Sucrose Metabolism and Exopolysaccharide Production in Wheat and Rye Sourdoughs by *Lactobacillus sanfranciscensis*

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The exopolysaccharide (EPS) produced from sucrose by *Lactobacillus sanfranciscensis* LTH2590 is predominantly composed of fructose. EPS production during sourdough fermentation has the potential to affect rheological properties of the dough as well as the volume, texture, and keepability of bread. Its in situ production by *L. sanfranciscensis* LTH2590 was demonstrated during sourdough fermentation after the hydrolysis of water soluble polysaccharides. In wheat and rye doughs with sucrose addition the concentration of fructose in the hydrolysate of polysaccharides was significantly higher than that in the hydrolysate of control doughs or doughs without sucrose addition. EPS production by *L. sanfranciscensis* in wheat doughs was confirmed by the determination of δ ^{13}C values of water soluble polysaccharides after the addition of naturally labeled sucrose, originating from C_3- and C_4-plants. In rye doughs, evidence for EPS production with the isotope technique could be demonstrated only by the determination of δ ^{13}C values of fructose from water soluble polysaccharides. In addition to EPS formation from sucrose, sucrose hydrolysis by *L. sanfranciscensis* in wheat and rye sourdoughs resulted in an increase of mannitol and acetate concentrations and in accumulation of glucose. It was furthermore observed that flour arabinoxylans were solublized during the fermentation.

Keywords: *Lactobacillus sanfranciscensis; exopolysaccharide; polyfructan; sourdough; arabinoxylan; carbon isotope ratio*

INTRODUCTION

Polysaccharides from plant source material and their modified forms, for example, starch, carageenans, and arabic gum, as well as microbial polysaccharides are important additives in the food industry (*1*). These polysaccharides improve the textural properties and shelf life of bread and therefore are commonly used as additives for bread production (*2*). Exopolysaccharide (EPS) production by lactic acid bacteria (LAB) during food fermentation allows the replacement of these additives (*3*). EPSs are extracellularly secreted microbial polysaccharides; their amount and chemical structure depend on the microorganism and carbon substrate. They can be classified on the basis of their composition in homo- and hetero-polysaccharides. Homo-polysaccharides consist of only one monosaccharide, such as dextran from *Leuconostoc mesenteroides* and fructan from S*treptococcus salivarius*. Hetero-polysaccharides consist of several different monosaccharides, such as xanthan produced by *Xanthomonas campestris* (*4*, *5*). The properties of EPSs depend on their molecular size, charge, monosaccharide composition, degree of branching, and types of glycosidic linkages. Whereas the application of EPS produced by lactic starter cultures is common practice in the dairy industry, the EPS production in dough in amounts relevant for bread quality has not been described. *Lactobacillus sanfranciscensis* belongs to the microbial flora of traditionally prepared wheat and rye sourdoughs (*6*). The strain *L. sanfranciscensis* LTH2590 was shown to produce EPS from sucrose. This EPS is a high molecular mass fructan homopolymer of the levan type (*7*, *8*). Among the numerous EPS-producing strains of LAB, the sourdough-related species *L. sanfranciscensis* and *Lactobacillus reuteri* contain strains known to produce homo-polysaccharides of the fructan type (*9*). Fructans such as levan, inulin, and the corresponding fructo-oligosaccharides are metabolized by bifidobacteria (*10*). Fructo-oligosaccharides are therefore applied as a prebiotic because they selectively favor the growth of bifidobacteria in the human intestinal tract. It was recently shown that intestinal bifidobacteria are also able to metabolize the high molecular weight fructan produced by *L. sanfranciscensis* LTH2590 (*8*). Analyses of batch cultures inoculated with human feces using denaturing gradient gel electrophoresis and selective culturing techniques demonstrated that fructan from *L. sanfranciscensis* LTH2590 selectively stimulated the growth of bifidobacteria. This finding is corroborated by our finding that this EPS from *L. sanfranciscensis* is degraded by several species of bifidobacteria (unpublished results).

