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Maximizing the Concentrations of Wheat Grain Fructans in Bread by Exploring Strategies To Prevent Their Yeast (*Saccharomyces cerevisiae*)-Mediated Degradation

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ABSTRACT: The degradation of endogenous wheat grain fructans, oligosaccharides with possible health-promoting potential, during wheat whole meal bread making was investigated, and several strategies to prevent their degradation were evaluated. Up to 78.4 ± 5.2% of the fructans initially present in wheat whole meal were degraded during bread making by the action of yeast (*Saccharomyces cerevisiae*) invertase. The addition of sucrose to dough delayed fructan degradation but had no effect on final fructan concentrations. However, yeast growth conditions and yeast genotype did have a clear impact. A 3-fold reduction of fructan degradation could be achieved when the commercial bread yeast strain was replaced by yeast strains with lower sucrose degradation activity. Finally, fructan degradation during bread making could be prevented completely by the use of a yeast strain lacking invertase. These results show that the nutritional profile of bread can be enhanced through appropriate yeast technology.

KEYWORDS: *Fructans, wheat, yeast, Saccharomyces cerevisiae, bread making*

INTRODUCTION

Fructans are oligomers and polymers that consist mainly or exclusively of fructose.[1] Although the Codex Alimentarius Commission makes no decision on whether oligomers with a degree of polymerization (DP) in the range of 3−9 are included in the dietary fiber definition, all fructans are generally considered to be dietary fiber.[2] Furthermore, certain fructan types, such as fructo-oligosaccharides (FOS) and inulin, have a positive health effect by selectively changing the composition and/or activity of the gastrointestinal microbiota. In other words, they meet the criteria of a prebiotic.[3] The consumption of these prebiotics can have a positive effect on satiety regulation and mineral absorption and may even play a role in the prevention of colorectal cancer.[3]

The potential health benefits of fructans explain the interest in their use as functional food ingredients.[4] However, the addition of fructans to food products is not always straightforward because fructans are often degraded during food processing. When bread is enriched in inulin-type fructans, the added fructans are partly degraded by yeast during dough fermentation, and fructan losses of more than 75% have been reported.[5] The extent of this degradation strongly depends upon the average degree of polymerization (avDP) of the applied fructan mixture.[6] When inulin-type fructans are added, a higher avDP is generally preferred to maximize fructan retention. Nevertheless, fructan addition can also affect bread quality, and this effect also depends upon the DP distribution. The addition of high avDP inulin generally reduces bread volume,[7,8] while Peressini and Sensidoni[8] demonstrated that the addition of a low avDP inulin product (number avDP of 10) to certain flour types increased bread volume. Besides loaf volume, fructan addition may also affect the rheological properties of the dough, the bread crumb hardness, and crumb color.[9]

Until today, research has mainly focused on the effect of fructan addition on dough properties and bread organoleptic quality and the degradation of the added inulin-type fructans. However, wheat whole meal in itself already contains between 0.7 and 2.9% fructans,[10] and fructan concentrations of 30% on kernel dry weight basis have been reported for immature kernels.[11,12] In addition, Shimbata et al.[13] showed that fructans can form 7.2% of the dry matter of the whole meal of sweet wheat, which lacks functional granule-bound starch synthase I and starch synthase IIa. Because wheat is by far the most important fructan source in the Western diet,[14] endogenous wheat fructans may have a significant impact on gut health.[15] Despite the high consumption of wheat-based products, little information is available on the degradation of wheat grain fructans during processing. Nilsson et al.[16] observed that yeast invertase is capable of hydrolyzing wheat grain fructans, despite the fact that wheat grain fructans are thought to have a complex, branched structure with both $\beta(2-1)$- and $\beta(2-6)$-linked fructose units.[17] These authors made dough with 1% yeast (on a dry basis), followed by boiling under reflux in 80% ethanol and analysis with size-exclusion chromatography (SEC). On the basis of the SEC profiles, it was estimated that about half of the initially present fructans were degraded.[16] This implies that a significant part of wheat grain fructans and their potential nutritional benefits are lost during the bread making process. However, the high consumption of bread

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makes it worthwhile to minimize this degradation to unlock the full benefit of this promising source of dietary fiber and its potential prebiotic effects.

Therefore, this study aimed to accurately quantify the loss in wheat grain fructans during bread making and to discover promising strategies to prevent this fructan degradation. The impacts of sucrose addition to dough, the yeast growing conditions, and the used yeast strain on fructan degradation during bread making were investigated. In addition, the effect of yeast invertase was examined by analyzing dough samples made with an invertase negative yeast strain and comparing to dough samples made with the corresponding wild-type yeast.

