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Microbiological Properties of Some Wheatmeal Sourdough Starters

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Summary

The microbiological properties of two Dutch commercial wheatmeal sourdough starters A (wheatmeal/water ratio 1.25, no salt) and B (wheatmeal/water ratio 1.54, 1.5% NaCl) were investigated.
A contained **Lactobacillus brevis** var. **lindneri** II and **L. sanfrancisco** (log N lactobacilli 7.7) and **Saccharomyces exiguus** (log N 7.0). In B, only **L. brevis** var. **lindneri** II (log N 9.0) was detected, and a small population of **Sacch. exiguus** (log N 1.5-4.5).
After 7 hours fermentation at 25°C, both starters reduced the dough pH till 3.8-4.0, with lactate/acetate ratios of 3.7 and 3.8, resp.
The microbiological stability of starter B against contaminations with commercial and maltose-grown baker's yeast (**Sacch. cerevisiae**) was studied. **Sacch. cerevisiae**, when added at a level equal to that of the resident sourdough yeast, could be detected after fermentation of the contaminated dough, but was eliminated after fermentation of a dough made with the contaminated dough as a starter.
Successive populations of **Lactobacillus** spp. and yeasts were identified during a 10-week development period of a new wheatmeal sourdough starter. The initial community containing **L. plantarum, L. brevis, L. büchneri, L. cellobiosus, Sacch. cerevisiae** and **Torulaspora delbrückii**, was reduced to only three species after 7 weeks (20 dough transfers), viz.: **L. brevis** var. **lindneri** II, **L. sanfrancisco** and **Torulaspora delbrückii**.

Mikrobiologische Eigenschaften einiger Weizenvollkorn-Sauerteigstarter

Zusammenfassung: Die mikrobiologischen Eigenschaften von zwei holländischen, handelsüblichen Weizenvollkorn-Sauerteigen A (Weizenvollkornmehl-/Wasserverhältnis 1,25; ohne Kochsalz) und B (Weizenvollkornmehl-/Wasserverhältnis 1,54; 1,5% NaCl) sind untersucht worden.
A enthielt **Lactobacillus brevis** var. **lindneri** II und **L. sanfrancisco** (log N Lactobacillen 7,7) und **Saccharomyces exiguus** (log N 7,0). In B wurden nur **L. brevis** var. **lindneri** II (log N 9,0) und eine unbedeutende Population von **Sacch. exiguus** (log N 1,5-4,5) entdeckt.
Die pH-Werte der Teige wurden durch die beiden Starter nach 7 Stunden Fermentationszeit bei 25°C auf 3,8-4,0 herabgesetzt (Laktat/Acetat-Verhältnisse 3,7 bzw. 3,8).
Die mikrobiologische Stabilität des Starters B gegen Kontamination durch handelsübliche und auf Maltose gezüchtete Bäckerhefe (**Sacch. cerevisiae**) wurde getestet.
Falls Sauerteig mit **Sacch. cerevisiae** im Mengenverhältnis der resistenten Sauerteighefe beimpft wurde, konnte **Sacch. cerevisiae** nach Ablauf der Fermentation noch nachgewiesen werden. Wenn aber dieser Teig als Starter für die nächste Fermentation benützt wurde, war **Sacch. cerevisiae** nach Ablauf dieser Fermenta-

tion verschwunden.
Aufeinanderfolgende Populationen von **Lactobacillus**- und Hefe-Arten wurden in frischem Weizenmehl-Sauerteig während einer Reifungszeit von 10 Wochen nachgewiesen. Die Anfangsgemeinschaft enthielt **L. plantarum, L. brevis, L. büchneri, L. cellobiosus, Sacch. cerevisiae** und **Torulaspora delbrückii**. Sie wurde nach 7 Wochen (20 Teigbereitungen) auf nur drei Arten, nämlich **L. brevis** var. **lindneri II, L. sanfrancisco** und **Torulaspora delbrückii**, reduziert.

