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Variability in yeast invertase activity determines the extent of fructan hydrolysis during wheat dough fermentation and final FODMAP levels in bread

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Abstract

Consumption of fructan-containing cereal products is considered beneficial for most people, but not for those suffering from irritable bowel syndrome (IBS), as they should avoid the consumption of fermentable oligosaccharides, disaccharides, monosaccharides and polyols (acronym: FODMAP). Controlling fructan levels in cereal products is not trivial. However, controlling yeast invertase-mediated hydrolysis of fructan during dough fermentation might offer a handle to modulate fructan concentrations. In this work, the variability in invertase activity and substrate specificity in an extensive set of industrial *Saccharomyces cerevisiae* strains is investigated. Analysis showed a high variability in the capacity of these strains to hydrolyse sucrose and fructo-oligosaccharides. Industrial yeast strains with a high activity towards fructo-oligosaccharides efficiently reduced wheat grain fructans during dough fermentation to a final fructan level of 0.3% dm, whereas strains with a low invertase activity yielded fructan levels around 0.6% dm. The non-bakery strains produced lower levels of CO₂ in fermenting dough resulting in lower loaf volumes. However, CO₂ production and loaf volume could be increased by the addition of 3% glucose. In conclusion, this study shows that

variation in yeast invertase activity and specificity can be used to modulate the fructan content in bread, allowing the production of low FODMAP breads, or alternatively, breads with a higher soluble dietary fibre content.

1. Introduction

Fructans are sucrose derived oligo- and polymers of fructose, containing no more than one glucose unit (Lewis, 1993). In plants, fructans serve as a reserve carbohydrate and play a role in cold, drought and salt tolerance. Fructan levels in wheat range from 0.7 to 2.9% (Huynh et al., 2008) and similar concentrations are found in spelt (Fretzdorff and Welge, 2003). The highest fructan levels among cereal grains are found in rye with levels up to 6.6% (Andersson et al., 2009). Haska et al. (2008) observed fructan levels in wheat flour that were clearly lower than those in the corresponding wheat bran fraction (Haskå et al., 2008). This suggests that whole wheat flour contains higher fructan levels compared to wheat flour, which was later confirmed by Verspreet et al., (2012) and Pejcz et al., (2019). While inulin, the most studied fructan-type, has a linear structure, wheat grain fructans have a more branched structure and belong to the graminan-type or neo-type fructans (Verspreet et al., 2015; Verspreet et al., 2017). Around 50% of the fructans in wheat flour have a degree of polymerisation (DP) of 3, 4 or 5 (Nilsson et al., 1986), with an average DP of 4 (Verspreet et al., 2017; Verspreet et al., 2012).

Cereal grain fructans are relevant from a technological as well as a nutritional point of view. Firstly, fructan is an important source of fermentable sugars during breadmaking. Struyf et al. (2017) showed that the hydrolysis products of wheat grain fructans account for a significant part of the CO₂-production during the first stages of bread dough fermentation (Struyf et al., 2017a). Fructan hydrolysis occurs during the mixing phase and dough fermentation and is mediated by yeast invertase (Verspreet et al., 2013). Second, fructan is not digested in the upper parts of the gastrointestinal tract and thus is considered dietary fibre (McCleary, 2010; Roberfroid et al., 2010). Moreover, inulin-type fructans are generally accepted as prebiotics since they have a positive health

effect by selectively stimulating beneficial gut bacteria (Roberfroid et al., 2010). Although less information is available about the effects of wheat grain fructans, it can be hypothesized that also these fructans are beneficial for health. Indeed, Jenkins et al., (2011) showed that graminan-type fructan has similar in vitro fermentation characteristics as inulin-type fructan.

For a small part of the population, fructans can cause negative health effects since they belong to the FODMAP (Fermentable Oligosaccharides, Disaccharides, Monosaccharides and Polyols) family. FODMAPs are small, osmotically active molecules that are poorly absorbed in the upper gastrointestinal tract and are rapidly fermented in the colon (Muir et al., 2019). These functional properties cause serious gastrointestinal symptoms in sensitive subjects, especially patients suffering from irritable bowel syndrome (IBS), a chronic functional gastrointestinal disorder (Gibson and Shepherd, 2010). Prevalence of IBS depends on the country and the diagnostic criteria used. Lovell and Ford (2012) compared 80 different studies and calculated an average prevalence of 11.2%. Recently, the Rome IV diagnostic criteria were introduced, which reduced the prevalence to 5% (Palsson et al., 2020; Van den Houde et al., 2019). Importantly, several studies showed that a diet low in FODMAPs can reduce the symptoms in around 70% of IBS patients (Gibson, 2017; Gibson and Shepherd, 2010; Marsh et al., 2016).

