R. Pahl, F.-J. Methner, J. Schneider, J. Kowalczyk, S. Hausmanns and A. Radowski

Study on the Applicability of Isomaltulose (Palatinose™) in Beer and Beer Specialties, and its remarkable Results

The functional disaccharide isomaltulose (Palatinose™) was tested to determine its suitability as an ingredient in beer and beer specialties, and its potential fermentability by typical beer contaminants, and typical beer production yeasts. It was shown that all tested beer contaminants were in the majority of the cases completely unable to ferment isomaltulose. The same was true for most of the tested brewing yeasts. As a result of the non-fermentability, promising results were obtained with respect to the microbiological stability, and to the sensorial profile and taste of beer and beer specialties.

Descriptors: microbiological stability, sensorial profile, functional, isomaltulose

1 Introduction

Isomaltulose is a sucrose isomer, in which glucose and fructose are 1,6-linked (6-O-α-D-glucopyranosyl-D-fructofuranose) (7). Isomaltulose is a sugar that naturally occurs in honey, and as a metabolite of microorganisms such as *Protaminobacter rubrum*, *Klebsiella planticola*, and organisms of the species *Serratia* and *Erwinia* (6, 4, 19, 28). On industrial scale isomaltulose is produced by the enzymatic isomerization of sucrose by *Protaminobacter rubrum* (25). Apart from its chemical properties, this sugar has a number of specific characteristics that make it worthwhile to be used in human nutrition. Compared to sucrose, it exhibits only about 40 % of the sweetness (14, 17). Kashimura et al found that isomaltulose helps to improve mental performance (12), and it is slowly metabolized in the small intestine which leads to it being the only disaccharide with a glycemic index (GI) that is considered very low (11, 13, 18, 2, 27). If the GI of glucose is set at 100, isomaltulose has a GI of 32 (27, 18). Isomaltulose received GRAS status in the United States in 2006, has been authorized as novel food in the European Union since 2005 (27, 7), and has been widely used in Japan since 1985 (14).

The motivation of evaluating isomaltulose in beer and beer specialties stems in great part from its proven non-cariogenicity. Many research works have focused on this aspect (23, 8, 14, 17, 15, 22, 21, 3, 10), and it has been repeatedly shown that it is difficult for oral bacteria to metabolise isomaltulose (20, 5, 26, 1, 9).

Authors: Pahl, R., VLB Berlin; Methner, F.-J., TU-Berlin; Schneider, J., University for Applied Science Lippe/Höxter; Kowalczyk, J., Südzucker AG; Hausmanns, S., Radowski, A. BENEO-Palatinit GmbH

Figures see Appendix

Since it is known that isomaltulose cannot be used as a substrate by oral lactic acid bacteria, the question of whether lactic acid bacteria that are commonly known beer contaminants can live on this non-cariogenic sugar gains a very particular importance.

Moreover, if it is demonstrated that lactic acid bacteria cannot ferment isomaltulose, then the question arises as to what sort of effect other beer spoilage bacteria could have on isomaltulose. If it is found worthwhile to use isomaltulose in beer production, then of course another important question needs to be addressed: how will brewing yeasts (saccharomyces yeasts) react to the presence of isomaltulose in the wort? And finally, what impact will that have on the finished product?

2 Experimental

For the fermentability tests, at first an aqueous solution containing 4.5 % isomaltulose as the only carbohydrate, 3.35 % yeast nitrogen base, and additional 2 % of a peptone solution (for the evaluation on lactic acid bacteria) was created. Solutions of isomaltulose and yeast nitrogen base were sterilized separately prior to mixing. Various lactic acid bacteria types, other typical beer spoilage bacteria, common brewing yeasts as well as beer spoilage yeasts were tested, for example Lactobacillus brevis, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus coryniformis, Lactobacillus lindneri, Lactobacillus buchneri, Pediococcus damnosus, Pectinatus frisingensis, Megasphaera cerevisiae Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Saccharomyces diastaticus, Schizosaccharomyces pombe. In all cases, the model solution was incubated with 1,5 x 10^6 cells/ml model solution in test tubes, and kept for 7 days, 28 °C under anaerobe conditions. The amount of isomaltulose was measured using an HPLC analysis.

