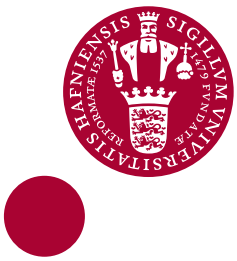

Flavour Production and Acidification of Sourdoughs in Relation to Starter Culture and Fermentation Temperature

Hansen, Åse Solvej; Lund, Bente; Lewis, M J

Published in:
Lebensmittel - Wissenschaft und Technologie

Publication date:
1989

Lebensm.-Wiss. u.-Technol., 22, 145–149 (1989)

Flavour Production and Acidification of Sourdoughs in Relation to Starter Culture and Fermentation Temperature

Å. Hansen, B. Lund and M. J. Lewis

Å. Hansen and B. Lund: Department of the Technology of Plant Food Products, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C (Denmark)
M. J. Lewis: Department of Agricultural Science, Institute of Arable Crops Research, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF (U.K.)
(*Received June 21, 1988; accepted November 30, 1988*)

Three heterofermentative and two homofermentative lactic acid bacteria were used as starter cultures for semi-fluid rye wholemeal sourdoughs produced at 25, 30, 35 and 40°C in a laboratory fermenter. The production of acids and volatile compounds in the sourdoughs was investigated. The fastest increase in acidity was generally seen at 35°C with stable acidification levels reached after 16–20 h of fermentation. Of the 10 alcohols, 10 esters and 6 carbonyls identified in the sourdoughs, ethyl acetate, 2-methyl-1-propanol, 2/3-methyl-1-butanol, ethyl n*-hexanoate and diacetyl varied considerably with starter culture and/or fermentation temperature used.*

Introduction

In the manufacture of rye bread, sourdough is used in the dough to achieve the desired texture and flavour of the bread. The aroma of a rye bread crumb is influenced by flavour precursors or flavours from the rye wholemeal, developed during the fermentation of the sourdough, the leavening of the dough and the baking process. Little is known about the influence of the sourdough fermentation process on rye bread aroma (1), but it is one of the factors giving rye bread a different and more intense aroma than wheat bread (1,2).
In order to optimize the control of sourdough fermentation, there has been an increased interest in using starter cultures. Spicher and co-workers have done many profound investigations concerning identification of the microflora (lactic acid bacteria and yeasts) from different types of sourdoughs, both spontaneously started sourdoughs and in commercial starter cultures for sourdoughs (3,4). Their work has also included investigations of the influence of temperature and dough yield on the production of lactic acid and acetic acid in sourdoughs started with lactic acid bacteria, and the baking performance of sourdoughs fermented with some of the lactic acid bacteria. These sourdoughs have been prepared according to German traditions with the use of low extraction rye flour, or from a mixture of rye and wheat flour. In Denmark sourdoughs and rye bread are primarily made from rye wholemeal. The extraction rate of the rye flour has been shown to influence the production of acids in the sourdoughs (5).
Few investigations have been made on identification of volatile flavour compounds in sourdoughs (6,7). The aim of this study was to investigate the influence of starter cultures (three heterofermentative and two homofermentative lactobacilli) on the production of acids and volatiles in rye wholemeal sourdoughs at different temperatures.

Materials and methods

Materials
Freeze-dried starter cultures of pure bacterial strains originating from rye sourdoughs as below.

Heterofermentative lactobacilli. L. sanfrancisco (L.sf) (earlier *L. brevis* var. *lindneri* according to Bergey's Manual, 1986), DDSF, Denmark; L.62 (named *L. brevis*), Chr. Hansen's Laboratories A/S, Denmark; L.h. (unidentifiable), Clas Lönner, University of Lund, Sweden (4).

Homofermentative lactobacilli. L.22 (named *L. delbrueckii*), Chr. Hansen's Laboratories A/S, Denmark; L.73 (named *L. plantarum*), Chr. Hansen's Laboratories A/S, Denmark.