Rye and wheat flours contain 0.8 and 0.6% sucrose, respectively (*11*), and sucrose is a common part of dough formulas. Therefore, the application of EPS-producing sourdough lactobacilli is promising to optimize the technological and nutritional properties of sourdough and bread, respectively. However, it is difficult to estimate the contribution of EPS to the total water

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Table 1. Composition of the Wheat and Rye Doughs (All Amounts Are in Grams)

	wheat doughs										rye doughs				
	W I	W II	W III	W IV	W V	W VI	W VII	W VIII	W IX	W X	R I	R II	R III	R IV	R V
flour[a]	100	100	100	100	100	100	100	100	100	100	200	200	200	200	200
water	100	100	100	100	100	100	100	100	100	100	200	200	200	200	200
C_3-sucrose[c]	0	0	4	8	12	12	0	0	0	0	0	0	0	16	0
C_4-sucrose[c]	0	0	0	0	0	0	4	8	12	12	0	16	16	0	16
chloramphenicol	0	0.02	0	0	0	0.02	0	0	0	0.02	0	0.04	0.04	0	0
erythromycin	0	0.01	0	0	0	0.01	0	0	0	0.01	0	0.02	0.02	0	0
lactic acid to pH 4	−	+	−	−	−	+	−	−	−	+	−	−	+	−	−
starter culture[b]	+	−	+	+	+	−	+	+	+	−	+	−	−	+	+

[a] Wheat flour type 550 for wheat doughs and wholemeal rye flour for rye doughs. [b] *L. sanfranciscensis* LTH2590 washed in tap water and inoculated to a cell count of 1×10^7 CFU g^{-1}. [c] Sucrose additions correspond to 20, 38, and 57 g kg^{-1} or 53, 106, and 160 mmol kg^{-1}.

soluble polysaccharides in dough because rye and wheat flours naturally contain polysaccharides. Consequently, the aim of this study was to determine the sucrose metabolism of *L. sanfranciscensis* in wheat and rye sourdoughs and to provide evidence for in situ EPS production during dough fermentation with this organism. In addition to the determination of substrates and products of microbial metabolism as well as the composition of water soluble polysaccharides, natural abundance stable isotope labeling was used to qualitatively trace sucrose metabolites of *L. sanfranciscensis* in dough.

MATERIALS AND METHODS

Organism and Culture Conditions. *L. sanfranciscensis* LTH2590 was used as the EPS-producing strain (*7*). This strain was isolated from rye sourdough as strain D1 by Böcker GmbH (Minden, Germany) and is deposited as strain LTH2590 and TMW 1.392 in the Lebensmitteltechnologie Hohenheim and Technische Mikrobiologie Weihenstephan strain collections, respectively. The organism was cultured at 30 °C in MRS (*12*) modified to meet the growth requirements of *L. sanfranciscensis* (Su-MRS). Su-MRS contained the following components per liter: peptone from casein, 10 g; yeast extract, 5 g; meat extract, 5 g; $K_2HPO_4 \cdot 3H_2O$, 2.6 g; KH_2PO_4, 4 g; cysteine−HCl, 0.5 g; NH_4Cl, 3 g; sucrose, 50 g; Tween 80, 1 mL/L. The pH was adjusted to 6.2, and the medium was sterilized at 121 °C for 20 min. Stock solutions containing $MgSO_4 \cdot 3H_2O$ (100 g/L) and $MnSO_4 \cdot 4H_2O$ (50 g/L) were autoclaved separately, and a vitamin mix containing biotin, folic acid, nicotinic acid, pyridoxal phosphate, thiamin, riboflavin, cobalamin, and panthothenic acid (0.2 g/L each) was sterilized by filtration. Each stock solution was added (1 mL/L) to the sterilized medium.

Preparation of Doughs. Wholemeal rye flour and wheat flour type 550 (ash content = 510−630 mg/100 g of wheat) were used to prepare doughs in a yield of 200 [dough yield; (mass of dough/mass of flour) × 100]. For inoculation of 200 g of dough, 10 mL of overnight culture of *L. sanfranciscensis* in Su-MRS was harvested by centrifugation at 8000*g* for 5 min, resuspended in 5 mL of physiological salt solution, and added to the dough. Control doughs were prepared with chloramphenicol (Serva) and erythromycin (Sigma) to inhibit microbial growth, and the pH was adjusted with 90% lactic acid to 4 (Riedel-deHaën). The dough formulas for wheat and rye doughs are given in Table 1. All doughs were incubated at 30 °C.

Determination of Colony-Forming Units (CFU). Cell counts were determined on mMRS4 agar (*13*). Appropriate dilution was plated using a spiral plater (IUL, Königswinter, Germany), and plates were incubated at 30 °C for 48 h under a controlled atmosphere (76% N_2, 20% CO_2, and 4% O_2).

Determination of Metabolites. Dough samples were taken for HPLC analysis. After the addition of 200% of water to the dough sample (w/w), solid constituents were removed by centrifugation at 8000*g* for 10 min. The concentrations of maltose, lactic acid, acetic acid, and ethanol in the supernatant were determined using Polyspher OA KC column (Merck); the mobile phase was 5 mmol/L H_2SO_4 and the temperature of the column 70 °C. Sucrose, glucose, fructose, xylose, arabinose, and mannitol concentrations were determined using a Polyspher CH PB column (Merck); the mobile phase was deionized H_2O and temperature of the column 80 °C. For detection a refractive index detector (Gynkotek) was used. The detection limits were 1 mmol L^{-1} for ethanol, 0.2 mmol L^{-1} for carbohydrates and mannitol, and 0.05 mmol L^{-1} for organic acids.