For the evaluation of fermentability of isomaltulose in beer, standard alcohol reduced lager type beer was brewed at the pilot plant of VLB Berlin using common brewing recipes. In case of the beer containing isomaltulose, the same was added 5 minutes prior to cast out. The wort was incubated with 20 x 10^6 cells per

ml of Saccharomyces carlsbergensis MJJ 11. The open fermentation took place at 12 °C followed by a cold storage for 14 days at 0 °C. The amount of isomaltulose as well as other carbohydrates was measured using an HPLC analysis.

For the evaluation of fermentability of isomaltulose in beer mix products a mix of 50 % beer and 50 % lemonade was produced, using commercial standard, alcohol reduced and low carb beer, and lemonades consisting of lemon flavor, water, sweetener (either sucrose, or isomaltulose or intense sweeteners (combination of aspartame, acesulfame, saccharine, and cyclamate)), and CO_2. The sweetness was adjusted to iso-sweet. Each product was incubated 1,5 x 10^6 cells/ml per 500 ml of mix, filled in PET bottles, kept for 14 days, 28 °C under anaerobe conditions. After the storage time the bottles were tested on turbidity using a Sigrist Process Photometer KTL30/21M, angles of measurement at 25 ° und 90 °, and an upper turbidity limit of 20 EBC, and formation of bulges by measuring the bottles with digital slide gauge.

All microorganisms used were taken from the central laboratories of VLB Berlin and Südzucker AG.

Sugars in solution were measured using HPLC analysis with an acetonitril/water mixture (acetonitril 60–75 %); main column: 250 mm x 4,6 mm; amino-phase injection-volume: 10 μl, flow rate: 1,0 –1,8 ml/min.

The sensorial evaluation of the specialty beers containing or not containing isomaltulose were carried out using a special spider web system, where each parameter had to be valued on a range from 1–5, but with 5 not necessarily being the ideal value. For sparkling quality, mouth-feel/body and bitterness, 3 was the optimal value, and since significant sweetness or wort-like aroma were unwanted with lager-type beers, the desired value therefore was as low as possible.

3 Results and Discussion

In screening trials with a model solution containing isomaltulose as the only carbohydrate, approximately 60 different brewing yeasts (*saccharomyces* yeasts) were tested for their ability to ferment isomaltulose. The trials showed that very few of these brewing yeasts were able to do so. Using the very simple parameters of formation of turbidity (cell multiplication) and formation of gas to gauge if fermentation of isomaltulose was carried out revealed that only three of the tested bottom fermenting yeasts and eight of the top fermenting ones were able to create life activity on the basis of isomaltulose. *Schizosaccharomyces pombe* was used as a cross reference, as it was known from literature that this yeast is able to ferment isomaltulose (6).

Yeasts that responded best (fastest formation of gas and biggest gas volumes) in these screening trials were taken into larger-scale fermentations. There, it was shown that these yeasts performed alcoholic fermentation on the basis of isomaltulose, but at very different rates, as shown in Figure 1 and Figure 2. In other words, even the yeasts known to be able to ferment isomaltulose differed significantly in their response to this sugar.

What was demonstrated in the screening trials – that most brewing yeasts did not react to isomaltulose – was verified on a larger scale, in brewing trials. For these trials, isomaltulose was added to the cast-out wort in the post-boil phase. Since the wort was kept circulating during the addition of isomaltulose, the solution of the sugar was complete and very fast with the side effect of an instant sterilisation of the sugar added since the wort still had boiling temperature. Isomaltulose was added in a concentration of 2 %.

Figure 3 shows the fermentation diagram of a wort containing isomaltulose that was produced using yeast that proved in the screening trials to be unable to ferment isomaltulose. There are slight differences in the speed of the uptake of the fermentable sugars, but when final attenuation was reached, the difference of 2 % in extract remained nutrient.

Consequently, the additional amount of isomaltulose contributes to an increase of the amount of extract in the finished beer.