Introduction

Microbiological, chemical and technological aspects of the use of sourdough starters as leavening agents in breadmaking have been reviewed recently [1,2]. Central European sourdough starters are traditionally based on rye meal or flour as a major raw material. German rye-based natural and commercially prepared sourdough starters were reported [3,4] to contain variable and diverse microbial communities including homo- and heterofermentative lactobacilli (**L. casei, L. fermentum, L. plantarum, L. acidophilus, L. fructivorans, L. farciminis, L. brevis** var. **lindneri, L. brevis**), **Pediococcus** spp. and yeasts (**Sacch. cerevisiae, Candida krusei, Torulopsis holmii, Pichia saitoi**).
On the other hand, wheat-based sourdough starters, employed in e.g. the manufacture of San Francisco sourdough bread, were reported to contain a microbial community limited to one **Lactobacillus** sp., for which the name **L. sanfrancisco** was suggested [5] and two yeasts, viz.: the imperfect stage of **Sacch. exiguus** (= **Torulopsis holmii**), and **Sacch. inusitatus** [6], the latter now being classified as **Sacch. cerevisiae** [7].
Sourdough breadmaking in the Netherlands is carried out at a relatively small scale, sometimes in conjunction with the manufacture of yeast-leavened products. Little is known concerning the microbiological composition of the sourdough starters used in the Netherlands, which are prepared entirely with wheatmeal and water (sometimes salt is added) and can be maintained during several years by regular dough transfers. In addition, the use of sourdough starters alongside with baker's yeast prompted an investigation of the microbiological stability of a sourdough starter against contaminations with baker's yeast.

Materials and Methods

The sourdough starters used in this study were obtained from commercial sourdough bakeries and had both been maintained by 2-6 transfers per week during several years.
During the experiments, they were transferred using similar conditions, including the use of the same batches of wheatmeal as used in the respective bakeries of origin.
Sourdough starter A was made with 50 g whole wheatmeal, 40 mL tapwater of 30°-35°C and 5 g of previous starter. Sourdough starter B was made with 50 g whole wheatmeal, 0.75 g NaCl, 32.5 mL tapwater of 30°-35°C and 5 g of previous starter. Both starters were fermented at 25°C for 7 hours before use or storage. Storage was at 4°C; new starters were prepared by the above procedure every 2-4 days.
Microbiological counts were performed using the following media and conditions: **total aerobic count**: plate count agar (Oxoid CM 325), 3 days at 30°C; **total anaerobic count**: Schaedler agar (Oxoid CM 437), 3 days at 30°C in anaerobic jars; **Lactobacillus** spp.: Rogosa agar (Oxoid CM 627) with pH 5.4 and 10 ppm filter-sterilised cycloheximide (Pfalz and Bauer), and/or M.R.S. agar (Oxoid CM 361) + 1,000 ppm filter-sterilised pimaricine (Natamycin, Gist-Brocades), 3-4 days at 30°C (overlay and aerobic incubation); **yeasts and moulds**: oxytetracyclin-glucose-yeast extract agar base (Oxoid CM 545) + 100 ppm filter-sterilised oxytetracyclin (Terramycin, Pfizer), and/or modified rose bengal medium [8], and/or wort agar (Oxoid CM 247), 3-5 days at 25°C.
Identification of **Lactobacillus** spp. was based on morphology of Gram-stained smears, catalase reaction, ability to grow at 15°C, 30°C, and 45°C, gas-production from glucose, and assimilation of 49 carbohydrates (API 50 CH). Identification of yeast spp. according to Lodder [9] was based on morphology, capability of sporulation on vegetable juice agar, yeast extract-malt extract (YM) agar, or malt extract (ME) agar,

assimilation of 19 carbon sources (API 20 C-aux), and ability to grow in the presence of 10 ppm cycloheximide (actidione). Enzymatic assays of L-lactic acid, D-lactic acid and acetic acid were carried out with Boehringer test-kits cat. no. 139084, 106941, and 148261, resp.