EPS Isolation and Purification. EPSs produced in Su-MRS were isolated after cells were removed by centrifugation and the addition of 2 volumes of chilled 95% ethanol to the supernatant. After incubation for 3 h at 4 °C, the precipitate was collected by centrifugation (8000*g*, 10 min). EPSs were dissolved in deionized water, dialyzed (molecular weight cutoff of 12000−14000, Serva) against deionized water at 4 °C for 24 h and then lyophilized. For isolation of water soluble polysaccharides and EPSs from dough, two parts of water were added to one part of dough (w/w) and after centrifugation (8000*g*, 10 min), the resulting supernatant was treated as described above. EPSs in doughs were partially purified from contaminating polysaccharides using gel permeation chromatography (GPC) at room temperature. Samples were injected on a Superdex 200 HR column (Amersham Pharmacia Biotech, Uppsala, Sweden) and eluted at a flow rate of 0.4 mL/min with 50 mM NaH_2PO_4 (pH 6) as a mobile phase. Polysaccharides were detected with a refractive index detector. Purified EPS from Su-MRS was injected on a Superdex 200 HR column to determine its elution volume. EPS from *L. sanfranciscensis* eluted at 6 mL, corresponding to an apparent molecular weight of $\sim 2 \times 10^6$ Da. Gel filtration HMW and LMW calibration kits (Amersham Pharmacia) were used for calibration of the GPC column.

Hydrolysis of Polysaccharides. Hydrolyses of dried EPS and flour polysaccharides were carried out by incubating the samples for 2 h in 1 M H_2SO_4 at 80 °C. It was verified by GPC that these hydrolysis conditions quantitatively converted EPS and flour polysaccharides to monosaccharides. Monosaccharides were determined using HPLC as described above. For determination of δ ^{13}C values of fructose in rye water soluble polysaccharides, fructose eluting from CH PB column was collected and lyophilized.

Determination of Carbon Isotope Ratio. The δ ^{13}C value of a compound indicates its content of the ^{13}C isotope relative to the V-PDB standard ($[^{13}C]/[^{12}C] = 0.0112372$). Because the ^{12}C isotope is enriched during photosynthesis, carbohydrates have negative δ ^{13}C values. In accordance with the different photosynthetic pathways employed by C_3- and C_4-plants, the δ ^{13}C values of carbohydrates from these plants differ (*14*). The ^{13}C analysis was performed on CO_2 directly obtained by quantitative combustion of the samples. δ ^{13}C values were then determined using isotope ratio mass spectrometry with a double gas inlet system, which measures in alternation the sample and a standard gas (*15*). The amount of the sample was 2−3 mg. The laboratory working standard was wheat starch (δ ^{13}C value = −24.60), which was calibrated with NIST-22 with a δ ^{13}C value of −29.80 (*16*). To determine the δ ^{13}C values of glucose and fructose in C_3- and C_4-sucrose, a solution (20 g/L) of each sucrose was hydrolyzed with 1 M H_2SO_4 and

Table 2. Concentrations of Substrates and Products in Wheat Sourdoughs (Millimoles per Kilogram)[a]

cough	time (h)	pH	sucrose	maltose	glucose	fructose	mannitol	lactate	acetate	ethanol
W I	0	6.20	3.6	17	1.6	0.2	1.1	0.0	0.0	0.0
	24	3.87	1.2	54	29	0.4	13	72	10	64
W II	0	3.96	3.5	16	2.0	1.0	1.4	50	0.0	0.0
	24	4.05	3.3	63	34	8.4	1.5	48	0.0	0.0
W III	0	6.31	46	14	0.0	0.8	0.0	0.0	0	0.0
	24	3.81	7.8	54	60	1.1	37	63	19	44
W IV	0	6.31	104	15	0.0	0.0	0.0	0.0	0.0	0.0
	24	3.78	42	49	80	6.0	43	60	21	39
W V	0	6.27	152	13	0.0	0.0	0.0	0.0	0.0	0.0
	24	3.85	75	50	82	6.6	43	61	24	36
W VI	0	3.96	159	13	0.0	0.0	0.0	55	0.0	0.0
	24	3.84	130	39	33	14	0.0	57	0.0	0.0

[a] Shown are data for doughs without sucrose and with addition of C_3-sucrose. Results obtained from doughs to which C_4-sucrose was added (doughs W VII, W VIII, W XI, and W X) correspond within experimental error to the results from doughs W III, W IV, W V, and WVI, respectively.

monosaccharides were obtained after preparative separation with HPLC, lyophilized, and measured as described above.