Hydrolysis of fructans is catalysed by yeast invertase, the same enzyme that also hydrolyses sucrose and raffinose. The amount of fructan hydrolysis during breadmaking ranges from 30 to 80% dependent of the yeast strain used, the fermentation time and the yeast dosage (Gélinas et al., 2016; Knez et al., 2014; Verspreet et al., 2013). In the common baker's yeast *Saccharomyces cerevisiae* (*S. cerevisiae*), invertase is encoded by a family of different, closely related *SUC* genes (Carlson and Botstein, 1983; Sainz-Polo et al., 2013). An individual strain often contains multiple *SUC* copies (paralogs) (Carlson and Botstein, 1983). The *SUC2* gene, which is the best characterized *SUC* paralog, encodes two differently regulated mRNAs, of which the larger one (1.9 kb) encodes the secreted and glycosylated form of invertase, whereas the smaller transcript (1.8 kb) encodes the intracellular,

nonglycosylated form (Carlson and Botstein, 1982). The expression of the secreted form is regulated by glucose repression, while the intracellular form is constitutively expressed at low levels (Carlson and Botstein, 1982). Secreted *Saccharomyces* invertase is believed to be a highly glycosylated octameric protein which is trapped in the cell wall due to its high molecular weight (Esmon et al., 1987; Sainz-Polo et al., 2013).

Using natural diversity in yeast invertase activity and specificity can be an interesting way to control fructan hydrolysis during breadmaking. Indeed, it was already shown that the extent of fructan hydrolysis depends on the yeast strain used for fermentation (Fraberger et al., 2018; Struyf et al., 2017b; Verspreet et al., 2013). The goal of this study was to get insight in the variability in invertase activity and specificity in yeast and to use this variability as a tool to modify fructan levels during bread fermentation. For this purpose, we examined a representative subset of industrial *S. cerevisiae* strains and investigated if invertase activity is correlated with the industrial background of the yeast. Based on invertase activity, we selected four yeast strains, analysed their performance during dough fermentation and evaluated the possibility of influencing fructan hydrolysis through yeast selection. To the best of our knowledge, this is the first study that uses invertase activity as a criterium to select strains to change fructan hydrolysis in bread. Retaining high fructan levels, and thus dietary fibre levels, in bread by selecting strains that hydrolyse less wheat grain fructans during fermentation is beneficial since dietary fibre intake, in general, is too low. Decreasing fructan levels in bread by selecting strains that can hydrolyse more wheat grain fructans during fermentation can be relevant for IBS patients. In the end, we assessed whether selected non-bakery *S. cerevisiae* strains had potential for use in breadmaking by investigating their CO₂ production capacity and their saccharide production and consumption dynamics in whole wheat flour dough.

2. Materials and methods

2.1. Materials

2.1.1. Yeast strains

One *S. cerevisiae* lab strain (*S. cerevisiae* s288c) and 27 *S. cerevisiae* strains from different industrial applications were obtained from the collection of the VIB Laboratory for Systems Biology (KU Leuven, Belgium). The set of industrial strain contains 6 beer strain (B1-B6), 3 bioethanol strains (BE1-BE3), 5 bread strains (Br1-Br5), 1 cocoa strain (Co), 3 sake strains (S1-S3), 1 spirits strain (Sp), 2 wild strains (Wi1-Wi2) and 6 wine strains (W1-W6).

2.1.2. Whole wheat flour and gluten

Winter wheat (*Triticum aestivum*) from the variety Terroir was grown at the experimental site of the Université de Liege (Agro-bio Tech, Gembloux, Belgium). After removing chaff and other impurities, roller milling with a Buhler MLU-202 laboratory mill (Buhler, AG, Uzwil, Switzerland) was used to produce white flour, shorts and bran. Shorts and bran were further reduced in size (<500 µm) using a Cyclotec 1093 sample mill (FOSS, Höganäs, Sweden). Whole wheat flour was then produced by mixing the bran, shorts and white flour fractions back together, in the proportions in which they were obtained by milling. Vital gluten was provided by Tereos Syral (Aalst, Belgium) and added to the whole wheat flour bread making recipe because the presence of bran dilutes the gluten compared to when only white flour is used and disturbs the breadmaking process, hence reducing the bread volumes.

2.2. Chemicals and reagents

Yeast extract and balanced peptone were from Lab M (Brussels, Belgium). Glucose and inulin-type fructo-oligosaccharides (FOS) were from Acros organics (Geel, Belgium) and Beneo (Orafti P95, Mannheim, Germany), respectively. All other chemicals and reagents were purchased from Sigma-Aldrich and were of analytical grade.