MATERIALS AND METHODS

Materials. All chemicals, solvents, and reagents were purchased from Sigma-Aldrich (Bornem, Belgium) and were of analytical grade, unless specified otherwise. 1-Kestose was purchased from Megazyme (Bray, Ireland); FOS was a commercial product from Béghin Meiji (Thumeries, France); and yeast extract and balanced peptone were purchased from International Medical (Watermaal-Bosvoorde, Belgium). Bifurcose, isolated from wheat culm,[18] and neokestose, purified from a *Xanthophyllomyces dendrorhous* culture broth,[19] were kindly provided by Prof. W. Van den Ende (Laboratory of Molecular Plant Physiology, KU Leuven, Belgium).

Four yeast strains were used: a commercial bread strain (Koningsgist, AB Mauri, Merelbeke, Belgium), a commercial beer strain, a laboratory strain with W303 background (W303 MATa {leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 [phi+]}, further referred to as W303), and an invertase negative strain with a W303 background (W303 MATa {leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15 suc2Δ::NatMX4}, further referred to as SUC2Δ). The last three strains were kindly provided by Prof. K. Verstrepen (VIB Laboratory for Systems Biology and CMPG Laboratory for Genetics and Genomics, KU Leuven, Belgium) and Dr. J. Koschwanez (FAS Center for Systems Biology, Harvard University, Cambridge, MA). Wheat kernels (*Triticum aestivum* L. var. Fortis, 2011) were obtained from AVEVE (Landen, Belgium) and were milled on a Bühler MLU-202 laboratory mill (Uzwil, Switzerland), with a milling yield of 76.6%. The bran and short fractions were further reduced in size (<500 μm) with a Cyclotec 1093 sample mill (FOSS, Hillerød, Denmark), after which they were added to the flour fraction in their original proportions. The obtained wheat whole meal was used for the bread making experiments.

Bread Making Procedure. Dough samples were prepared according to the procedure by Shogren and Finney[20] to examine fructan degradation. Wheat whole meal (10.0 g, 14.0% moisture base), 0.53 g of compressed commercial yeast, 0.15 g of salt, and 6.10 mL of water were mixed with a 10 g pin mixer (National Manufacturing, Lincoln, NE) for 225 s. Fermentation and proofing were performed in a fermentation cabinet (30 °C and 90% relative humidity, National Manufacturing) for 90 and 36 min, respectively. Dough samples were punched after 52, 77, and 90 min of fermentation. Baking was performed for 13 min at 232 °C in a rotary oven (National Manufacturing). At several time points during the bread making process, dough samples were taken, frozen using liquid nitrogen, lyophilized, and ground with a laboratory mill (model A10, IKA-Werke GmbH and Co. KG, Staufen, Germany). The resulting lyophilized powder was analyzed for mono- and disaccharides and fructans. The same procedure was followed using different amounts of sucrose (0.20 and 0.60 g) in the dough recipe to study the effect of sucrose addition.

To study the influence of the yeast growth conditions, the used yeast strain, and yeast invertase, similar experiments were performed without salt addition because the fermentation capacity of these strains reduced markedly when salt was added. Furthermore, yeast strains grown and harvested as described below were used instead of compressed commercial yeast. Dough samples were only analyzed at the start of fermentation and after 15, 40, and 120 min of fermentation. To study the impact of the yeast growing conditions, dough samples were only analyzed at the start of fermentation and after 120 min of fermentation.

Carbohydrate Analysis. The lyophilized dough powders (50 mg) were heated in ethanol (1.0 mL, 90 °C) until all added ethanol was evaporated to inactivate yeast invertase. After the addition of 500 μL of the internal standard rhamnose (8.0 mg/mL), samples were extracted with hot water for 60 min (15 mL, 80 °C). The same extraction but with only 5.0 mL of water was executed for samples for which low sugar concentrations were expected. Carbohydrates were analyzed with high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC−PAD) on a Dionex ICS3000 chromatography system (Sunnyvale, CA). Rhamnose, glucose, fructose, sucrose, and melibiose were quantified using isocratic elution conditions (90 mM NaOH, 1.0 mL/min, 15 min), while fructan concentrations were determined after mild acid hydrolysis as described previously.[21] The avDP was calculated as the ratio of fructose and glucose released from fructan hydrolysis plus one.[21] All carbohydrate concentrations are expressed on a dry wheat whole meal basis. The elution conditions described by Vergauwen et al.[22] were used for the analysis of raffinose and the fructan distribution in dough extracts. The HPAEC−PAD retention time of 6-kestose was determined by comparison of the elution profiles to that of the sucrose hydrolysis products of yeast invertase as described by Farine et al.[23]

Yeast Growth and Growth Media. Yeast cultures were, unless specified otherwise, grown in YPD (medium with 1.0% yeast extract, 2.0% balanced peptone, and 2.0% dextrose). First, one colony was grown overnight in 5 mL of YPD at 30 °C in a shaking incubator (250 rpm). The overnight culture was then 1:200 diluted and grown in 250 mL of YPD at 30 °C, and the optical density (OD) at 595 nm was measured with a microplate reader (Bio-Rad Laboratories, Nazareth, Belgium) at several time points to follow up the yeast growth. Unless specified otherwise, yeast was harvested when it reached the stationary growth phase by centrifugation (600*g*, 3 min, room temperature, EBA 21 centrifuge, Hettich, Tuttlingen, Germany) and removal of the supernatant. The amount of water retained by the yeast pellet was taken into account when yeast was added for dough fermentation.