Statistical Analysis of the Data. The overall experimental error (variation of microbial metabolic activity, flour enzymatic activity, as well as extraction and quantification of substrates and fermentation products) was determined through fermentations under identical conditions,that is, sourdoughs containing equal amounts of C_3- and C_4-sucrose. The coefficient of variation between two fermentations was generally in the range of 5−15%. The extraction of polysaccharides from dough and the determination of δ ^{13}C values were reproducible with a coefficient of variation of 2%.

RESULTS

EPS Production in Su-MRS and δ ^{13}C Values of Sucrose and EPSs Derived Thereof. To verify EPS production from sucrose by *L. sanfranciscensis* LTH2590, as well as to demonstrate the changes of δ ^{13}C values through the EPS production in doughs, δ ^{13}C values of C_3-sucrose (sugar beet), C_4-sucrose (sugar cane), and purified EPS were measured. C_3- and C_4-sucrose had δ ^{13}C values of −25.65 and −11.64, respectively. EPS consists predominantly of fructose, so it was important to know whether the δ ^{13}C value of sucrose is the same in its constituents glucose and fructose. Glucose and fructose from C_3-sucrose had δ ^{13}C values of −25.11 and −25.87, respectively, and glucose and fructose from C_4-sucrose had δ ^{13}C values of −12.23 and −11.40, respectively. Thus, the differences in δ ^{13}C values between fructose and glucose in either C_3- or C_4-sucrose are very small. The δ ^{13}C values of EPS produced by *L. sanfranciscensis* during growth in culture media with C_3- and C_4-sucrose followed by dialysis were −26.69 and −17.28, respectively. Comparison of these values with the δ ^{13}C values of sucrose shows that the ^{12}C carbon isotope was enriched in the fructose moieties used by *L. sanfranciscensis* for EPS formation, probably because of isotopic partitioning between EPS and other products of sucrose metabolism. During growth of *L. sanfranciscensis* in Su-MRS, the fructose moiety of sucrose is either incorporated into EPS or reduced to mannitol. The glucose moiety is used as carbon source or left unused and accumulated in the medium (Korakli et al., 2000, data not shown). The amount of EPS during growth of *L. sanfranciscensis* in Su-MRS was 14 g L^{-1}, corresponding to a yield of 57% based on the fructose content of sucrose. The EPS yield from sucrose and the difference between δ ^{13}C values of EPS from C_3- and C_4-sucrose were therefore large enough to expect an impact of EPS production from C_3- or C_4-sucrose in dough on the δ ^{13}C values of overall water soluble polysaccharides.

Carbohydrate Metabolism in Wheat Sourdoughs. To monitor the microbial metabolism in inoculated and control doughs, substrates and fermentation products were determined (Table 2). The concentration of metabolites in doughs with C_4-sucrose addition (doughs W VII−W X, data not shown) was equivalent to the concentration in the corresponding doughs with C_3-sucrose addition (doughs W III−W VI). It was verified by plate counts that the fermentation flora was identical with that of the starter culture. Contaminants were not detectable and thus accounted for <0.1% of the total microflora. In control doughs without inoculum, the total cell counts were $<10^4$ CFU g^{-1} throughout the fermentation, excluding an effect of microbial metabolism on carbohydrates in dough. In control wheat doughs, maltose and glucose were accumulated after 24 h in all doughs because of the amylase and α-glucosidase activities of flours. In doughs with sucrose contents of 3.5, 46, 104, and 152 mmol/kg of dough, the amounts of sucrose metabolized were 2.5, 38, 62, and 77 mmol/kg of dough, respectively. The strain LTH2590 is unable to use fructose as a carbon source but converts fructose to mannitol with concomitant oxidation of NADH to NAD^+. However, in doughs containing additional sucrose, the amount of sucrose used was greater than the amount of mannitol formed. The accumulated fructose in dough does not account for the molar difference between sucrose metabolized and mannitol produced. Therefore, the differences between sucrose consumed and metabolites formed, 2.0, 13, and 27 mmol kg^{-1} of fructose in doughs containing 20, 38, and 57 g kg^{-1} of sucrose, respectively, indicate EPS production from sucrose. Apparently increased sucrose concentration led to increased EPS production, and the proportion of sucrose hydrolyzed to the alternative end product fructose was decreased. The lactate and acetate concentrations varied between 58 and 63 and between 19 and 24 mmol/kg of dough, respectively, resulting in pH of 3.78−3.85. The metabolic activity was stopped at this pH, although substrates were still available. In dough without added sucrose, the release of fructose from flour oligo- and polysaccharides allowed for the formation of 13 mmol kg^{-1} mannitol. Enzymatic liberation of 8.4 mmol kg^{-1} fructose during the fermentation was observed in the control dough without sucrose addition (dough W II). Reduction of fructose to mannitol allows *L. sanfranciscensis* to produce acetate instead of ethanol in a molar ratio of 2 mol of mannitol to 1 mol of acetate. Accordingly, increased mannitol concentration in dough with sucrose addition was accompanied by increased