Rye wholemeal having a falling number of 140 Sec (ICC standard No. 107) (8), titratable acidity (S°) of 4.5 (3) and an ash content of 1.43% of d.m. (9) was used.

Methods

Sourdough fermentation conditions were as follows.

Mother sponge. A 'mother sponge' was prepared from a rye meal–water mixture (200 g rye meal and 400 g water) by adding freeze dried starter culture (to give a cell content of 10^8 per gram dough) and then incubating for 24 h at 30°C.

Sourdough. 1500 g rye wholemeal, 3000 g water and 450 g fermented 'mother sponge' were mixed. The dough was transferred to a Chemap Laboratory fermenter (type CF 7 l) and fermented at 25, 30, 35 or 40°C.
The stirring speed was initially 600 rpm, after 12 h it was

0023-6438/89/040145 + 05 $03.00/0

decreased to 400 rpm and after 24 h to 200 rpm (as the sourdough viscosity decreased during the fermentation). Samples were removed after 0, 7, 12, 16, 20, 24 and 30 h and analysed for pH, titratable acidity, bacteria and yeast content. 'Mature' sourdoughs were analysed for content of acetic and lactic acid and volatile compounds in general. 'Mature' sourdough is here defined as a sourdough where the change in titratable acidity between two sample collections is less than 1.5S°. Sourdoughs were run twice under each condition.

Acid content analysis. pH and total titratable acidity (S°) were determined according to (3). Acetic and lactic acid contents were determined enzymatically using Boehringer Mannheim kits.

Microbial content analysis. The number of bacteria were counted on MRS agar (Merck) as surface growing colonies when incubated at 30°C for 2–5 days. The number of yeasts were counted on Wort agar (Merck) after incubation at 25°C for 3–5 days. Possible bacterial contamination was controlled by isolation of randomly selected colonies and testing these for air production, colony- and cell-morphology, Gram-staining, catalase and oxidase reaction, while a few were also examined using carbohydrate fermentation kits (API Systems S.A., France).

Content of volatile compounds

Volatile compounds in the 'mature' sourdoughs were collected by a Headspace technique and analysed by gas chromatography with identification based on GC-retention times for reference compounds and GC–mass spectrometry.

Headspace procedure. The headspace collection procedure was modified after (10). A gas washing bottle containing 50 g rye sourdough diluted with 50 ml water was added 1 ml 50 ppm 4-methyl-1-pentanol as internal standard. It was placed in a 40°C water bath with magnetic stirring. After 20 min, nitrogen gas (60 ml/min) was bubbled through the content in the bottle, exiting through a glass tube (6.8 × 0.4 cm) containing 300 mg Porapak Q (50–80 mesh). After 1 h gas stripping, the Porapak Q tube was removed, and the retained volatiles were eluted from the polymer using double-distilled diethylether. After collecting 180 mg ether in a V-bottom vial, the eluate was concentrated to 40 mg by gently blowing nitrogen gas over the ether surface.

Gas chromatographic procedure. Concentrated eluate (0.5 μl) was injected on the gas chromatograph (Hewlett–Packard 5890 A Chromatograph with Flame Ionisation Detector FID, and HP 3392 Integrator) using a Carbowax 20M Capillary column (50 m × 0.31 mm) operated at a helium gas flow of 2 ml/min and the following temperature program; 10 min at 50°C, 50–180°C at 3°C/min, 180°C isothermal. Injection port temperature was 200°C, while FID temperature was 220°C. The split was 1:10. The samples were analysed in duplicate.

Gas chromatography–mass spectrometry (GC–MS) procedure. GC–MS analyses were performed on a Kratos MS 80 RFA mass spectrometer. The conditions for the gas chromatograph were as mentioned above. The column was connected directly into the source. Samples were injected via a split/splitless injector, with the split valve operated after 30 s. The ionisation voltage was 70 eV, mass range was 27–500 amu and scan speed was 1 s/decade. The source temperature was 200°C and the interface temperature was 250°C.