2.3. Whole wheat flour characterization

Moisture content was determined using AACC International Method 44-15.02 (AACC, 2000). Protein content ($N \times 5.7$) was determined using an automated Dumas protein analysis system (EAS, VarioMax N/CN, Elt, Gouda, The Netherlands) following an adaptation of the AOAC method 990.03 (AOAC, 1995). Baking absorption was determined using a Farinograph (Brabender, Duisburg, Germany) according to AACC International Method 54-21.02 (AACC, 2000). Optimal mixing time was determined with a Mixograph (National Manufacturing, Lincoln, NE) according to AACC International Method 54-40.02 (AACC, 2000).

2.4. Preparation and growth of yeast cells

Yeast cultures were grown by suspending a yeast colony in 5.0 mL of YPSuc (1.0% w/v yeast extract, 2.0% w/v balanced peptone, and 2.0% w/v sucrose). Precultures were obtained by shaking this suspension (250 rpm) overnight at 30 °C. For dough and breadmaking experiments, 2.0 mL of the preculture was used to inoculate 200 mL YPSuc in an Erlenmeyer. The culture was shaken (250 rpm) overnight at 30°C. After 16 h, the optical density (OD) at 595 nm was measured with a microplate reader (Bio-Rad laboratories, Nazareth, Belgium). If an OD of 1.2 ± 0.2 was reached, yeast cells were harvested by centrifugation (3 min, 870g) using a benchtop centrifuge (model EBA 21, Hettich Lab Technology, Massachusetts, USA) and subsequently washed with 30 mL sterile deionized water to remove residual growth media.

2.5. Measurement of yeast invertase activity

One *S. cerevisiae* lab strain (S288c) and 27 industrial *S. cerevisiae* strains were analysed for their capacity to hydrolyse sucrose and FOS. The invertase activity of whole yeast cells towards sucrose and inulin-type fructo-oligosaccharides (FOS, DP 2-8) was determined using an adapted version of the method described by Harkness et al. (2014). Yeast cultures were prepared in triplicate as described in paragraph 2.4. In the next step, the cells were diluted to the same amount of biomass. Therefore, the preculture was diluted in YP to obtain an OD₅₉₅ of 0.1 or 0.5 for experiments with

sucrose or FOS, respectively. An aliquot of this yeast suspension (200 μ L) was centrifuged (10 min, 3000g) and the pellet was washed twice with 200 μ L sodium azide (10 mM) to inactivate the yeast cells. The washed pellets were resuspended in 50 μ L sodium acetate buffer (50 mM, pH 5.0) and pre-incubated for 5 min at 50°C. Next, 12.5 μ L of a sucrose or FOS solution (150 g/L) was added and the samples were incubated for different times at 50°C. For samples containing sucrose, the reaction was stopped after 5, 10 and 15 min by adding 75 μ L of 0.2 M K_2HPO_4 . Samples containing FOS were incubated for 15, 30 and 60 min. The samples were stored on ice and properly diluted. Glucose and fructose concentration in the samples were analysed with an enzymatic method using UV-detection for the analysis of D-fructose (Megazyme, Bray, Ireland).

2.6. Dough preparation and sampling

Dough was prepared in triplicate, using three biological replicates of each yeast strain, according to an adapted version of the straight dough method described by Shogren and Finney (1984). The following formula was used: 10.0 g of whole wheat flour (on a 14.0% moisture basis), 3.0% (w/w) gluten, 1.5% (w/w) sodium chloride, 5.3% (w/w) freshly harvested yeast and 7.4 mL deionized water. The ingredients were mixed in a 10 g pin bowl mixer (National Manufacturing, Lincoln, NE, USA) for 3 min. The resulting doughs were fermented in Risograph canisters (National Manufacturing, Lincoln, NE, USA) at 30°C for 180 min. Immediately after mixing the ingredients and after 60, 120 and 180 min of fermentation dough samples were taken from each canister, frozen in liquid nitrogen, lyophilized and grounded with a laboratory mill and mortar to a powder (particle size < 250 μ m).

2.7. Breadmaking

Doughs were prepared in triplicate using three biological replicates as described in the paragraph above. Fermentation and final proofing were performed in a fermentation cabinet (National Manufacturing, Lincoln, NE, USA) at 30 °C and 90% relative humidity for 90 and 36 min, respectively. The fermenting doughs were punched at 52, 77, and 90 min. After the last punching step, doughs were moulded and placed in a baking tin for a final proofing period 36 min. Finally, the doughs were

baked for 13 min at 232°C in a rotary oven (National Manufacturing). Loaves of bread were cooled for 2 hours and then their volumes were determined with a Volscan Profiler (Stable Micro Systems, Godalming, Surrey, United Kingdom). They were frozen in liquid nitrogen, lyophilized and ground with a laboratory mill and mortar to a powder (particle size < 250 µm).