The growth of the W303 and SUC2Δ strains on media with different carbohydrates was assayed using the Bioscreen C (Oy Growth Curves Ab, Ltd., Helsinki, Finland). First, yeast was grown overnight at 30 °C in 5 mL of YPD. Next, this culture was 1:5 diluted with YP and then 1:50 diluted in a well plate with YP containing 2.0% of either sucrose, raffinose, FOS, or inulin (fructose/glucose ratio ≥ 25). These yeast cultures were placed in the Bioscreen C and incubated for 71 h at 30 °C with constant shaking. The OD was measured every 15 min at 600 nm.

Statistical Analysis. Statistical analysis of the results was performed using SAS software 9.3 (SAS Institute, Inc., Cary, NC). One-way analysis of variation (ANOVA) ($p < 0.05$) was performed to compare sugar concentrations in wheat whole meal and in the unyeasted control at the end of the baking process. In addition, different experiments were performed to study the effect of time and either (i) sucrose addition, (ii) yeast growing conditions, or (iii) type of yeast strain on the sugar concentration in dough. In each experiment, time and one of the three other variables were treated as fixed factors in a general linear model and a two-way ANOVA was performed. After a positive omnibus test, post-hoc analyses were conducted to detect differences among experimental settings. A Tukey multiple comparison procedure was used with a 5% family significance level.

RESULTS

Quantification of Fructan Degradation during Bread Making. Fructan degradation was studied during bread making with wheat whole meal (2.06 ± 0.05 g of fructan/100 g of dry wheat whole meal) with no or 5.3% added yeast. With 5.3% yeast addition, 78.4 ± 5.2% of the initially present fructans were degraded by the end of the baking procedure. This degradation occurred during mixing and fermentation and could be attributed to yeast activity in these phases because the decrease

Table 1. Fructan, Sucrose, Glucose, and Fructose Concentrations[a] in the Initial Wheat Whole Meal and in the Unyeasted Control at the End of the Baking Process

	fructan (g/100 g of dry meal)	sucrose (g/100 g of dry meal)	glucose (g/100 g of dry meal)	fructose (g/100 g of dry meal)
whole meal	2.06 ± 0.05 a	1.08 ± 0.14 a	0.12 ± 0.02 a	0.10 ± 0.01 a
unyeasted control	1.93 ± 0.12 a	0.81 ± 0.05 b	0.62 ± 0.01 b	0.25 ± 0.05 b

[a]Values are averages with standard deviations on at least triplicate measurements. Values within columns with the same lowercase letter are not statistically different.

