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In situ production and analysis of *Weissella confusa* dextran in wheat sourdough

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ABSTRACT

Several lactic acid bacteria belonging to the genera *Leuconostoc*, *Lactobacillus*, and *Weissella* have been introduced to wheat sourdough baking for *in situ* production of exopolysaccharides. This is considered a novel method for improving the shelf-life, volume and nutritional value of bread without additives. However, *in situ* production of exopolysaccharides during sourdough fermentation is challenged by simultaneous acidification due to metabolic activities of the bacteria, which may significantly diminish the positive technological impact of exopolysaccharides. In this study, the growth, activity and *in situ* production of dextran by *Weissella confusa* VTT E-90392 in wheat sourdoughs were investigated. Furthermore, the influence of dextran-enriched sourdoughs, at the addition level of 43%, on the subsequent bread quality was established. *W. confusa* efficiently produced dextran from the added sucrose in wheat sourdough without strong acid production. A new specific enzyme-assisted method for *in situ* analysis of dextran in sourdoughs was developed. With this method, we could for the first time proof significant (11–16 g/kg DW) production of polymeric dextran in sourdoughs. Concomitant formation of shorter isomaltooligosaccharides by *W. confusa* was also detected. The produced dextran significantly increased the viscosity of the sourdoughs. Application of dextran-enriched sourdoughs in bread baking provided mildly acidic wheat bread with improved volume (up to 10%) and crumb softness (25–40%) during 6 days of storage. Hence, *W. confusa* is a promising new strain for efficient *in situ* production of dextrans and isomaltooligosaccharides in sourdoughs without strong acidification.

1. Introduction

Cereal fermentation by yeast and lactic acid bacteria (LAB), commonly referred to as sourdough or yeasted preferment, is a traditional and natural method for improving the flavour, texture and shelf-life of bakery products (Clarke and Arendt, 2005). Furthermore, recent results have demonstrated the effectiveness of fermentation in modifying the nutritional value of cereal products (Liukkonen et al., 2003). The positive influence of sourdough on the textural properties of wheat bread is based on increased acidity, which modifies the solubility of proteins, cell wall components and enzyme activities of flour (e.g. inhibits alpha-amylase and activates proteases). All these changes influence the rheological properties of dough, such as extensibility and softness (Clarke and Arendt, 2005). Utilisation of traditional sourdough, even in optimised conditions, improves bread volume and softness in general by 10–20% (Katina et al., 2006a). However, sourdough technology is still challenged by the use of baking aids such as enzymes that can improve bread volume and softness up to 50% (e.g. Katina et al., 2006b).

Microbial dextrans are used in sourdough baking. Dextrans are produced by *Leuconostoc*, *Lactobacillus* and *Weissella* species, which are found indigenously in cereals. The addition of pure dextran has been reported to have both negative (Ross et al., 1992) and positive effects on bread quality (JP-07055124b2 and U.S patent 2,983,613). Key features of dextrans for positive impact on sourdough bread quality appear to be a low degree of branching and a high molecular weight (Lacaze et al., 2007). The required molecular weight has been reported to be from 2×10^6 to about 4×10^6 Da (U.S Patent 6,399,119). The structure of dextrans (chain length, degree of branching) is dependent on the producer strain and to some extent on fermentation conditions and available nutrients (Heinze et al., 2006).

Liquid sourdough containing dextran has been reported to improve the volume (up to 12%), moist feel in the mouth and softness of rye breads and rye mixed breads (Lacaze et al., 2007). In wheat breads, dextrans have been shown to improve mouth feel and freshness after storage (Cappelle, 2004), and to increase bread volume compared with sourdough breads without dextran (Di Cagno et al., 2006). Since sourdoughs or preferments are

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generally used at the level of 10–40% (depending on the sourdough type), the content of dextran produced during fermentation should be high to obtain a technological impact on the final bread dough. However, the technological benefits of dextran produced *in situ* may be challenged by the simultaneous increase in acidity during sourdough fermentation. As shown by Kaditzky et al. (2008), the pattern and intensity of acidification can override the positive technological effects of levan produced *in situ*.

Another challenge in *in situ* production is to ensure that dextrans are produced efficiently and that their structure remains consistent from day to day. This requires a specific and reliable method for quantifying dextran in sourdough, which is currently not available. So far, dextran production has mostly been investigated by comparing the monosaccharide content and composition of the water-extracted polysaccharides from sourdoughs with those of acidified control samples (Korakli et al., 2001; Tieking et al., 2003; Di Cagno et al., 2006). This is an unspecific method and is also easily affected by changes in the water-solubility of flour polysaccharides during sourdough fermentation. Furthermore, the water-solubility of dextrans is affected by their chemical structure; e.g. dextrans with more α-(1→6) linkages are more water-soluble than dextrans with less α-(1→6) linkages (Jeanes et al., 1954).

The aim of this work was to study the potential of a novel dextran-producing *Weissella confusa* strain in wheat sourdough fermentation. In addition, we aimed to develop a simple enzyme-assisted method for direct *in situ* analysis of the amount and type of polymeric dextran present in *W. confusa* sourdoughs. The technological functionality of dextran-containing sourdough in wheat baking was also established.