Table 3. Composition of Water Soluble Polysaccharides in Wheat Doughs

	mmol kg^{-1}			
sample	glucose	fructose	xylose	arabinose
0 h, all doughs	2.4	2.4	10.8	6.6
24 h, W I	3.3	2.0	19.5	16.8
24 h, W II	2.0	1.5	21.5	16.3
24 h, W V	1.8	14.7	18.6	16.0
24 h, W IX	1.9	17.2	20.5	16.0
24 h, W X	1.7	2.2	19.2	17.5

acetate and decreased ethanol production, and the molar ratio of mannitol to acetate was ~2:1 in all doughs. In doughs containing sucrose, glucose concentrations were substantially higher than in control dough. This indicates that a part of the glucose liberated from sucrose by *L. sanfranciscensis* was not used as carbon source but accumulated in doughs.

Composition of Water Soluble Polysaccharides in Wheat. The hydrolysis of water soluble polysaccharides following HPLC analysis demonstrated that these polysaccharides are composed of glucose, arabinose, xylose, and fructose. The concentrations of arabinose and xylose were increased during the fermentation in all doughs (Table 3). Polysaccharides in flour have a high degree of polymerization (DP); during the incubation the long chains are broken into more water soluble chains. This degradation of polysaccharides explains the increase of the concentrations of arabinose and xylose during the incubation. This increase was also observed in the control doughs with antibiotics and, therefore, this solubilization could be attributed to the activity of flour enzymes. In doughs W V and W IX with sucrose addition (Table 3) the concentration of fructose at t = 24 h in the hydrolysate of water soluble polysaccharides was substantially higher than in control doughs. In doughs W V and W IX, cereal and microbial fructans accounted for up to 30% of water soluble polysaccharides. This increased fructose content of water soluble polysaccharides indicated the production of EPS from sucrose.

δ ^{13}C Values of Wheat Dough Soluble Polysaccharides. The δ ^{13}C values of dialyzed water soluble polysaccharides from wheat sourdoughs are shown in Table 4. Addition of either C_3- or C_4-sucrose to control doughs (W VI and W X) did not affect the δ ^{13}C values of water soluble polysaccharides; therefore, shifts in δ ^{13}C values of water soluble polysaccharides are attributable to microbial conversion of sucrose to high molecular weight EPS. At t = 0 the mean of δ ^{13}C values of water soluble polysaccharides was -25.37 ± 0.16 in all doughs, as expected for C_3-plants. The difference in δ ^{13}C values between t = 0 and t = 24 h in the control doughs was -1.12 ± 0.05 (mean of the three control doughs). In fermented doughs, decreased δ ^{13}C values of water soluble polysaccharides were observed if C_3-sucrose was added. The difference was greater in the dough with 57 g kg^{-1} sucrose added (W V) compared to the dough with 20 g kg^{-1} sucrose added (W III), indicating increasing production of EPS at higher sucrose concentrations. Accordingly, in fermented doughs with C_4-sucrose addition, δ ^{13}C values of water soluble polysaccharides were higher than those of the control doughs. An enrichment of EPS from the total water soluble dough polysaccharides was achieved by GPC. Fructan from *L. sanfranciscensis* LTH2590 has a molecular mass of ~2 $\times$ 10^6 Da, and water soluble wheat polysaccharides were distributed over the range of 10^4–(5 $\times$ 10^6) Da (data not shown). The elution volume of 5–7 mL, corresponding to the elution volume of EPS produced in Su-MRS, was collected for further analysis. After this enrichment of the EPS in the water soluble polysaccharides, the differences in δ ^{13}C values of the fermented doughs with C_3 and C_4 addition relative to the control doughs were more pronounced. The δ ^{13}C value of the dough with C_3-sucrose was more negative than that without sucrose and had nearly the same δ ^{13}C value as EPS isolated from Su-MRS. Accordingly, the δ ^{13}C value was higher in dough with C_4-sucrose addition. As described above, the δ ^{13}C values of EPS from C_3- and C_4-sucrose were -26.69 and -17.28, respectively, and the δ ^{13}C value of the contaminating polysaccharides of wheat was -25.84 (Table 4). Therefore, when the same amounts of EPS in doughs with C_3- and C_4-sucrose are produced, the change in δ ^{13}C value with C_4-sucrose will be more significant than that one with C_3-sucrose.