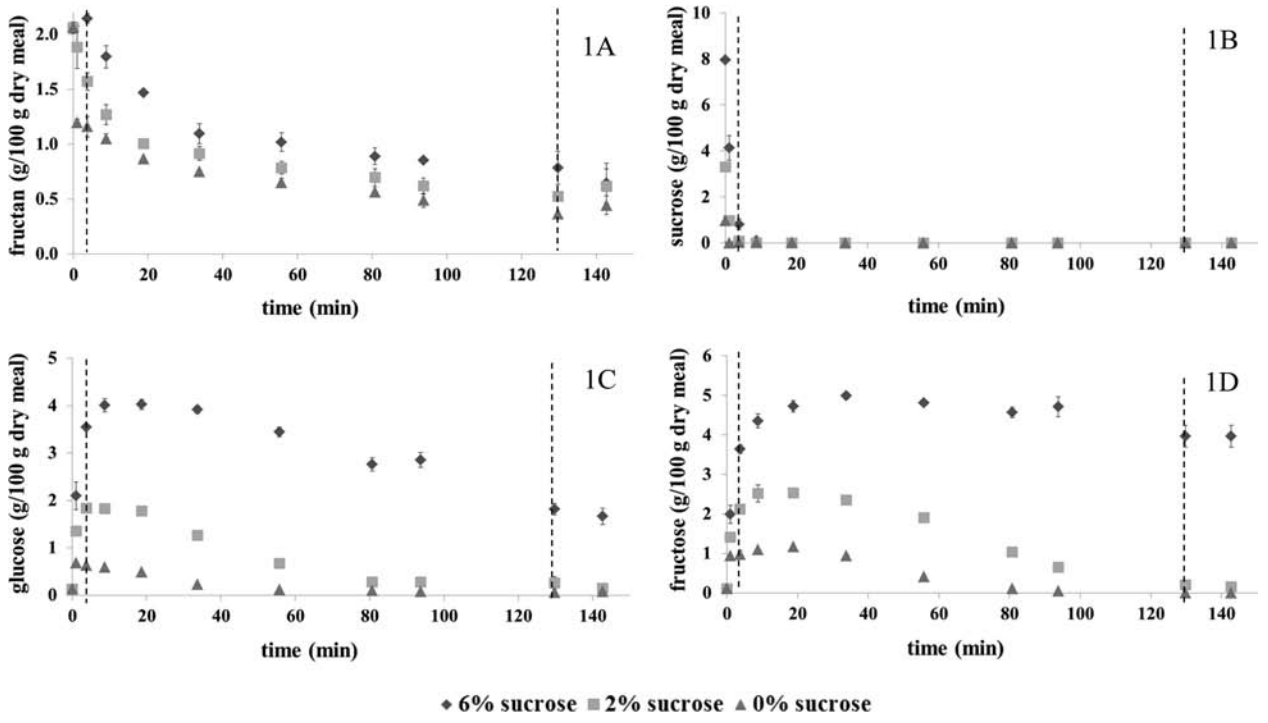

Figure 1. Carbohydrate concentrations in dough samples with different amounts of added sucrose. Evolution of (A) fructan, (B) sucrose, (C) glucose, and (D) fructose contents during bread making with 5.3% commercial yeast and either 0% (dark gray triangle), 2% (light gray square), or 6% (black diamond) added sucrose. The first time point (t = 0) was defined as the moment when mixing was started. Error bars are standard deviations on triplicate measurements. Vertical dashed lines separate the three consecutive steps of bread making: mixing, fermentation, and baking. The first time point (t = 0) shows the concentrations in the wheat whole meal or, in the case of sucrose, the theoretical concentration.

in the fructan concentration during bread making in the unyeasted control (Table 1) was not significant. However, limited enzymatic activities in the unyeasted control could not be excluded because small amounts of sucrose were degraded and glucose and fructose were set free (Table 1). The calculated average DP of fructan increased slightly during the first 30 min of fermentation (from 5 to 6) but did not change significantly thereafter.

Impact of Sucrose Addition on Fructan Degradation by Commercial Yeast. The evolution of fructans, sucrose, glucose, and fructose was studied in dough samples prepared with different concentrations of added sucrose (0, 2, and 6%) and 5.3% yeast from a commercial yeast block (Figure 1). Fructan degradation mainly took place during the first minutes of mixing in dough without added sucrose and during the first 30 min of fermentation in dough with 6% added sucrose (Figure 1A). Indeed, the more sucrose that was added, the less rapidly fructan was degraded. However, the final fructan concentration in bread was not significantly affected by sucrose addition (68.7 ± 9.3% fructan degradation with 6% sucrose addition and 78.4 ± 5.2% fructan degradation in bread making without sucrose addition). Sucrose was degraded completely during the first minutes of mixing and fermentation, even with 6% sucrose addition (Figure 1B). Simultaneously, glucose and fructose were set free at the start of fermentation, proportional to the amount of sucrose added (panels C and D of Figure 1). Later on, at around 20 min of fermentation, the glucose concentration decreased because of its consumption by yeast, while fructose was only consumed when the glucose concentration was low. The final 13 min baking step had no significant effect on the fructan concentrations.

With 2% sucrose addition, the calculated average DP of fructan in the wheat whole meal increased during the first 15 min of fermentation from 5 to 6, similar to the dough samples without added sucrose. However, the avDP did not differ significantly later on during fermentation or when 6% sucrose had been added for dough making (results not shown). Bifurcose, neokestose, and 1-kestose were degraded during dough fermentation, whereas 6-kestose was formed in the mixing step when 6% sucrose had been added (Figure 2). The formed 6-kestose was hydrolyzed again later during fermentation, and the formation of 6-kestose was not seen in dough without added sucrose.

Influence of Yeast Growing Conditions on Fructan Degradation. The W303 laboratory yeast strain was grown and harvested under four different conditions (Table 2) and