The content of compounds is expressed as relative peak areas = (peak area of compound/peak area of internal standard) × 100.

Results

All cultures acidified the sourdoughs well and no contamination with other bacteria was observed apart from L.22 at 40°C, where acidification did not occur before 12–14 h. Yeast propagated especially in the homofermentative cultures. Differences in aroma of the sourdoughs were pronounced. The development in titratable acidity (S°) is shown in **Fig. 1**. The analytical data for the 'mature' sourdoughs are given in **Table 1**. Results of the analysis for volatile compounds are given in **Table 2** and **Fig. 2**.

Table 1 Souring characteristics for mature cultures of *L. sanfrancisco*, L.62, L.h, L.22 and L.73. (a) Heterofermentative. (b) Homofermentative

(a)

	L. sanfrancisco				L.62				L.h			
Temp. (°C)	25	30	35	40	25	30	35	40	25	30	35	40
Time (h)	16	16	16	16	20	20	20	20	20	20	20	20
pH	3.5	3.6	3.5	3.9	3.7	3.7	3.7	3.7	3.9	3.8	3.7	3.9
S° (ml)	14.4	16.3	16.4	16.2	15.0	17.7	18.0	17.6	18.0	20.0	19.5	20.7
Log cfu MRS	9.5	9.4	8.8	8.0	9.5	8.6	9.4	8.0	9.8	9.7	9.4	9.2
Log cfu WORT	3.3	3.0	3.3	3.0	7.2	6.3	3.0	3.8	>2.0	3.7	2.0	2.7
% L-lactic acid	0.61	0.60	0.57	0.58	0.59	0.60	0.61	0.50	0.82	0.84	0.87	0.69
% D-lactic acid	0.29	0.30	0.28	0.31	0.21	0.29	0.35	0.36	0.12	0.23	0.24	0.15
% lactic acid	0.90	0.90	0.85	0.88	0.81	0.89	0.96	0.86	0.95	1.07	1.12	0.84
% acetic acid	0.12	0.15	0.11	0.12	0.16	0.22	0.19	0.20	0.12	0.29	0.23	0.31

(b)

	L.22				L.73			
Temp. (°C)	25	30	35	40	25	30	35	40
Time (h)	20	20	20	—	16	16	20	16
pH	3.6	3.6	3.6	—	3.6	3.6	3.6	3.7
S° (ml)	13.6	16.4	16.6	—	12.6	13.9	17.2	15.1
Log cfu MRS	8.7	8.6	8.6	—	9.3	9.2	9.1	8.5
Log cfu WORT	4.0	6.0	7.6	—	5.3	5.6	7.3	7.0
% L-lactic acid	0.94	1.16	1.16	—	0.22	0.22	0.45	0.50
% D-lactic acid	0.00	0.00	0.00	—	0.65	0.68	0.64	0.34
% lactic acid	0.94	1.16	1.16	—	0.87	0.90	1.09	0.84
% acetic acid	0.02	0.01	0.02	—	0.00	0.02	0.03	0.03