The alcohol content of less than 3 %vol in these beers was decisively lower than that of normal beers (5–5,5 %vol) because only a certain amount of malt was used in order to end up with an original gravity of about 7 %. Subsequently only little amounts of fermentable sugars remained after a normal short mashing procedure. Since isomaltulose was still present in nearly its original concentration of 2 %, a positive effect of its continued presence on the palatefulness and mouth-feel of the produced beer can be proved, as Figure 4 shows.

Many lite beers on the market suffer from a lack of body, and for this reason are often judged negatively in general. In the tasting scheme utilized, the ideal value for the parameters bitterness, sparkling quality and palatefulness were set “ideal” at a value of 3 since it is possible not only to undercut but also to exceed the desired impression in these parameters. For total quality and quality of smell, the maximum value of 5 was, of course, considered ideal. The other taste impressions depend on the beer style: if the tested beer is a lite lager/pilsner type beer, as in this case, then they would be expected to be low (between 0 and 2). A significant improvement for the beer containing isomaltulose in the parameter mouth-feel and palatefulness was observed, which then led to an improvement of total quality.

Concerning various other quality parameters that are important to beer it can be stated that, based on the research results achieved, isomaltulose does not influence for example foam stability and stability against turbidity in any way.

It was reported that isomaltulose is not easily fermented by brewing yeasts. In addition to that, this sugar was investigated to determine if it was nutritive to beer spoilage microorganisms. As shown in Figure 1, it could be proved that in a model solution, none of the tested beer spoilage microorganisms was able to use the isomaltulose provided. The yeasts *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* MJJ2 were used deliberately as a cross check, to prove that isomaltulose was fermentable in the set-up used. *Saccharomyces cerevisiae* MJJ2 was one of the very few brewing yeasts that can utilize isomaltulose. But in Figure 1,

it can be clearly seen that in the same set-up and trial time, this yeast performs a much slower fermentation of the isomaltulose than *Schizosaccharomyces pombe*.

The results shown in Figure 5 were obtained by repeating the same test using different strains of each microorganism tested, and it was possible to verify the results for each of them. It is especially interesting to note that *Saccharomyces diastaticus* as well as *Lactobacillus brevis* are not able to ferment isomaltulose. There are even hints that the presence of isomaltulose inhibits the use of other available carbohydrates by *Saccharomyces diastaticus*.

Figure 6 shows an exemplary result of an extensive series of tests conducted on shandy type beer products. For the shandy type beer, standard pilsner type beer was mixed 1:1 with lemonade, the lemonade part varying only in the type of sweetener used: sucrose, isomaltulose or high intense sweeteners. Amounts of the sweeteners were adjusted in a way to keep the sweetness levels of the drinks comparable. The produced beer-mixes were then incubated with different beer spoilage microorganisms. Occurring turbidity was used to measure spoilage.

It can be clearly seen that the beer-mixes with the sucrose-sweetened lemonade were spoiled very quickly, with the maximum turbidity of 20 EBC reached on the second day of incubation. Interestingly, the drinks with the high intense sweeteners were also spoiled (occurring turbidity) rather quickly. Since high intense sweeteners are not nutritive, it can be assumed that *Saccharomyces diastaticus* performed cell multiplication on the dextrins that were present in the beer part of the mix. Surprisingly, the turbidity rose much more slowly in the drinks containing isomaltulose. It may be assumed that the isomaltulose inhibited the usage of dextrins; otherwise, the spoilage here should have been as fast as in the drinks containing high intense sweeteners.

Additionally, a shandy made from lite beer that did not contain any carbohydrates, was tested as shown for example in Figure 7. By using this special beer, it was possible to exclude any carbohydrates being brought in by the beer part, leaving only the sugars of the lemonade part in the drink. It was shown that none of the typical beer spoilage microorganisms was able to spoil the beer-mixes when high intense sweeteners or isomaltulose were used in the lemonade.

Of course, these trials were also accompanied by taste tests, which showed that isomaltulose-lemonade based shandy showed a higher sensorial acceptance than shandy with high intense sweeteners, and that isomaltulose as a sweetener ranges at the same quality level as the sucrose.