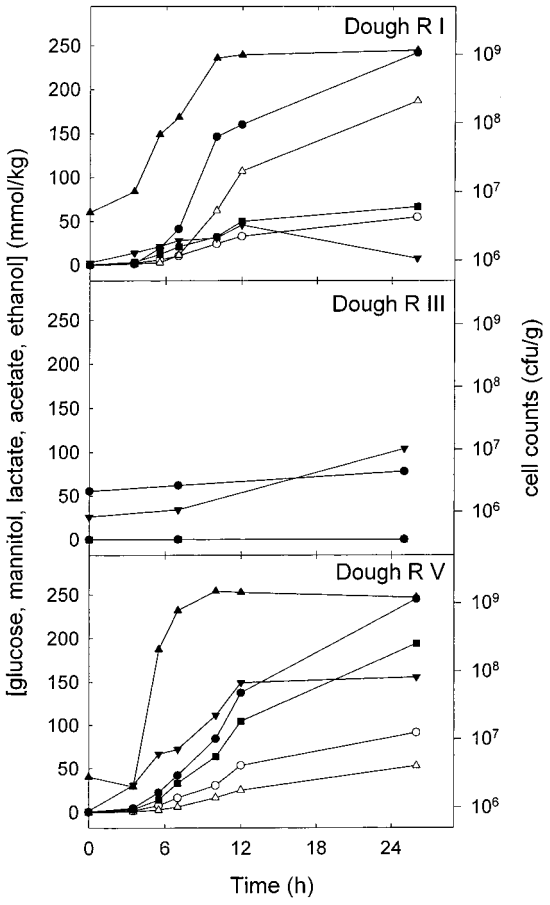

Figure 1. Kinetics of microbial growth and fermentation products in rye sourdoughs: (▼) glucose; (■) mannitol; (●) lactate; (○) acetate; (△) ethanol; (▲) CFU. Dough RI, fermented dough without sucrose addition; dough RIII, control dough with C_4-sucrose added; dough R V, fermented dough with addition of 56 g kg^{-1} C_4-sucrose.

Carbohydrate Metabolism in Rye Sourdoughs. Wholemeal rye flour has a higher pentosan and polysaccharide content than wheat flour. To estimate the influence of these polysaccharides on the sourdough fermentation, the kinetics of the main fermentation products in wholemeal rye doughs were determined. The results for doughs R I (no sucrose addition), R III, and R V (addition of C_4-sucrose) are shown in Figure 1. The kinetics of substrates and fermentation products in the dough with added C_3-sucrose (R IV) was comparable with that of C_4-sucrose, and the differences were within

Table 4. δ ^{13}C Values (‰ V-PDB) of Water Soluble Polysaccharides from Wheat Sourdoughs

	dough									
	W I	W II	W III	W IV	W V	W VI	W VII	WVIII	W IX	W X
δ ^{13}C, 0 h	−25.15	−25.29	−25.60	−25.52	−25.28	−25.35	−25.25	−25.44	−25.23	−25.60
δ ^{13}C, 24 h	−26.59	−26.30	−26.69	−26.81	−27.18	−26.54	−25.77	−25.66	−25.90	−26.75
δ $^{13}C_{24h}$ − δ $^{13}C_{0h}$	−1.44	−1.01	−1.09	−1.29	−1.90	−1.19	−0.52	−0.22	−0.67	−1.15
after enrichment of EPS by GPC										
δ ^{13}C, 24 h	−25.84	ND	ND	ND	−26.44	ND	ND	ND	−21.21	ND

Table 5. Composition of Water Soluble Polysaccharides in Rye Doughs

	mmol kg^{-1}			
sample	glucose	fructose	xylose	arabinose
0 h, all doughs	2.7	0.0	6.3	2.5
26 h, R I	0.4	0.2	30.8	17.7
26 h, R II	0.7	0.2	25.0	15.5
26 h, R III	0.4	0.6	29.2	15.4
26 h, R IV	0.2	6.8	23.4	13.8
26 h, R V	0.3	7.5	24.1	13.9