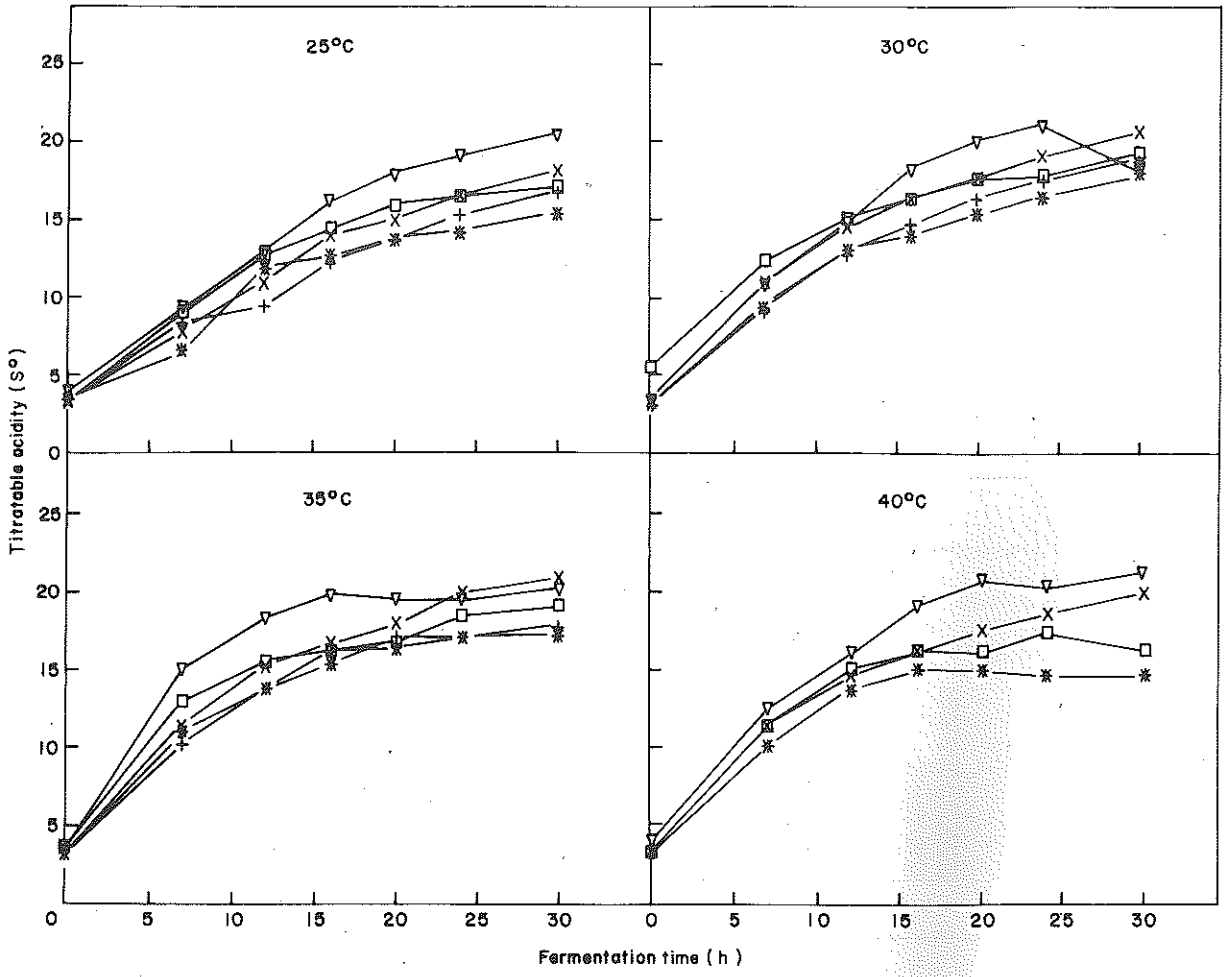

Fig. 1 Titratable acidity of sourdoughs fermented with: □, *L. sanfrancisco* (L.sf); ×, L.62; ▽, L.h; *, L.73; and +, L.22; at 25, 30, 35 and 40°C

Table 2 Compounds identified in sourdoughs with *L. sanfrancisco*, L.62, L.h, L.73 and L.22 at 25, 30, 35 and 40°C

Alcohols	Esters	Carbonyls	Others
ethanol**	ethyl acetate***	3-methyl-1-butanal^t	2-pentylfuran**
n-propanol**	ethyl *n*-propanoate^t	diacetyl*	6 unknowns
2-methyl-1-propanol***	butyl acetate**	*n*-hexanal**	
n-butanol*	2-methylbutyl acetate**	2-heptanone**	
1-penten-3-ol*	butyl *n*-propanoate^t	*n*-nonanal^t	
2/3-methyl-1-butanol***	*n*-pentyl acetate**	benzaldehyde***	
n-pentanol***	ethyl *n*-hexanoate**		
n-hexanol***	*n*-hexyl acetate***		
n-heptanol**	ethyl lactate**		
n-octanol^t	ethyl *n*-octanoate**		

Key to identification: ***, very good correlation with literature spectra; **, good correlation with literature spectra; *, reasonably sure correlation with literature spectra; and ^{t}, tentative correlation with literature spectra.

