]	A	B	*S. cer*	*C. mill*	0[a]	A	B	*S. cer*	*C. mill*
Alcohols:																					
Ethanol	12010	13000	16411	16090	25038	13813	10243	7683	21779	14758	33604	19651	16772	10925	17878	16388	20365	39457	9475	14097	22961
n-Propanol	154	82	77	67	124	108	87	70	111	94	322	76	78	54	80	78	107	199	59	93	112
2-Methyl-1-propanol	1258	852	1046	879	1275	1223	842	662	947	802	2258	1086	1213	899	1173	1158	1099	2146	806	1022	1114
n-Butanol	6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3	6	N.D.	8	N.D.	17	11	3	N.D.	N.D.	8	7
2/3-Methyl-1-butanol	688	1609	1743	1586	1801	1921	1216	942	1269	1058	2633	1750	1824	1386	1801	1564	7	8	5	7	7
n-Pentanol	7	6	N.D.	N.D.	6	6	N.D.	3	3	4	12	5	N.D.	N.D.	7	N.D.	7	8	5	7	7
n-Hexanol	8	9	5	8	12	5	3	3	6	5	17	N.D.	8	10	11	11	12	14	10	13	11
Esters:																					
Ethyl acetate	17	15	10	12	11	20	N.D.	21	N.D.	31	87	22	23	24	20	22	20	32	17	18	20
Ethyl	N.D.	3	N.D.	1	8	N.D.	N.D.	2	N.D.	9	9	5	6	3	7	4	4	6	N.D.	8	5
Ethyl *n*-hexanoate	3	4	N.D.	5	5	7	N.D.	2	N.D.	N.D.	8	5	N.D.	5	6	7	4	7	N.D.	7	N.D.
Carbonyls:																					
2,3-Butandione	10	9	N.D.	9	N.D.	14	N.D.	N.D.	N.D.	N.D.	33	17	17	10	20	22	N.D.	N.D.	N.D.	11	N.D.
Acids:																					
Acetic	6	55	41	123	37	37	31	30	32	46	56	165	176	300	344	447	96	48	41	122	60
2-Methylpropanoic	34	92	82	250	121	81	70	62	66	43	60	85	140	108	152	119	94	96	89	77	65
Butanoic	3	8	8	17	7	7	6	5	6	7	6	6	8	6	10	13	8	8	9	7	6
3-Methylbutanoic	29	36	91	122	84	52	39	33	34	48	35	44	109	75	124	63	49	64	66	42	36
Aromatic compounds:																					
Benzaldehyde	7	4	3	4	4	5	3	3	2	3	5	3	N.D.	3	3	3	4	7	4	4	N.D.
Furfurylalcohol	3	5	6	5	5	6	N.D.	N.D.	N.D.	2	4	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	9	3	5	4
Benzyl alcohol	6	4	3	3	3	259	N.D.	N.D.	N.D.	N.D.	N.D.	11	N.D.	N.D.	N.D.	N.D.	8	15	20	3	256
2-Phenylethanol	153	210	557	742	482	243	125	106	68	158	184	151	761	184	159	235	205	308	371	202	214

[a] 0 = no addition of sourdough yeast

The amounts of volatile compounds are expressed as relative area of the peak area = (area of the peak of compound/area of the peak of internal standard) × 100

Alcohols, carbonyls and esters were detected in the headspace analysis, acids and aromatic compounds in extraction analysis

Table 4 Descriptive words for sensory analysis of sourdough bread crumb. The bread dough was supplemented with 15% sourdough fermented with *L. plantarum* or *L. sanfrancisco*

Control bread	Sourdough bread		
	L. plantarum	*L. sanfrancisco*	*L. plantarum* + *S. cerevisiae*[a]
Odour:			
Flour	Very sour	Mild sour	Mild sour
Sweet	Very pricking	Mild pricking	Mild pricking
Yeasty	Buttermilk	Appetizing	Fresh
			Aromatic
			Yeasty
Taste:			
Bread	Very sour	Mild sour	Mild sour
Sweet	Metallic	Fresh	Aromatic
Dumpling	Bitter	Mild	Spicy
	Aftertaste	Home-made bread	Aftertaste
		Salty	

[a] The sourdough was also supplemented with the sourdough yeast *Saccharomyces cerevisiae*

propanoic acid was seen in sourdough bread fermented with *L. plantarum* and yeast strain B. Thus, the amounts of flavour compounds in the sourdough bread were influenced by the starter culture used, but interactions between starter cultures and sourdough yeasts were seen for some of the flavour compounds. The different content of the volatile compounds in the sourdoughs [10] could generally not be recognized in breads to which 15% sourdough was added.

Sensory evaluation

The results of the ranking of acidity odour and taste are given in Table 1. In the ranking tests the judges found that greater addition of sourdough increased the acidity in the loaves. The judges preferred loaves made with an addition of 5% to 10% sourdough fermented with *L. plantarum* and loaves made with the addition of 5% to 15% sourdough fermented with *L. sanfrancisco*. The results of the descriptive sensory evaluation of loaves with the addition of 15% sourdough can be seen in Table 4. This table shows that there is a difference between the sourdough breads with regard to bread odour and bread taste. The control bread had a sweet yeasty odour and a sweet bread taste. Culture *L. sanfrancisco* resulted in a sourdough bread with a mild, pricking and sour odour and a home-made bread taste. The loaves had a salty taste compared with the other loaves. Lilja et al. [17] showed that it was possible to increase the intensity of saltiness of both wheat and rye bread by increasing the acidity.

Loaves based on the sourdoughs fermented with *L. plantarum* were described as having a strong, sour and unpleasant odour and a metallic sour taste with an aftertaste. This difference in the perception of sour odour and taste between the sourdough breads fermented with *L. sanfrancisco* and *L. plantarum* might be due to the difference in the content of acetic acid. The production of acetic acid in sourdough fermented with *L. sanfrancisco* was 0.08% compared with 0.01% in sourdoughs fermented with *L. plantarum* [10], and acetic acid may act as an enhancer for the other flavour compounds [8] giving a milder bread flavour.

Some judges could recognize a buttermilk odour (diacetyl) in bread with the addition of sourdough fermented with *L. plantarum*. But the level of diacetyl was low and not higher than in control bread. When *S. cerevisiae* was added to sourdough fermented with *L. plantarum* the bread acquired the most aromatic odour and taste. The strong sour metallic taste was replaced by a more aromatic wheat bread flavour. The contents of 2/3-methyl-1-butanol, 2-methylpropanoic acid, 3-methylbutanoic acid, and 2-phenylethanol were significantly higher in sourdough bread fermented with *L. plantarum* plus *S. cerevisiae* than sourdough bread fermented with *L. plantarum* alone, and the higher content of those compounds might, in part, contribute to the more intense bread flavour.

In contrast to Richard-Molard and co-workers [8] we did not find that higher contents of iso-acids, such as 2-methylpropanoic acid and 3-methylbutanoic acid, had a negative influence on the bread flavour.

In conclusion, it may be advantageous to introduce sourdough into the wheat bread-making process. The bread volume can be increased and the bread flavour might be enhanced depending on the starter culture and sourdough yeast used.

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