2.8. Gas production measurements

Gas production in dough as a function of fermentation time was measured using a Risograph device (National Manufacturing, Lincoln, NE, USA). Doughs were prepared as described earlier and were fermented in the Risograph canisters at 30°C for 300 min. Gas production was measured every minute. Experiments were performed in triplicate using biological replicates of each yeast strain.

2.9. Quantification of mono-, di-, trisaccharides and fructan

Mono-, di- and trisaccharides were extracted from whole wheat flour or lyophilized dough powder as described by Struyf et al. (2017). The extracts were diluted and analysed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a Dionex ICS 5000 chromatography system (Sunnyvale, CA, USA). Saccharides were separated on a CarboPac PA-100 column (4 x 250 mm), equilibrated with 90 mM NaOH, by applying a sodium acetate gradient (0–6 min, 10 mM; 6–16 min, 10–100 mM; 16–26 min, 100–175 mM). An internal standard and standard solutions were used to identify and quantify saccharides. Fructan concentrations in whole wheat flour and in the lyophilized dough powders were determined after mild acid hydrolysis as described by Verspreet et al. (2013). Measurements in wheat flour were done in triplicate. Measurements of saccharides levels in dough and bread were performed in triplicate starting from three biological replicates of each yeast strain.

2.10. Statistical analysis

Statistical analysis was done using the statistical software JMP Pro 14. Significant differences were detected by performing a one-way ANOVA followed by a comparison of the mean values using a Tukey test ($\alpha = 0.05$).

3. Results and discussion

3.1 Whole wheat flour characterisation

The wheat variety Terroir was selected because earlier research showed a high fructan level in Terroir whole wheat flour (Struyf et al., 2017a). In this study, the Terroir whole wheat flour obtained after milling had a moisture content of 13.5% and a protein content of 14.6% on dry matter (dm) base. Baking absorption and optimal mixing time were respectively 7.4 mL and 3 min. The fructan content of the whole wheat flour was 1.42 ± 0.01 % dm. This is significantly lower than the level reported by Struyf et al. (2017a), which can be explained by a different harvest year. Initial levels of sugars on dry matter base were $0.05 \pm 0.00\%$ for glucose, $0.04 \pm 0.01\%$ for fructose, $1.03 \pm 0.02\%$ for sucrose, $0.22 \pm 0.01\%$ for raffinose and $0.07 \pm 0.00\%$ for maltose.

3.2 Invertase activity and specificity of industrial *Saccharomyces cerevisiae* strains

Sucrose and FOS-hydrolysing capacities were quantified by measuring the fructose release during an incubation period of 5 min or 15 min, respectively. We first verified whether these incubation times led to a response within the linear part of the progress curve (data not shown) by measuring the fructose release at different incubation times. Inulin with a degree of polymerization (DP) ranging from 2 to 8 was used as FOS substrate, which is similar to the DP of wheat grain fructans. This substrate was selected because earlier research showed that the capacity of a yeast strain to hydrolyse fructan in dough was correlated with its FOS-hydrolysing capacity in buffer (Struyf et al., 2017b).

Figure 1 shows the invertase activity of the different *S. cerevisiae* strains towards sucrose (Fig. 1A) and FOS (Fig. 1B) as a substrate. The different yeast strains showed a wide range in invertase

activities, with fructose release per minute from sucrose varying from 0.18 ± 0.03 to 2.49 ± 0.17 g/L. Bioethanol strain 3 (BE3) released the highest amount of fructose from sucrose. The wine 3 (W3), lab (L) and cocoa (Co) strain were examples of strains with a relatively low invertase activity towards sucrose. The fructose release per minute from FOS ranged from 0.015 ± 0.002 to 0.39 ± 0.03 g/L. Concerning FOS as substrate, bioethanol strain 1 (BE1) showed a significant ($p < 0.05$) higher invertase activity than the other strains. In general, the invertase activity towards FOS was around 10 times lower than towards sucrose, suggesting that yeast strains have more difficulties to hydrolyse longer substrates. Indeed, earlier research already showed that *S. cerevisiae* invertase activity is negatively correlated with the DP of the substrate (Nilsson et al., 1987; Sainz-Polo et al., 2013).