Results and Discussion

Microflora of commercial starters

Table 1 summarises the major microbial components of the investigated sourdough starters. Counts below 10/g were obtained for Leuconostocs, Streptococci, Staphylococci, Micrococci, coliform bacteria, *E. coli* and bacterial endospores, and were not included in this table. In contrast to the diverse microflora present in the wheatmeal used as a raw material for their preparation and maintenance, the sourdough starters contained only a limited number of dominant populations. Lactobacilli were found in large numbers in both starters, and their fastidious character is clearly shown by their poor growth on plate count agar or Schaedler agar.
On the other hand, yeasts were found in smaller numbers in the relatively dry and salt-containing starter B, whereas they were abundant in starter A.
Nevertheless, leavening activities of both A and B were of the same order of magnitude (not shown here), indicating that the lactobacilli must contribute significantly to the production of leavening gas during the fermentation.
Some characteristics of the **Lactobacillus** spp. isolated from sourdough starters **A** and B are presented in Table 2. Based on their morphology, growth at different temperatures, and ability to assimilate carbohydrates, only two species could be distinguished, viz.: **L. brevis** var. **lindneri** II, and **L. sanfrancisco**. All isolates were classified as heterofermentative although some did not produce gas in Gibson's semi-solid tomato-juice medium [10] which is generally used to detect heterofermentative strains. However, all isolates produced gas abundantly in M.R.S.-broth.
Growth of isolates in M.R.S.-broth was initially slow but improved after transfer; their growth on M.R.S.-agar was poor, also with overlay or under microaerobic conditions.
Both species have been reported and their characteristics described by earlier workers: **L. brevis** var. **lindneri** II in rye sourdough [11], and **L. sanfrancisco** in wheat sourdough [5].
The yeasts in sourdough starters **A** and B were predominantly **Sacch. exiguus**. Although the imperfect stage of this species (**Torulopsis holmii**) has been found in both rye [4] and wheat sourdoughs [6], the perfect stage has not been reported earlier in this type of product. The isolated strains of **Sacch. exiguus** did not assimilate maltose, and

Table 1 Dominant microflora of two aged wheatmeal sourdough starters

(log N/g)	sourdoughs	
	A[1]	B[2]
total aerobic count	7.2	3.5
total anaerobic count	7.1	3.5
Lactobacillus spp.	7.7	9.0
yeasts	7.0	1.5 - 4.5
moulds	2.2	2.1

[1] A: wheatmeal/water ratio 1.25, no salt added

[2] B: wheatmeal/water ratio 1.54, with 1.5% NaCl (based on meal weight)

Table 2 **Lactobacillus** spp. isolated from aged wheatmeal sourdough starters

	L. sanfrancisco	**L. brevis** var. **lindneri** II
Number of isolates:		
sourdough A	3	6
sourdough B	-	5
growth at: 15°C	+	+
30°C	+	+
45°C	-	-
Assimilation of carbohydrates (API 50 CH system):		
maltose	+	+
glucose	-	+
all other 47 carbohydrates	-	-

Table 3 Acid production in wheatmeal doughs, fermented 7 hours at 25°C

	A[1]	B[2]
pH	3.8	4.0
titratable acid (mL 0.1 N NaOH/10 g dough)	18.2	17.1
L-lactate (% w/w)	0.58	0.60
D-lactate (% w/w)	0.16	0.19
Acetate (% w/w)	0.20	0.21
Lactate/acetate ratio	3.7	3.8

[1]A: sourdough starter A 5 g, wheatmeal 50 g, tapwater (30°-35°C) 40 mL

[2]B: sourdough starter B 5 g, wheatmeal 50 g, NaCl 0.75 g, tapwater (30°-35°C) 32.5 mL

could grow in the presence of 10 ppm cycloheximide.

Acid production in commercial starters

Wheatmeal doughs were formulated and fermented as carried out under commercial conditions. The acid production was measured after 7 hours fermentation at 25°C. Table 3 shows that similar acidifications took place in doughs A and B. According to the standards of Spicher et al. [1] for rye sourdoughs, the acidification in doughs A and B is strong; likewise, the lactate/acetate ratio can be classified as medium, corresponding with a somewhat sour and hearty taste of the resulting bread.

Microbiological stability of commercial starter

The fate of added contaminations of **Sacch. cerevisiae** was investigated in sourdough B since this has only a small resident yeast-population and might therefore prove more sensitive than sourdough A. Contamination levels of half, and the same size of the resident yeast population were added at the time of preparing a fresh dough using the formula shown in Table 3. Counts of total yeasts, the relative proportion of **Sacch. cerevisiae** in the yeast count plates, and of **Lactobacillus** were made after 7 hours fermentation at 25°C (Table 4, fermentation time 1) and after a similar fermentation of fresh doughs

Table 4 Contamination of sourdough starter B with bakers' yeast

		ratio **Sacch. cerevisiae** : **Sacch. exiguus**		total yeasts (log N/g)		lactobacilli (log N/g)	
contamination level**	fermentation time***	control*	contaminated	contr.	contam.	contr.	contam.
bakers' pressyeast:							
2.5	1	0:15	0:15	3.8	3.8	9.3	9.5
	2	0:15	0:15	3.9	3.9	9.4	9.5
4.4	1	0:15	7: 8	3.7	4.3	9.0	8.9
	2	0:15	0:15	4.4	4.4	9.3	9.1
Maltose-grown bakers' yeast:							
2.6	1	0:15	1:14	4.4	4.2	9.3	9.2
	2	0:15	0:15	5.0	4.9	9.3	9.6
4.6	1	0:15	3:12	4.4	4.3	9.3	9.1
	2	0:15	0:15	5.0	5.3	9.3	9.4