Discussion

In general S° increased fastest at 35°C and slowest at 25°C (**Fig. 1**). The fastest increase in S° at a given temperature was seen in heterofermentative cultures, especially of L.h and *L. sanfrancisco*, but the differences between species were small. The highest S° was observed in cultures of L.h. No general relationship between lactobacilli cell count and acidification of the sourdough could be seen. The increases in cell number varied between the cultures and were generally lowest at 40°C. This could possibly be due to the aerobic cultivation of sublethally damaged bacteria. The propagation of yeasts was higher in homofermentative cultures (*c.* 10^8) than in heterofermentative cultures (*c.* 10^4) evidently due to the inhibiting effect of the acetic acid content on the latter. The time at which the yeast count increased varied from batch to batch.

The sourdoughs were 'mature' after 16–20 h of fermentation, with *L. sanfrancisco* and L.73 most rapidly reaching a stabilisation of titratable acidity (**Table 1**). In relation to acid production, L.22 produced only L-lactic acid, whereas the other cultures produced varying proportions of L-lactic and D-lactic acid. The content of acetic acid was, as expected, higher in heterofermentative cultures than in homofermentative cultures. Based on weight, acetic acid accounted for the following % of total content of lactic and acetic acid: 12% in *L. sanfrancisco* cultures, 18% in L.62, 19% in L.h, 2.1% in L.73