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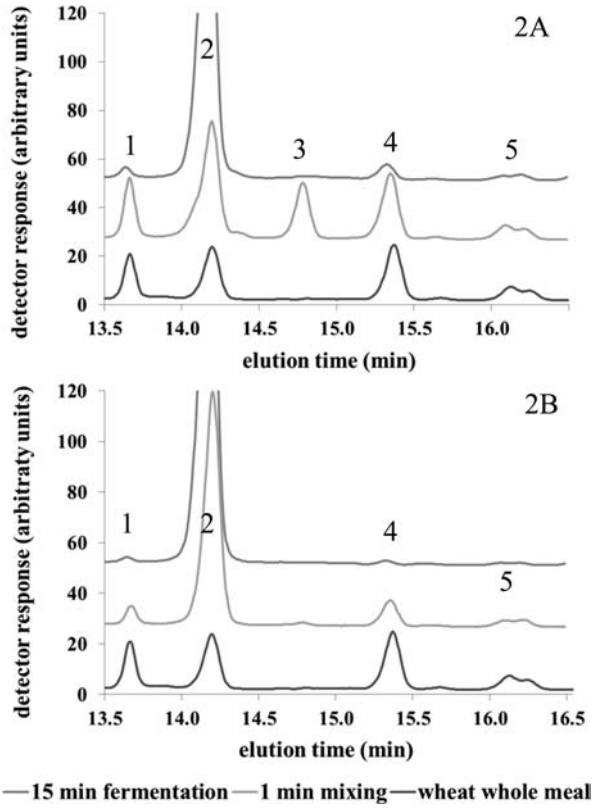

Figure 2. HPAEC–PAD profiles of dough extracts. HPAEC–PAD profiles of extracts of wheat whole meal (black line) and dough samples after 1 min of mixing (light gray line) and 15 min of fermentation (dark gray line) with either (A) 6% added sucrose or (B) no added sucrose: (1) 1-kestose, (2) maltose, (3) 6-kestose, (4) neokestose, and (5) a double fructan peak, of which the first is bifurcose.

Table 2. W303 Laboratory Strain Was Grown and Harvested under Four Different Conditions

	description of the growth conditions
I	growth in YPD, harvest in the stationary phase
II	growth in YP with 20 g of sucrose/L and 1.08 g of glucose/L, harvest in the stationary phase
III	growth in YP with 20 g of sucrose/L and 0.09 g of glucose/L, harvest in the stationary phase
IV	growth in YP with 20 g of sucrose/L and 0.09 g of glucose/L, harvest in the exponential phase and incubation for 6 h in YP with 0.09 g of glucose/L

used for dough fermentation with 3.0% added glucose, after which the evolution of fructans, sucrose, glucose, and fructose in dough was determined (Table 3). For conditions I, II, and III, the total degradation of fructans after 2 h of fermentation was each time around 25–30%. For condition IV, however, around 70% of the fructans initially present in wheat whole meal was degraded. In addition, the consumption of sucrose was more rapid than for the other three growth conditions.

Influence of the Used Yeast Strain on Fructan Degradation. The fructan degradation capacity of three yeast strains was compared. A commercial bread yeast strain, a lab strain, and a commercial beer yeast strain were grown in YPD, and their carbohydrate degradation capacities in dough with 3.0% added glucose were compared (Table 4). The commercial bread strain, W303, and the commercial beer strain degraded 78.9 ± 3.2, 26.3 ± 2.7, and 61.2 ± 4.0%, respectively, of the fructans initially present in the wheat whole meal. The rapid degradation of sucrose by the commercial yeast strains also suggests that these strains have a high invertase activity. These two strains had degraded all sucrose after the mixing step, while sucrose was still present in the dough with W303 after 40 min of fermentation. All of the used strains were good fermenters, as indicated by the almost complete consumption of glucose after 2 h of fermentation.

Impact of Yeast Invertase on Fructan Degradation. The growth and fermentation properties of the invertase negative strain, SUC2Δ, were compared to those of the corresponding wild-type yeast with W303 background on several media with different carbohydrate sources. This comparison was performed to verify the effectiveness of the SUC2 mutation in SUC2Δ and whether the wild-type yeast has a good invertase activity (Figure 3). As expected, W303 grew well on media with sucrose (Figure 3A) or raffinose (Figure 3B) as the only carbohydrate source, while SUC2Δ did not. Similarly, W303 grew well on a FOS medium in contrast to the invertase-lacking strain (Figure 3C). However, in the inulin medium, no or only a limited growth was observed for SUC2Δ and W303 (Figure 3D). The small OD increase of the medium with SUC2Δ and inulin may be due to the presence of small amounts of contaminating glucose, fructose, and sucrose in the commercial inulin sample.

Because yeast invertase appeared to play a crucial role in the degradation of inulin-type fructans during yeast growth, the invertase effect was also studied for wheat grain fructans during dough fermentation. Both the W303 and SUC2Δ were grown in YPD, harvested, and used for dough fermentation with 3.0% added glucose (Table 4). After 2 h of fermentation, the wild-type yeast had degraded 26.3 ± 2.7% of the fructans initially present in the wheat whole meal and all sucrose. The invertase negative strain on the contrary had no significant impact on the dough fructan concentration, and the effect on sucrose was of equal magnitude as for the unyeasted control (0.81 ± 0.01 and 0.81 ± 0.05 g of sucrose/100 g of dry wheat whole meal at the end of fermentation in the dough with SUC2Δ and the unyeasted control, respectively). Likewise, SUC2Δ had no effect on the raffinose concentration (0.13 ± 0.01 g of raffinose/100 g of dry wheat whole meal in both the initial wheat whole meal and the dough after 2 h of fermentation), while W303 degraded 43.8 ± 8.9% of this trisaccharide. Both W303 and SUC2Δ consumed the majority of the added glucose. In dough with W303 but not with SUC2Δ, fructose concentrations increased because of fructan hydrolysis (Table 4).