Variation in invertase activity of the yeast strains can be explained by different factors. Firstly, the amount of *SUC* genes and the ploidy of the strain might have an impact on its invertase activity. The presence of multiple *SUC* genes can result in more invertase production (Naumov and Naumova, 2010) and the same goes for a higher ploidy. Indeed, it is suggested that amplification of *SUC* genes is an adaptive mechanism of yeasts to increase their fitness in specific industrial environments (Codón et al., 1998). Secondly, a higher expression level of a *SUC* gene might result in more invertase production and hence a higher overall activity. Finally, the *SUC* gene sequence, the genetic and the industrial background of the yeast strain also influence its invertase activity. The effect of the industrial background of a yeast strain on its invertase activity was further investigated by summarizing the data in box plots (Fig. 2). As shown in Figure 2, the mean invertase activities for both sucrose (Fig. 2A) and FOS (Fig. 2B) are significantly higher for the bioethanol and bread strains compared to the lab, cocoa, sake, spirits and wine strains used in this study. Interestingly, these differences in invertase activity correlate with the ecological niches of the different yeast strains. Wine strains are likely not selected for high invertase activity since wine must contains high amounts of glucose and fructose (Códon et al., 1998). In contrast, high invertase activity may be advantageous for bioethanol and bread strains since they are typically grown in molasses, which contains a high amount of sucrose (Códon et al., 1998). However, invertase activities also vary significantly between

strains that share the same industrial background. For example, the different bioethanol strains analysed in this study show high variability in their capacity to hydrolyse sucrose and FOS (Fig. 2).

The FOS specificity factor of the different yeast strains was calculated by dividing the fructose release per min from FOS by the fructose release per min from sucrose (Fig. 1C). By calculating a FOS specificity factor, absolute activities governed by factors such as the amount of *SUC* genes, expression level and ploidy are eliminated from the equation. As shown in Figure 1C, FOS specificity factors were ranging from 0.04 ± 0.01 to 0.24 ± 0.02 . The most striking result emerging from this data was the high FOS specificity of BE1. The FOS specificity factor of BE1 was almost 2 times higher compared to the other strains. Factors that might contribute to differences in FOS specificity are *SUC* gene sequence, genetic background and industrial background. However, based on these results, the impact of industrial background seems rather limited. This might be explained by the fact that yeast strains commonly adapt to their industrial environments by amplifying *SUC* genes (Codón et al., 1998) resulting in more *SUC* genes in a specific strain. This improves the invertase activity of a yeast strain but does not affect its substrate specificity.

Based on these results, four yeast strains with a different industrial background that could control fructan levels during dough fermentation were selected. On the one hand, BE1 and Br4 were selected since they showed high activity and specificity for FOS. Hence, using these yeast strains for breadmaking could result in more fructan hydrolysis and fructose consumption during fermentation and thus in low FODMAP breads. On the other hand, the Co and B1 strains were selected because of their low capacity to hydrolyse FOS, as we hypothesized that these yeasts degrade fewer fructans during bread fermentation, resulting in breads with more fermentable dietary fibre. Further dough and breadmaking experiments were performed to evaluate the impact of different yeast strains on the CO₂ production, the sugar dynamics and the final bread volumes.

3.2. CO₂ production profiles of the selected yeast strains in whole wheat dough

Since a sufficient CO₂ production during fermentation is important to obtain an optimal loaf volume, the CO₂ production profiles of the selected strains in Terroir whole wheat dough were determined by using a Risograph device. Van der Maelen and co-workers showed a reduced fermentation capacity of one in six *S. cerevisiae* strains (Van Der Maelen et al., 2019). However, as shown in Figure 4A, the selected strains were able to ferment in the whole wheat dough, but the CO₂ production rate in dough varied according to the yeast strain used. After 20 min of fermentation, Br4 reached a CO₂ production rate of 1.1 mL/min. Around 50 min, the CO₂ production rate dropped slightly after which it reached its maximal rate of 1.3 mL/min. Be1 also reached a CO₂ production rate of 1.1 mL/min, but only after 50 min of fermentation. A clear drop in the CO₂ production rate occurred after approximately 100 min of fermentation followed by again an increase in CO₂ production rate until 0.95 mL/min. B1 and Co both reached an initial CO₂ production rate of 0.9 mL/min. After a drop, the CO₂ production rate of B1 again increased until 1.0 mL/min whereas the CO₂ production rate of the cocoa strain kept decreasing.

As already described by Struyf et al. (2017a), a standard CO₂ production profile of a *S. cerevisiae* bakery strain consist of two stages. In a first fermentation phase, yeast cells consume their preferred sugars glucose and fructose that are both released from sucrose and fructan by the action of invertase (Struyf et al., 2017a). During this phase, Br4 and the BE1 reached a higher CO₂ production rate than the other two strains. This observation might be explained by the higher invertase activity of these strains. Indeed, a higher invertase activity results in more glucose and fructose release from sucrose and fructan and thus in more preferred fermentable sugars available for the yeast cells. This finding is consistent with that of Struyf et al. (2017b). In a second phase, yeast cells switch to maltose consumption which is typically characterized by a lag phase in CO₂ production because of the glucose repression mechanism (Higgins et al., 1999; Struyf et al., 2017a). The CO₂ production profile of Co did not show a second fermentation phase suggesting that this strain was unable to shift to maltose consumption in the timespan of this fermentation.