Table 6. δ ^{13}C Values (‰ V-PDB) of Water Soluble Polysaccharides and of Fructose in Water Soluble Polysaccharides Isolated from Wholemeal Rye Sourdoughs

	dough			
	R I	R III	R IV	R V
δ ^{13}C, 26 h, after dialysis	−24.65	−24.62	−23.84	−23.14
δ ^{13}C, 26 h, after enrichment with GPC	−23.69	−23.33	−23.33	−20.34
δ ^{13}C, 26 h, of fructose	−21.44	−20.06	−22.00	−19.14

experimental error (data not shown). In the control dough R III acidified to pH 4, glucose accumulation was observed (Figure 1), whereas nearly no glucose was accumulated in the control dough R II (data not shown). The initial pH of dough R II was 6.36, which fell to 6.13 after 27 h of incubation. In fermented doughs, the cell number of *L. sanfranciscensis* reached 10^9 CFU g^{-1} within 8 h, independent from sucrose addition. In wholemeal rye dough without sucrose (dough R I) more mannitol was produced than in the comparable wheat dough, and this is attributed to the higher content of fructans in wholemeal rye compared to in wheat flour type 550. In rye dough with C_4-sucrose addition (dough R V) more mannitol and acetate were produced than in dough R I, in which lactate and ethanol were the main end products. In the control dough (R III) glucose accumulation could be observed due to the activity of flour enzymes. The glucose liberated during the fermentation was higher in doughs with sucrose addition (R V).

***δ* ^{13}C Values of Rye Dough Soluble Polysaccharides.** Water soluble wholemeal rye polysaccharides have a composition comparable to that of wheat polysaccharides (Table 5). Polysaccharides from rye doughs were composed of <15% fructans. In doughs R IV and R V, the fructan concentrations were higher than in the control doughs, indicating EPS production. This could also be confirmed by determination of δ ^{13}C values of water soluble polysaccharides after dialysis (Table 6). However, the differences in δ ^{13}C values in dialyzed water soluble polysaccharides did not indicate a clear shift in δ ^{13}C values with the exception of dough R V, and even after enrichment of EPS with GPC, a significant change was observed only in the dough with C_4-sucrose (R V). The evidence for EPS production in rye doughs from C_3- and C_4-sucrose was provided by acid hydrolysis of the water soluble polysaccharides followed by preparative separation of the fructose. The δ ^{13}C value of fructan from doughs with C_3-sucrose was lower than the corresponding values of the control and the dough without sucrose addition. The difference in δ ^{13}C value of fructan in dough with C_4-sucrose compared to control dough was more significant.

DISCUSSION

Natural abundance carbon isotope labeling could be used as an analytical tool to demonstrate in situ EPS production by *L. sanfranciscensis* LTH2590 in a complex food matrix. This technique is a common tool used in ecosystem studies (*17*) and has been applied to detect food adulterations (*14*). The use of natural abundance labeling of carbon isotopes to trace metabolic fluxes in food fermentations is preferable over ^{13}C and ^{14}C carbon isotopes because the substrates are cheap, no safety precautions have to be met, and the products are suitable for sensory evaluation.

Fructan production by lactobacilli may replace the use of plant polysaccharides that are commonly incorporated in baking improvers as conditioners, crumb softeners, and antistaling agents. Furthermore, the use of EPS-producing sourdough starters will meet the strict requirements of the modern baking technology for "clean labels" and consumer demands for a reduced use of additives. The formation of a fructan from sucrose by *L. sanfranciscensis* LTH2590 was previously described (*7*), but the production of microbial polyfructan from sucrose cannot be directly demonstrated in wheat and rye doughs. The high content of polysaccharides, including fructans, in rye and wheat interferes with the quantification of microbial polysaccharides. Carbon balances of microbial metabolism are difficult to estimate in dough fermentations because maltose, glucose, and other substrates are continuously released during fermentation by cereal enzymes. Qualitatively, the use of natural ^{13}C-labeled C_3- and C_4-sucrose followed by the determination of δ ^{13}C values of water soluble polysaccharides of the dough samples demonstrated EPS production from sucrose. Two parameters may be used to estimate the amount of EPS in dough: (i) Sucrose is not formed by cereal enzymes in unstarted doughs and is hydrolyzed to a limited extend only. Sucrose metabolism by *L. sanfranciscensis* LTH2590 results in the formation of glucose and fructan or glucose and fructose. The latter is further converted to mannitol. The difference between the amount of sucrose metabolized and the amounts of fructose and mannitol formed, corrected by the mannitol production in doughs without added sucrose, therefore is attributable to EPS formation. (ii) The water soluble polysaccharides in fermented doughs without sucrose addition and in control doughs were predominantly composed of xylose and arabinose, and only small amounts of glucose and fructose were detected. An increased proportion of the fructose content in water soluble polysaccharides upon sucrose addition