2. Material and methods

2.1. Raw materials

Sourdough experiments were carried out in two series, A and B (Table 1). The characteristics of the wheat flour (Oululaisen Mylly, Finland) used in series A were: ash content 0.77% DW, protein content 13.2% and falling number 259. Those of the wheat flour (Raisio, Finland) in series B were: ash content 0.52% DW, protein content 13.6% and falling number 250. In both series, the flour samples were first heat-treated for 6 h at 100 °C prior to the sourdough fermentation in order reduce the endogenous α-amylase activity. After heat treatment, the falling number of flours A and B was 338 and 446, respectively.

Table 1
Recipes for different sourdoughs and sourdough breads.

Sourdoughs for series A	1.	2.	3.	4.
Raw materials	*g*	*g*	*g*	*g*
Heat-treated wheat flour[a]	1400	1260	1400	1260
Sucrose		140		140
Water	2100	2100	2100	2100
Target LAB number	10^6–10^7	10^6–10^7	10^6–10^7	10^6–10^7
Total	3500	3500	3500	3500
Fermentation time: 24 h				
Fermentation temperature: 25 °C	Static	Static	Static	Static
Sourdoughs for Series B	5.	6.	7.	8.
Heat-treated wheat flour[b]	1143	1236	1080	1080
Sucrose		137	120	120
Water	1799	2059	1799	1799
Fructose	57			
Target LAB[2] number	10^6–10^7	10^6–10^7	10^6–10^7	10^6–10^7
Total	3000	3433	3500	3500
Process	Static	Static	Shaking	Static
Fermentation time: 17 h				
Fermentation temperature: 25 °C				

[a] Flour was heat-treated for 6 h at 100 °C.
[b] LAB = lactic acid bacteria.

2.2. LAB strains for sourdough experiments

LAB strains were obtained from the VTT Culture Collection. Based on a preliminary screening of about 100 LAB strains originating from sourdoughs, cereals and vegetables, *Weissella confusa* VTT E-90392 (E392) was selected for the sourdough application due to its ability to produce high amounts of dextran with few branch linkages (Maina et al., 2008). *Leuconostoc mesenteroides* VTT E-062512 (NRRL B-512F) used in the commercial production of dextran was used as an EPS (exopolysaccharide)-positive control strain. In addition, lyophilised *Lactobacillus brevis* L-62 sourdough starter (Lallemand, France) was used as an EPS-negative reference strain. LAB were routinely cultivated in MRS broth (de-Man-Rogosa-Sharpe, Oxoid, Basingstoke, UK) with 2% sucrose at 30 °C for 24 h in a CO_2 atmosphere (Anaerocult C, Merck).

2.3. Preparation of sourdoughs

Eight types of wheat sourdoughs were studied in two series (Table 1). In series A ($n = 2$), *in situ* production of dextran by *W. confusa* and the technological impact of *W. confusa* sourdoughs was compared with EPS-negative *Lb. brevis* sourdoughs. The series consisted of four sourdoughs: 1) *W. confusa* fermented sourdough without sucrose (EPS-negative sourdough), 2) *W. confusa* fermented sourdough with 10% sucrose (EPS-positive sourdough), 3) *Lb. brevis* fermented sourdough (EPS-negative sourdough), and 4) *Lb. brevis* fermented sourdough with 10% sucrose (EPS-negative sourdough).

In series B, preliminary studies ($n = 1$) were carried out on the influence of mixing and shortening of the fermentation time (17 h) on the production of dextran by *W. confusa. L. mesenteroides* was used for comparison in this series. Series B consisted of four sourdoughs: 5) *W. confusa* fermented sourdough with fructose (5% of flour weight), static incubation (EPS-negative sourdough); 6) *W. confusa* fermented sourdough with 10% sucrose, static incubation, (EPS-positive sourdough); 7) *W. confusa* fermented sourdough with 10% sucrose, horizontal shaking at 150 rpm, (EPS-positive sourdough); and 8) *L. mesenteroides* fermented sourdough with 10% sucrose, static incubation (EPS-positive control sourdough). In both series, the performance of *W. confusa* sourdoughs with sucrose was compared with that of *W. confusa* sourdoughs without sucrose. In series B, sourdough 5 was supplemented with fructose (amount calculated based on the results of series A) to eliminate the possible technological impact of unused fructose in sucrose-supplemented doughs on the final bread quality.

For sourdough fermentations, *W. confusa* and *L. mesenteroides* strains were cultivated twice in succession in MRS broth (Oxoid) with 2% (w/v) sucrose. The cells were harvested from the liquid cultures by centrifugation (20,000*g*, 15 min, 8 °C), washed once with sterile saline water and re-suspended in sterile tap water for a cell density of 5×10^8–1×10^9 cfu per ml. The sourdoughs were prepared by mixing 2100 g of tap water, 1260–1400 g of wheat flour (140 g of sugar) and a cell suspension (target count 10^6–10^7 cfu/g dough) in a large beaker (5000 ml) and covered with aluminium foil. Commercial *Lb. brevis* was added as a lyophilised culture (1.2 g). The sourdoughs were fermented for 24 h at 25 °C in series A and 17 h at 25 °C in series B. Samples of the sourdoughs were stored at −20 °C and freeze-dried for later measurements of acidity, soluble arabinoxylans, dextran and sugars. Sourdoughs applied in baking were transferred to a cooling cabinet (4 °C) and used in subsequent baking without delay (maximum storage time 1 h at 4 °C).