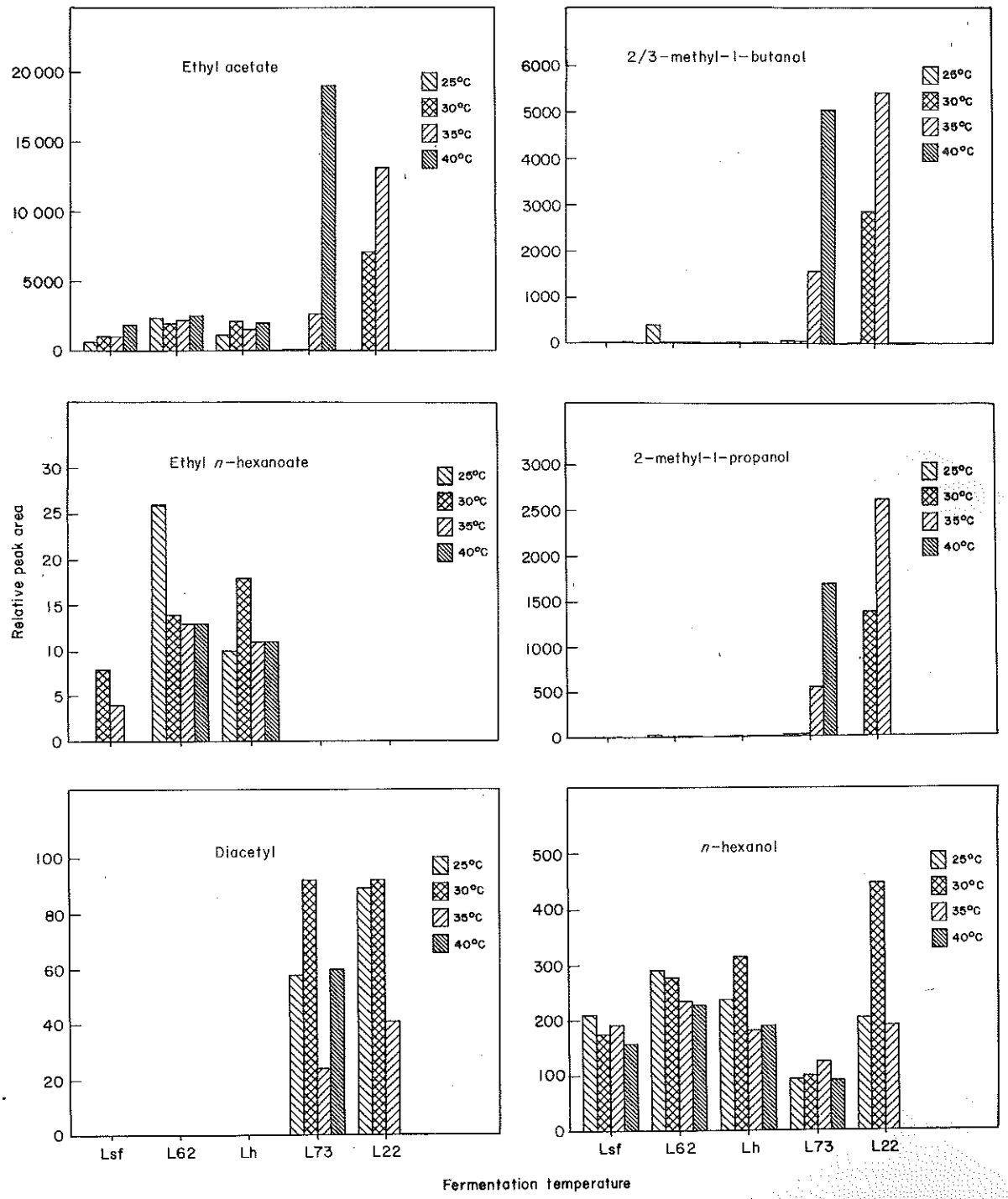

Fig. 2 Content of ethyl acetate, ethyl *n*-hexanoate, diacetyl, 2/3-methyl-1-butanol, 2-methyl-1-propanol and *n*-hexanol in sourdoughs fermented with *L. sanfrancisco* (L.sf), L.62, L.h, L.73 and L.22. Relative peak area = (peak area of compound/peak area of internal standard) × 100. Data for L.22 at 40°C are excluded due to contamination

and 1.5% in L.22 cultures. The total content of lactic and acetic acid was 0.9–1.3% (weight). These acids only accounted for *c.* 70% of the titratable acidity, with the rye wholemeal itself accounting for *c.* 10%.

The total content of volatile compounds—with the exception of the dominating content of ethanol—were generally lowest and most constant (independent of fermentation temperature) in the heterofermentative cultures, whereas it was fluctuating and less reproducible in homofermentative cultures. No direct connection with the yeast count could be seen. Of the 10 alcohols, 10 esters and 6 carbonyls identified, only the compounds with sizeable variations are shown in **Fig. 2**. The content of yeast fermentation products, ethyl acetate and the 'iso'-alcohols 2-methy-1-propanol and 2/3-methyl-1-butanol, followed each other. The two last mentioned compounds were not seen in large amounts in the heterofermentative cultures, whereas ethyl *n*-hexanoate was only seen in these, particularly in L.62 cultures. Diacetyl was only seen in the homofermentative cultures.

The starter cultures of lactobacilli themselves produced few volatile compounds, whereas the propagating yeast produced large quantities. Compounds such as *n*-hexanal, *n*-hexanol, 1-penten-3-ol, 2-pentylfuran and benzaldehyde could be oxidation products from unsaturated fatty acids originating from the rye wholemeal (11,12). The content of oxidation products in high extraction meal can be high (13).

In earlier investigations on bakery sourdoughs produced without starter cultures and under different processing conditions

(7) nearly the same compounds were found, with ethanol and ethyl acetate as the dominating peaks, and with different levels of 'iso'-alcohols and esters.

Conclusion

The five selected lactobacilli cultures (three heterofermentative and two homofermentative) did not have sharp optimum temperature intervals, but the fastest increase in titratable acidity was generally seen at 35°C. All cultures acidified the sourdoughs well at the temperatures 25, 30, 35 and 40°C (exception: one homofermentative culture at 40°C), and a stable acidification level was reached after 16–20 h of fermentation.