DISCUSSION

In this study, the extent of wheat grain fructan degradation during bread making was determined and several strategies were investigated to circumvent the loss of the endogenous fructans during bread making. Because almost 80% of the fructans initially present in wheat whole meal was degraded, it was indeed crucial to find strategies to prevent this degradation to benefit from the health effects of wheat grain fructans in bread.

In a first approach, the effect of sucrose addition on fructan degradation in dough was studied. Nilsson et al.[16] demonstrated that yeast invertase not only degrades sucrose but also wheat grain fructans. In theory, higher concentrations of sucrose, the primary invertase substrate, may therefore protect fructans from hydrolysis by yeast invertase. Our results indicate

Table 3. Evolution of Fructan, Sucrose, Glucose, and Fructose Contents[a] in Dough Fermented with 3.0% Added Glucose and the W303 Yeast Strain Grown under Four Different Conditions (Table 2)

fermentation time (min)	I	II	III	IV
		Fructan (g/100 g of Dry Meal)		
0	1.85 ± 0.03 Aa	1.73 ± 0.18 Aa	1.98 ± 0.05 Aa	1.43 ± 0.07 Ba
120	1.52 ± 0.02 Ab	1.43 ± 0.05 Bb	1.37 ± 0.04 Bb	0.63 ± 0.01 Cb
		Sucrose (g/100 g of Dry Meal)		
0	0.91 ± 0.02 Aa	0.67 ± 0.04 Ba	0.71 ± 0.04 Ba	0 Ca
120	0 Ab	0 Ab	0 Ab	0 Aa
		Glucose (g/100 g of Dry Meal)		
0	3.08 ± 0.18 Aa	3.03 ± 0.17 Aa	3.29 ± 0.09 Aa	3.31 ± 0.04 Aa
120	0.61 ± 0.16 Ab	0.82 ± 0.24 Ab	0.86 ± 0.10 Ab	0.12 ± 0.01 Bb
		Fructose (g/100 g of Dry Meal)		
0	0.15 ± 0.03 Aa	0.31 ± 0.09 Ba	0.20 ± 0.03 ABa	0.63 ± 0.01 Ca
120	0.46 ± 0.05 Ab	0.53 ± 0.05 Ab	0.50 ± 0.02 Ab	0 Bb

[a]Values are averages with standard deviations on triplicate measurements. Values within rows with the same uppercase letter are not statistically different, and values within columns with the same lowercase letter are not statistically different. The first time point ($t = 0$) was defined as the moment when mixing was completed.

Table 4. Evolution of Fructan, Sucrose, Glucose, and Fructose Contents[a] in Dough Fermented with Either a Commercial Bread Yeast Strain, a Commercial Beer Yeast Strain, W303, or SUC2Δ[b]

fermentation time (min)	bread strain	beer strain	W303	SUC2Δ
		Fructan (g/100 g of Dry Meal)		
0	1.44 ± 0.05 Aa	1.79 ± 0.11 Ba	1.85 ± 0.03 BCa	2.01 ± 0.05 Ca
15	1.27 ± 0.08 Ab	1.53 ± 0.02 Bb	1.88 ± 0.13 Ca	2.04 ± 0.07 Ca
40	0.99 ± 0.05 Ac	1.07 ± 0.14 Ac	1.84 ± 0.08 Ba	2.08 ± 0.04 Ca
120	0.43 ± 0.01 Ad	0.80 ± 0.06 Bd	1.52 ± 0.02 Cb	1.96 ± 0.11 Da
		Sucrose (g/100 g of Dry Meal)		
0	0 Aa	0 Aa	0.91 ± 0.02 Ba	0.99 ± 0.04 Ca
15	0 Aa	0 Aa	0.67 ± 0.05 Bb	1.01 ± 0.01 Ca
40	0 Aa	0 Aa	0.32 ± 0.02 Bc	1.00 ± 0.03 Ca
120	0 Aa	0 Aa	0 Ad	0.81 ± 0.01 Bb
		Glucose (g/100 g of Dry Meal)		
0	3.37 ± 0.06 ABa	3.56 ± 0.05 Aa	3.08 ± 0.18 Ba	3.29 ± 0.12 ABa
15	3.29 ± 0.07 Aa	3.39 ± 0.03 Ba	3.05 ± 0.16 Aa	3.13 ± 0.06 Aa
40	2.09 ± 0.12 Ab	2.22 ± 0.60 Ab	2.71 ± 0.08 Aa	2.64 ± 0.04 Ab
120	0.06 ± 0.01 Ac	0.38 ± 0.11 ABc	0.61 ± 0.16 Bb	0.50 ± 0.18 Bc
		Fructose (g/100 g of Dry Meal)		
0	0.71 ± 0.02 Ab	0.58 ± 0.09 Bb	0.15 ± 0.03 Cc	0.11 ± 0.01 Cab
15	0.84 ± 0.03 Aa	0.85 ± 0.05 Aa	0.27 ± 0.03 Bb	0.09 ± 0.01 Cb
40	0.86 ± 0.03 Aa	0.89 ± 0.03 Aa	0.43 ± 0.05 Ba	0.12 ± 0.01 Ca
120	0 Ac	0.45 ± 0.08 Bb	0.46 ± 0.05 Ba	0.12 ± 0.01 Aa