Overall, our results show that whereas all four yeast strains can ferment in whole wheat dough, there are some differences in CO₂ production which might lead to differences in loaf volumes of the final bread. Furthermore, Br4 was better adapted to the dough environment resulting in a faster start of the fermentation and a shorter lag phase.

3.3. Evaluation of saccharide production and consumption during fermentation of yeasted whole wheat dough

In a next set of experiments, we investigated the capacity of the selected yeast strains to ferment whole wheat doughs. During dough fermentation, samples were taken to follow up the saccharide evolution. Figure 3, panels A to E show, respectively, fructan, sucrose, maltose, fructose and glucose concentrations after mixing and during dough fermentation as a function of time for the doughs fermented with the different yeast strain.

Fructan concentrations decreased during mixing and fermentation (Fig. 3A). This decrease was more pronounced during the first hour of fermentation. As shown in Figure 3A, there was variation in the rate and the amount of fructan hydrolysed in doughs containing the different strains. BE1 and Br4 hydrolysed wheat grain fructans faster and to a greater extent than the other two strains. After 3h of fermentation, BE1 and Br4 could respectively degrade 92.3% and 82.3% of the wheat grain fructans while B1 and Co could only degrade 64.5% and 63.6%, respectively. Since no fructan was hydrolysed in the unyeasted dough, the hydrolysis of fructan can be fully attributed to yeast activity. Sucrose concentrations decreased very quickly in all the doughs (Fig. 3B). In doughs fermented with yeasts BE1 and Br4, all sucrose was already hydrolysed after mixing because of the high invertase activity of these strains. Surprisingly, also the sucrose concentration in the unyeasted dough decreased somewhat during fermentation. A possible explanation for this is the presence of low levels of contaminating enzymes in the whole wheat flour. Possible contaminating enzymes could be invertases or α -glucosidases from microbial origin. The presence and activity of microbial enzymes in wheat flour were demonstrated for arabinoxylan degrading enzymes by Dornez and co-workers

(2006). Some α -glucosidase activity was already shown to be present in flour (Struyf et al., 2016). Maltose concentrations increased quickly during mixing and the first hour of fermentation in all the dough samples (Fig. 3C) due to the action of amylases on damaged starch. In dough fermented with Br4, the maltose level decreased after two hours of fermentation because of maltose consumption. This might explain the shorter lag phase in CO₂ production as observed in Figure 4A. After 3h of fermentation, the maltose concentration in the doughs fermented with the BE1 and B1 also started to decrease, while no decrease in maltose content could be detected in dough fermented with the cocoa strain and in the unyeasted dough. This can explain the absence of a second fermentation phase (Fig. 4A) and thus supports the hypothesis that the cocoa strain is not able to consume maltose. Glucose and fructose levels increased after mixing in all the fermented doughs, but after two hours of fermentation, these sugars were almost completely consumed (Fig 3D-E). Furthermore, the fructose and glucose increase after mixing was the highest in dough fermented with BE1, followed by dough fermented with Br4. In unyeasted dough, the glucose and fructose content increased respectively from 0.04 to 0.94% dm and from 0.04 to 0.54% dm after 3h of fermentation. This increase can be attributed to sucrose hydrolysis by the contaminating enzymes in the whole wheat flour. As the glucose increase is higher than the fructose increase, there is probably also some α -glucosidase or glucoamylase activity present in the whole wheat flour, which has been observed in earlier research (Struyf et al., 2016). The small amount of raffinose in whole wheat flour was fully hydrolysed by invertase after 60 min of fermentation. After mixing, a small amount of raffinose was detected in the doughs fermented with BE1 (0.06%) and Br4 (0.08%) while raffinose levels in the doughs fermented with B1 (0.20%) and Co (0.19%) were significantly higher. These results again reflect the differences in invertase activity of the four strains.

These results can be linked with the invertase activity and the CO₂ production profiles of the selected yeast strains. As expected, the yeast strains that were selected because of their high invertase activity (BE1 and Br4) showed the fastest fructan and sucrose hydrolysis, resulting in a fast release of fructose and glucose and thus in a higher CO₂ production rate in the first fermentation phase (Fig.

4A). After 3h of fermentation, the fructan content was the lowest in the dough fermented with the strain showing the highest FOS specificity (BE1).

3.4. Evaluation of whole wheat bread made with different *S. cerevisiae* strains

Whole wheat breadmaking trials were performed to investigate how the selected strains influence the final fructan and sugar levels in bread and to correlate the CO₂ production rates with bread volumes.