is therefore attributable to fructan synthesis by *L. sanfranciscensis*. If the EPS production is calculated on the basis of the carbon balance of sucrose metabolism in dough, it can be estimated that ~5 g kg^{-1} EPS, or 1% expressed on a flour basis, was produced in wheat doughs containing 56 g kg^{-1} sucrose. The amount of EPS in the same wheat doughs calculated on the basis of the fructose content of water soluble polysaccharides can be estimated to range between 2.6 and 3.1 g kg^{-1}. The differences between the two methods for calculation relate to the difficulties in establishing an exact carbon balance for metabolism of *L. sanfranciscensis* in dough and possibly to losses of low molecular weight fructan during dialysis. Compared to an EPS yield from sucrose of 57% (fructose in EPS/fructose in sucrose) during growth of *L. sanfranciscensis* LTH2590 in Su-MRS, the EPS yield from sucrose in wheat doughs was only 15−20%, indicating that under the conditions of dough fermentation an increased proportion of sucrose is hydrolyzed to glucose and fructose rather than converted to glucose and fructan. Nevertheless, the fructan content of *L. sanfranciscensis* fermented wheat doughs exceeds that of hydrocolloids commercially applied as baking improvers. The addition of modified cellulose to sourdough bread at a level of 0.3% (flour basis) significantly affects dough rheology as well as bread texture and keepability (*2*, *18*). Although it remains to be established whether fructan produced by *L. sanfranciscensis* has an effect comparable to those of hydrocolloids commonly used in bread-making, the amount of fructan produced in dough argues in favor of an effect on textural dough and bread properties.

In wholemeal rye with a higher content of polysaccharides the evidence for EPS production through the determination of δ ^{13}C values of water soluble polysaccharides was more difficult to obtain than in wheat doughs. Rye flour contains more water soluble polysaccharides interfering with EPS determination. Evidence for EPS production in rye doughs could be provided on the basis of the fructose content of water soluble polysaccharides and by determination of the δ ^{13}C values of fructose released from these polysaccharides by acid hydrolysis.

In sourdoughs studied in this work, maltose and glucose were formed during the fermentation through starch hydrolysis by cereal amylases and then utilized by the lactobacilli. In doughs with sucrose addition, substantially more glucose was accumulated through the metabolism of sucrose by *L. sanfranciscensis* LTH2590. This accumulation of glucose may affect yeast metabolism in cocultures of yeast and lactobacilli. High glucose concentrations support the gas production by yeasts and thus contribute to dough leavening but repress maltose utilization in baker's yeast.

A correlation between acetic acid content and the shelf life of the bread was found by Salovaara et al. (*19*, *20*). On the other hand, spontaneous sourdough fermentation reportedly improved the volume, crumb grain, staling, and shelf life of bread more than doughs prepared with addition of organic acids (*21*). In rye and wheat sourdough with sucrose addition the concentration of acetate was substantially higher that in doughs without sucrose. Fructose released from sucrose is used as electron acceptor for the regeneration of NADH and results in increased acetate formation. In addition to the preservative effect of acetate, it affects the sensorial quality of the resulting bread (*22*).

In the class Monocotyledoneae the composition of the hemicelluloses in the endosperm varies greatly: wheat and rye contain mainly arabinoxylans, whereas β-glucans predominate in barley and oats (*1*). These arabinoxylans undergo a degradation process during the fermentation. The long chains of arabinoxylans exhibit less water solubility, and these chains are degraded by cereal enzymes in small more soluble chains during the sourdough fermentation. This solubilization of arabinoxylans was also reported by Escrivá et al. (*23*). These higher solubilities of arabinoxylans will play an important role in the improvement of the water binding capacity of the dough. Arabinoxylans could have furthermore a health-promoting effect according to Kontula et al. (*24*), who found that some lactobacilli isolated from human intestines such as *L. paracasei* could utilize arabinoxylan-oligosaccharides prepared from rye arabinoxylans.

In conclusion, it was shown by analysis of microbial metabolism, composition of water soluble polysaccharides, and an isotope labeling technique that fructan is produced by *L. sanfranciscensis* during sourdough fermentation. On the basis of the amount of EPS produced, 0.5−1% of flour basis, the use of EPS-producing sourdough lactobacilli in order to affect rheological properties of dough as well as the texture and shelf life of bread can be envisaged. Fructan produced during sourdough fermentation might furthermore open new vistas for novel cereal-based functional foods.

ABBREVIATIONS USED

CFU, colony-forming units; DP, degree of polymerization; EPS, exopolysaccharide; GPC, gel permeation chromatography; ND, not done; NIST, National Institute of Standards and Technology; V-PDB, Vienna International Standard Pee-Dee Belemnite.

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