The titratable acidity in sourdoughs fermented with heterofermentative lactobacilli reached a higher level than sourdoughs fermented with homofermentative lactobacilli and, as expected, the content of acetic acid was highest in the former.

The most pronounced differences between the homo- and heterofermentative cultures used were the higher yeast count and content of yeast fermentation products—such as ethyl acetate and 'iso'-alcohols—in homofermentative cultures. The total amount of volatiles fluctuated with the yeast activity in homofermentative cultures being dependent on fermentation temperature. In addition to alcohols, the content of ethyl acetate, ethyl *n*-hexanoate and diacetyl varied considerably with the starter cultures used—and especially if they used a homo- or heterofermentative fermentation pathway.

Acknowledgements

This work has been financially supported by the Danish Technical Research Council. We are grateful to Chr. Hansen's Laboratories a/s, The Danish Distilleries a/s (DDSF) and Clas Lönner, University of Lund for supplying the starter cultures. We thank Annemarie Petersen for technical assistance.

References

1 Rothe, M. *Aroma von Brot.* Berlin: Akadamie-verlag (1980)
2 Schieberle, P. and Grosch, W. Identification of volatile flavour compounds of wheat bread crust—comparison with rye bread crust. *Zeitschrift für Lebensmittel-Untersuchung und Forschung,* **180**, 474–478 (1985)
3 Spicher, G. and Stephan, H. *Handbuck Sauerteig. Biologie, Biochemie, Technologie.* 2. Auflage. Hamburg: BBV, p. 351 (1984)
4 Spicher, G. and Lönner, C. Die Mikroflora des Sauerteiges. XXI. Mitteilung: Die in Sauerteigen schwedischer Bäkereien vorkommende Lactobacillen. *Zeitschrift für Lebensmittel-Untersuchung und Forschung,* **181**, 9–13 (1985)
5 Gröman, S. and Möttönen, K. Production of acids in rye flour baking. In: Holas, J. and Kratochvil, J. (Eds), *Progress in Cereal Chemistry and Technology, Developments in Food Science* 5B. Elsevier, pp. 690–1250 (1983)
6 Marková, J., Honischová, E. and Hampl, J. Aromastoffe des Brotes und der Zwischenprodukte seiner Erzeugung. *Brot und Gebäch,* **24**, 166–175 (1970)
7 Hansen, Å. and Lund, B. Volatile compounds in rye sourdough. In: Martens, M., Dalen, G. A. and Russwurm, H. Jr. (Eds), *Flavour Science and Technology.* John Wiley, p. 656 (1987)
8 ICC (International Association for Cereal Chemistry). *Approved Methods of the ICC.* Vienna, Austria (1977)
9 AOAC (Association of Official Analytical Chemists). *Official Methods of Analysis,* 14th edn. The Association (1984)
10 Poll, L. Influence of storage temperature on sensory evaluation and composition of volatiles of McIntosh apple juice. *Lebensmittel-Wissenschaft und-Technologie,* **16**, 220–223 (1983)
11 Grosch, W. Lipid degradation products and flavours. In: Morton, I. D. and Macleod, A. J. (Eds), *Food Flavours, Developments in Food Science* 3A. Elsevier, p. 460 (1982)
12 Lund, B., Hansen, Å. and Lewis, M. J. The influence of yeast and sourdough fermentation on volatile compounds in rye bread crumb. In: Munck, L. (Ed.), *Cereal Science and Technology,* Proceedings from 23rd Nordic Cereal Congress. Copenhagen: The Danish Cereal Society (1988)
13 Galliard, T. Wholemeal flour and baked products: chemical aspects of functional properties. In: Blanshard, J. M. V., Frazier, P. J. and Galliard, T. (Eds), *Chemistry and Physics of Baking.* Royal Society of Chemistry, Special Publication No. 56, p. 276 (1986)