* not contaminated; ** **Sacch. cerevisiae** level added (log N/g fresh dough); *** 1 = 7 hours at 25°C and 2 = dough obtained using dough [1] as a starter, fermented 7 hours at 25°C; contr. = control; contam. = contaminated

prepared with the contaminated doughs (Table 4, fermentation time 2).
The results in Table 4 show that contamination of these magnitudes had no significant effect on the levels of total yeasts and **Lactobacillus** counts. Although the presence of **Sacch. cerevisiae** could still be detected at the end of the first fermentation, this was no longer possible after the second fermentation.
This experiment was repeated with the same strain of **Sacch. cerevisiae** grown in maltose broth prior to being used as a contaminant. The data in Table 4 indicate that prior cultivation of **Sacch. cerevisiae** in the presence of maltose did not improve its chances of survival in the sourdough.

Microbial succession in a new sourdough

To gain an insight concerning the period required to establish a limited and stable community of sourdough microorganisms, a new sourdough starter was initiated using a wheatmeal/tapwater ratio of 1.43 without salt. Table 5 summarises samples taken after 1, 3, 7, and 10 weeks, corresponding to 6, 12, 27 and 36 dough transfers, resp. The dominating yeast, **Torulaspora delbrückii**, has physiological properties similar to **Torulopsis holmii** and **Sacch. exiguus**, i.e. it cannot assimilate maltose and can grow in the presence of 10 ppm cycloheximide. Whereas **Sacch. cerevisiae** could be detected in the 1-week old sourdough as well, **Torulaspora delbrückii** remained the dominant yeast species in later stages.
During the investigated 10-week period, a succession of **Lactobacillus** populations was observed, resulting after 7 weeks in a climax community of **L. brevis** var. **lindneri** II and **L. sanfrancisco**. However, the ratios between these organisms after 7 and 10 weeks indicate that more time might be required to achieve a stable equilibrium of **Lactobacillus** populations.

Mechanisms of sourdough stability

The present data support the view expressed earlier by Wood [12] that prolonged maintenance of sourdoughs is required to establish stable communities of sourdough microorganisms. In addition there is an interesting similarity between the microbial communities of the investigated commercial starters and the 7- and 10-week-old stages of the freshly-made starter. Although in the latter starter neither **Torulopsis holmii** nor **Sacch. exiguus** were detected, the presence of a yeast with resembling properties, **Torulaspora delbrückii**, indicates a niche in this environment for maltose-negative,cycloheximide-resistant yeasts. Various factors have been proposed to contribute to the development and maintenance of stable sourdough communities, including the abscence of competition between lactobacilli and yeasts for maltose [2], the formation by lactobacilli of substances inhibitory to certain yeasts [6], and excretion by the

Table 5 Populations identified during the development of a new wheatmeal sourdough starter (wheatmeal/water ratio 1.43, no salt added)

Age (weeks) of sourdough starter	1	3	7	10
Corresponding number of dough transfers	6	12	27	36
Lactobacillus (log N/g)	9.5	9.4	9.3	9.3
Number of isolates identified:				
L. plantarum	5	7	-	-
L. brevis	2	3	-	-
L. büchneri	2	-	-	-
L. cellobiosus	1	-	-	-
L. brevis var. **lindneri** II	-	-	10	4
L. sanfrancisco	-	-	2	6
Yeasts (log N/g)	6.2	7.4	6.7	6.7
Number of isolates identified:				
Sacch. cerevisiae	3	-	-	-
Torulaspora delbrückii	5	10	10	10

yeasts of compounds promoting the growth of the lactobacilli [11].

Our experiments with **Sacch. cerevisiae** have shown that this maltose-positive, cycloheximide-sensitive organism is not able to co-exist or compete with a natural sourdough community, even after its maltose assimilation had been boosted by precultivation in a maltose broth. Although these results are not in disagreement with the factors mentioned earlier, they do not prove that resistance to antimicrobial substances is the only pre-requisite for yeasts to co-exist or compete with the sourdough community. It is to be expected that additional physiological factors including carbohydrate assimilation rates and growth kinetics of the members of different temporary and ultimate microbial populations contribute to the development of a stable microflora in sourdough as well.

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