[a]Values are averages with standard deviations on triplicate measurements. Values within rows with the same uppercase letter are not statistically different, and values within columns with the same lowercase letter are not statistically different. The first time point ($t = 0$) was defined as the moment when mixing was completed. [b]All strains were grown in YPD, and dough fermentation was performed with 3.0% added glucose.

that sucrose addition indeed delays fructan degradation but has no significant impact on the final fructan concentration in bread. The fraction of fructans degraded was high (between 68.7 ± 9.3 and 78.4 ± 5.2%), irrespective of the extent of sucrose addition. Furthermore, the 13 min baking step had no influence on fructan concentrations. Fructan concentrations were already leveling off at the end of fermentation, and therefore, fructan degradation by yeast probably remained low during baking. Thermal degradation during baking is also unlikely because the temperature inside bread never exceeds 100 °C[24] and the dough pH remains above 5 during a 2 h fermentation.[25] Indeed, Courtin et al.[26] showed that FOS is stable at neutral pH and 100 °C for at least 10 min, and L'Homme et al.[27] observed the same at pH 4.0 and 90 °C.

The average fructan DP did not increase or only slightly during dough fermentation. This suggests that, in this experiment, yeast has little preference with regard to DP when it degrades wheat grain fructans, despite previous reports showing that baker's yeast has a preference for low DP fructans during the degradation of long-chain inulin-type fructans.[6,7] However, the average DP of wheat grain fructans is rather small, and fructans with a DP between 3 and 5 have probably the largest share in the DP distribution of wheat grain fructans.[21,28,29] Consequently, the average fructan DP and DP range of wheat grain fructans may be too small to induce a high DP selectivity of yeast invertase and to induce a large absolute increase of the average fructan DP during fructan degradation.

In the next part of this study, the influence of the yeast growth conditions was investigated. The SUC genes encoding yeast invertase are known to be repressed by glucose;[30] therefore, it can be expected that a higher glucose concentration in the growth medium results in less fructan

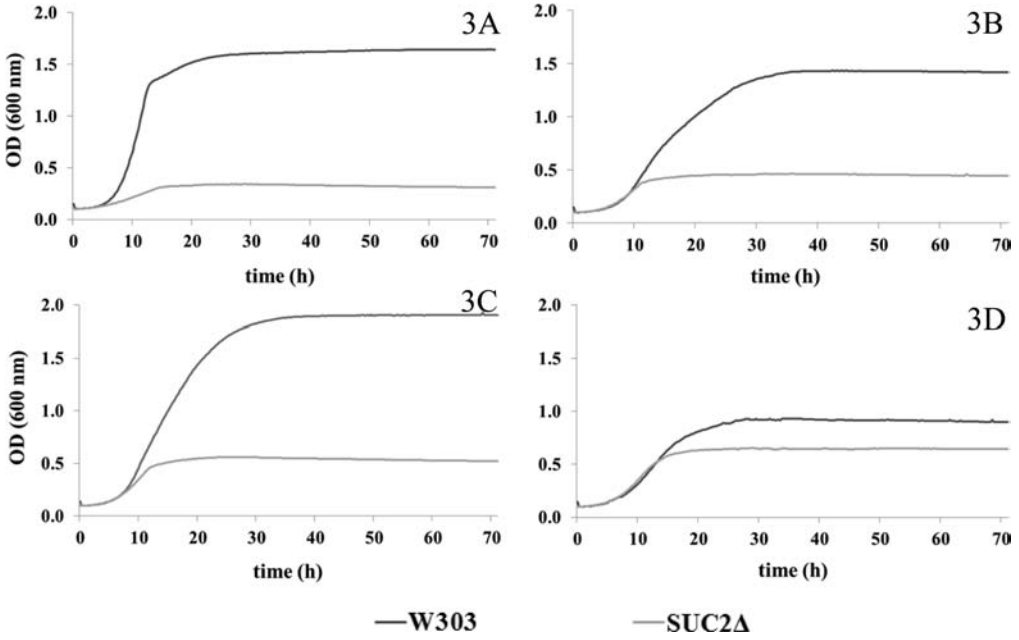

Figure 3. W303 and SUC2Δ growth on media with different carbohydrate sources. Yeast strains were grown in YP media with either (A) 2% sucrose, (B) 2% raffinose, (C) 2% FOS, or (D) 2% inulin as the only available carbohydrate source. The average OD, measured at 600 nm on four replications, is represented by the bold-colored line for W303 and by the light-shaded line for SUC2Δ.