Table 1A shows fructan and sugar concentrations in the resulting whole wheat breads. By using BE1 and Br4 for breadmaking, the final fructan levels were 0.31 ± 0.05 % dm and 0.28 ± 0.04 % dm, respectively. These fructan levels are around the low FODMAP cut-off value as defined by Varney et al. (2017). Significantly more fructan was measured in bread fermented with Co (0.63 ± 0.08 % dm) and B1 (0.60 ± 0.04 % dm). Sucrose concentrations are not mentioned in Table 1 since no sucrose was detected in the breads. Fructose was also almost fully consumed by the yeast cells. The glucose levels in the breads were low for all the different yeast strains. By contrast, the maltose content did show variation, with the lowest concentrations measured in the bread fermented with Br4 (0.91 ± 0.07), likely because of its fast switch to maltose consumption as discussed earlier. In the breads fermented with the BE1 and Co, the maltose levels were higher because the switch to maltose consumption did not occur during fermentation.

The specific bread volumes are depicted in Figure 4B. As expected, the bread volumes depended on the yeast strain used. The final bread volumes were correlated with the CO₂ production rate of the yeast strain during the proofing period (Fig. 4A). Figure 4A shows that the CO₂ production rate of Br4 was the highest during the proofing period. As a consequence, the doughs are still leavening when they are placed in the baking tin, resulting in a higher bread volume. Because Co cannot consume maltose, the CO₂ production had already dropped to low levels during the proofing step, resulting in a limited dough rise during proofing and thus in a lower bread volume.

To improve the bread volumes and to make them independent from the yeast strain, the breadmaking recipe was adapted by adding 3.0% glucose. In general, this increased the fermentation rate and prolonged the productive fermentation time (Fig. 4C). Consequently, there was more CO₂ production during the proofing period resulting in higher bread volumes that were independent of the used yeast strain (Fig. 4D). Since adding glucose could influence fructan hydrolysis and the final fructan and sugar levels in bread, the fructan and sugar concentrations in the breads prepared with this new recipe were determined. Table 1B shows that there was no significant difference in fructan content. However, glucose addition to the dough led to higher bread maltose, fructose and glucose levels. Glucose addition delayed the switch to maltose consumption, resulting in a limited amount of maltose consumption. Only Br4 consumed a significant amount of maltose during breadmaking. Although glucose and fructose are concomitantly used, consumption of glucose is faster when both sugars are present (Berthels et al., 2004). Hence, fructose consumption slowed down by adding glucose, resulting in a higher final fructose level. Furthermore, the fructose, as well as the glucose content, was significantly higher in the breads fermented with Br4 and BE1, again reflecting their high invertase activity. A high fructose content in the final breads is, however, not desirable as fructose in excess of glucose is considered as a FODMAP. By optimizing the amount of added glucose, excess fructose in the final bread can be prevented. Another approach is the addition of amyloglucosidase as demonstrated by Struyf et al. (2018). This enzyme releases a high amount of glucose from starch and thus can increase CO₂ production during proofing (Struyf et al., 2017). Since the inactivation temperature of amyloglucosidase (65-70°C) is higher than that of yeast (ca. 55°C), glucose will be still be released during the beginning of the baking phase without subsequent consumption by yeast (Arica et al., 1998; Struyf et al., 2018). However, strongly elevated bread glucose levels are not desirable since they might lead to a sweeter bread with a higher glycemic index.

4. Conclusion

The goal of this study was to explore the natural diversity in *S. cerevisiae* invertase activity and specificity, and to investigate if this variability can be used to modify fructan hydrolysis during dough fermentation. Our results reveal that different industrial yeast strains show different capacities to hydrolyse sucrose and FOS. Using 4 strains with varying invertase activities, we showed that this variability serves as a simple yet effective handle on fructan hydrolysis during breadmaking. Specifically, strains showing high invertase activities towards FOS, hydrolysed wheat grain fructans faster and to a greater extent, resulting in a lower final fructan level and thus a lower FODMAP level in bread. While strains with a high invertase activity towards FOS are useful to reduce FODMAP levels in bread, strains with a lower invertase activity are interesting to increase fermentable dietary fibre levels in bread by retaining more fructan during fermentation. Using non-bakery *S. cerevisiae* strains resulted in different CO₂ production profiles as well as different saccharide consumption and production dynamics compared to a reference bakery strain. Non-bakery *S. cerevisiae* strains are less adapted to the dough environment, resulting in lower CO₂ production and hence lower loaf volumes. However, these adverse effects can be prevented by the addition of glucose or amyloglucosidase as they both increase the CO₂ production during proofing and make the loaf volumes independent from the yeast strain used (Struyf et al., 2017). It should be noted that besides the selection of a yeast strain, also the choice of grain species and variety, proofing time and type of bread (e.g. white, whole wheat, sourdough) will have a significant impact on the final FODMAP content. Many factors can, hence, influence final FODMAP levels in bread, and combining elements could be an interesting way to modify these levels. Whereas our study lays the foundation the modification of FODMAP levels in fermented bakery products by using differences in invertase activity of yeasts, further research is needed to evaluate the impact of using non-bakery *S. cerevisiae* strains on end-product characteristics such as bread texture, aroma and taste.

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Figure 1: Invertase activity towards (A) sucrose and (B) fructo-oligosaccharides (FOS) of 27 industrial *Saccharomyces cerevisiae* strains and one lab strain. The invertase activity is expressed as fructose release per minute for a standardized amount of yeast cells. (C) FOS specificity factor of the investigated yeast strains calculated by dividing the fructose release from FOS (B) by the fructose release from sucrose (A). Horizontal bars represent standard deviations of biological triplicate measurements. Values with different letters are significantly different ($p < 0.05$).

Figure 2: Comparison of the invertase activity towards (A) sucrose and (B) fructo-oligosaccharides (FOS) between the different industrial yeast backgrounds. Grey dots represent the individual values for the invertase activity. Black dots represent the mean invertase activity of yeast strains from the same industrial background. Mean values with a different letter are significantly different ($p < 0.05$).

Figure 3: Fructan (A), sucrose (B), maltose (C), fructose (D) and glucose (E) concentrations in Terroir whole wheat dough fermented with the different selected yeast strains as a function of time. Saccharide concentrations were analysed after mixing ($t = 3$ min) and after 60, 120 and 180 min of fermentation and are expressed as weight percentages on flour dry matter base (% w/w dm). Saccharide concentrations at $t = 0$ refer to the concentrations in whole wheat flour. Unyeasted dough was analysed as a control. Vertical bars represent standard deviations of biological triplicate measurements.

Figure 4: CO₂ production rate (mL CO₂/min) of the selected yeast strains in Terroir whole wheat dough as a function of fermentation time measured using a Risograph device at 30°C with (A) No sugar added to the bread making recipe, (B) 3% glucose added to the bread making recipe. Vertical bars represent standard deviations of biological triplicate measurements. Relative bread volume of breads prepared with (C) Terroir whole wheat flour without added sugar and (D) Terroir whole wheat flour with 3.0% added glucose. Bread volumes are represented relative to those of bread fermented with the bread strain. Horizontal bars represent standard deviations of biological triplicate measurements. Means with different letters are significantly different ($p < 0.05$).

Table 1: Saccharide levels in whole wheat bread made with the selected yeast strains and (A) no sugar or (B) 3.0% glucose added to the bread making recipe. Saccharide concentrations are expressed as weight percentages on flour dry matter base (% w/w dm). Values are means with standard deviations of biological triplicate measurements. Values within columns with different letters are significantly different ($p < 0.05$).

	Yeast strain	Fructan content (w/w %dm)	Maltose content (w/w %dm)	Fructose content (w/w %dm)	Glucose content (w/w %dm)
A. No sugar	Bioethanol 1 (BE1)	0.31 ± 0.05 C	2.31 ± 0.23 B	0.00 ± 0.00 C	0.12 ± 0.01 E
	Bread 4 (Br4)	0.28 ± 0.04 C	0.91 ± 0.07 D	0.02 ± 0.02 C	0.11 ± 0.00 E
	Beer 1 (B1)	0.60 ± 0.04 B	1.72 ± 0.14 C	0.00 ± 0.00 C	0.12 ± 0.02 DE
	Cocoa (Co)	0.63 ± 0.08 AB	2.48 ± 0.03 B	0.00 ± 0.00 C	0.13 ± 0.00 DE
B. 3 % glucose	Bioethanol 1 (BE1)	0.27 ± 0.05 C	2.89 ± 0.12 A	0.56 ± 0.07 A	0.25 ± 0.02 B
	Bread 4 (Br4)	0.33 ± 0.03 C	2.53 ± 0.11 B	0.56 ± 0.03 A	0.35 ± 0.02 A
	Beer 1 (B1)	0.68 ± 0.04 AB	2.88 ± 0.10 A	0.32 ± 0.11 B	0.17 ± 0.01 CD
	Cocoa (Co)	0.75 ± 0.02 A	3.05 ± 0.07 A	0.32 ± 0.08 B	0.22 ± 0.04 BC

Highlights

S. cerevisiae strains show a high variability in invertase activity and specificity

Invertase activity determines fructan hydrolysis during dough fermentation

Saccharide production and consumption during fermentation is yeast dependent

Addition of sugar allows to make bread with maltose-negative yeast strain

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Figure 1

Figure 2

Figure 3

Figure 4