degradation by yeast. Nevertheless, the fructan degradation capacity of W303 grown in YPD (26.3 ± 2.7%) differed little from that of the same strain grown under conditions II and III in YPS (YP medium with 2% sucrose), 30.7 ± 3.6 and 33.4 ± 3.3%, respectively. When yeast is grown in YPS until the stationary growth phase, it will have the opportunity to degrade all added sucrose to glucose and fructose and, again, high glucose concentrations are present in the medium and may have a repressive effect on invertase. To demonstrate that W303 does have the potential to hydrolyze more fructans when grown in specific conditions, we performed a final fermentation trial based on the experiments by Koschwanez et al.[31] They found that, when yeast was harvested in the exponential growth phase and incubated for 6 h in a medium containing 0.5 mM glucose, the strain displayed the highest invertase expression. In accordance with these findings, W303 grown and harvested under the described conditions degraded 69.5 ± 3.1% of the fructans initially present, which is similar to the percentage of fructan degradation seen with the commercial yeast.

Another strategy to minimize fructan degradation is the use of a yeast strain with low invertase activity. Meyer and Peters[7] already demonstrated that yeast strains may differ in their inulin degradation capacity. The current study shows that the same holds for the effect of different strains on wheat grain fructans. A 3-fold reduction was observed in the degradation of wheat grain fructans when the W303 strain was used for dough fermentation instead of the commercial bread strain. The commercial brewing strain displayed a fructan degradation capacity intermediate to that of the commercial bread strain and W303. The slower degradation of sucrose initially present in the wheat whole meal also suggested that the laboratory strain has a lower invertase activity. A low invertase activity in laboratory strains in comparison to industrial strains was also seen by Meneses and Jiranek,[32] who compared the fermentation performances of brewing, baking, and laboratory strains. High invertase activity of commercial baking strains can be explained by their extended cultivation and production in sucrose-rich molasses.[33]

The final and most promising approach to tackle fructan degradation was the use of an invertase negative yeast strain. Growth experiments with an invertase negative strain and the corresponding wild-type yeast were performed. The wild-type strain but not the invertase mutant grew well on media with sucrose, raffinose, and FOS, which suggests that, in our experiments, invertase was the sole enzyme responsible for the hydrolysis of these carbohydrates. In contrast to raffinose and FOS, inulin (fructose/glucose ratio ≥ 25) was a poor growth substrate for both yeast strains tested. The limited growth of the wild-type yeast strain on inulin confirms the earlier reported preference of yeast for short-chain inulin-type fructans.[6,34] When dough fermentation experiments were performed, yeast invertase played again a crucial role in fructan degradation. In dough made with the invertase mutant, no significant fructan degradation took place and also raffinose concentrations remained constant. At the same time, the glucose consumption was similar to that of the wild-type strain. This indicates that breads can be made without fructan degradation occurring. The fermentation capacity of the invertase negative strain is not impaired by the invertase mutation. Surprisingly, yeast not only degraded fructans but also formed 6-kestose in dough with 6% added sucrose. This result demonstrates the possibility to increase fructan concentrations in bread via the stimulation of 6-kestose production by yeast. Indeed, yeast invertase not only hydrolyzes sucrose but also has a small transferase activity.[35] Lafraya et al.[36] already proved that 6-kestose formation can be stimulated by genetic engineering of yeast strains. They increased the *in vitro* synthesis of 6-kestose by *S. cerevisiae* markedly by mutation of the SUC2 gene. Hence, if 6-kestose production can be stimulated in yeast strains with low or no invertase hydrolyzing activity, the use of these strains should allow us to increase the fructan concentration in bread.

It can be concluded that, although yeast may hydrolyze almost 80% of the endogenous wheat grain fructans, this

degradation can be prevented in several ways. This study showed that the addition of sucrose to dough may delay fructan degradation but that it had no significant impact on the final fructan concentration in bread when dough is fermented for 2 h. However, the yeast growth conditions did have a significant impact on yeast fructan degradation capacity. Besides yeast growth conditions, also yeast genotype affected fructan degradation during bread making. Fructan loss decreased significantly when a laboratory strain or a commercial beer strain was used for fermentation instead of a commercial bread strain. The most effective way to prevent fructan loss during bread making was to use an invertase negative yeast strain, which lacks invertase and, therefore, has no effect on fructan concentrations.

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