4 Conclusion

Evaluations of the reducing disaccharide isomaltulose to determine its suitability in production of beer and beer specialties produced promising results. Extensive screening tests demonstrated that most common brewing yeasts are unable to ferment isomaltulose, suggesting that the brewing industry would profit from adding isomaltulose as early as possible in the brew house procedure. In the finished product, isomaltulose has a positive influence on the mouth-feel of alcohol reduced and alcohol free beers that usually lack of body. In beer specialties such as beer/lemonade mix drinks, it was found that isomaltulose cannot be fermented by a wide range beer contaminants including *Lactobacillus brevis* and *Saccaromyces diastaticus*. If isomaltulose is the only carbohydrate present in the beer-mix product an increase of microbiological stability can be achieved. Parameters like foam stability and stability against occurring turbidity were not influenced.

It can be concluded that the use of isomaltulose in beer and beer specialties will result in an improvement of the sensorial profile and taste as well as the product stability.

5 Literature

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Appendix

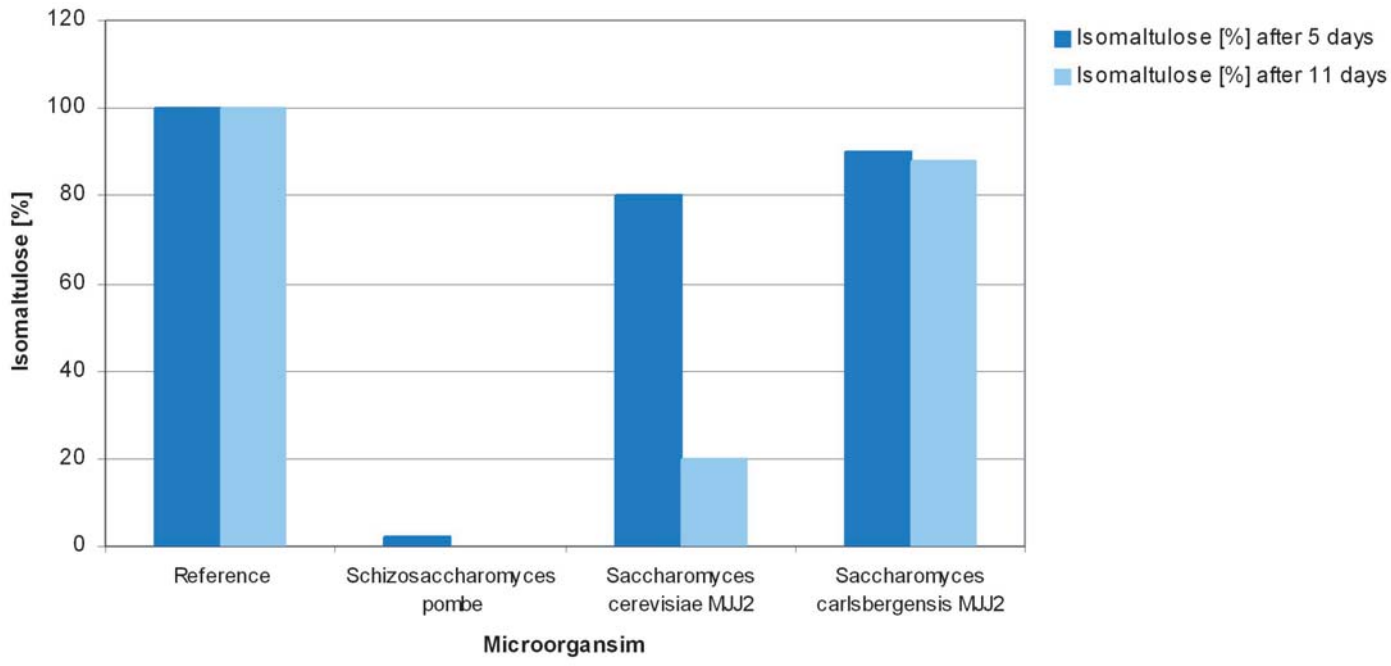

Figure 1 Amount of isomaltulose in model solution after 5 and 11 days of fermentation (appr. 1,5 x 106 cells on 500 ml, 28 °C, aerobic conditions)

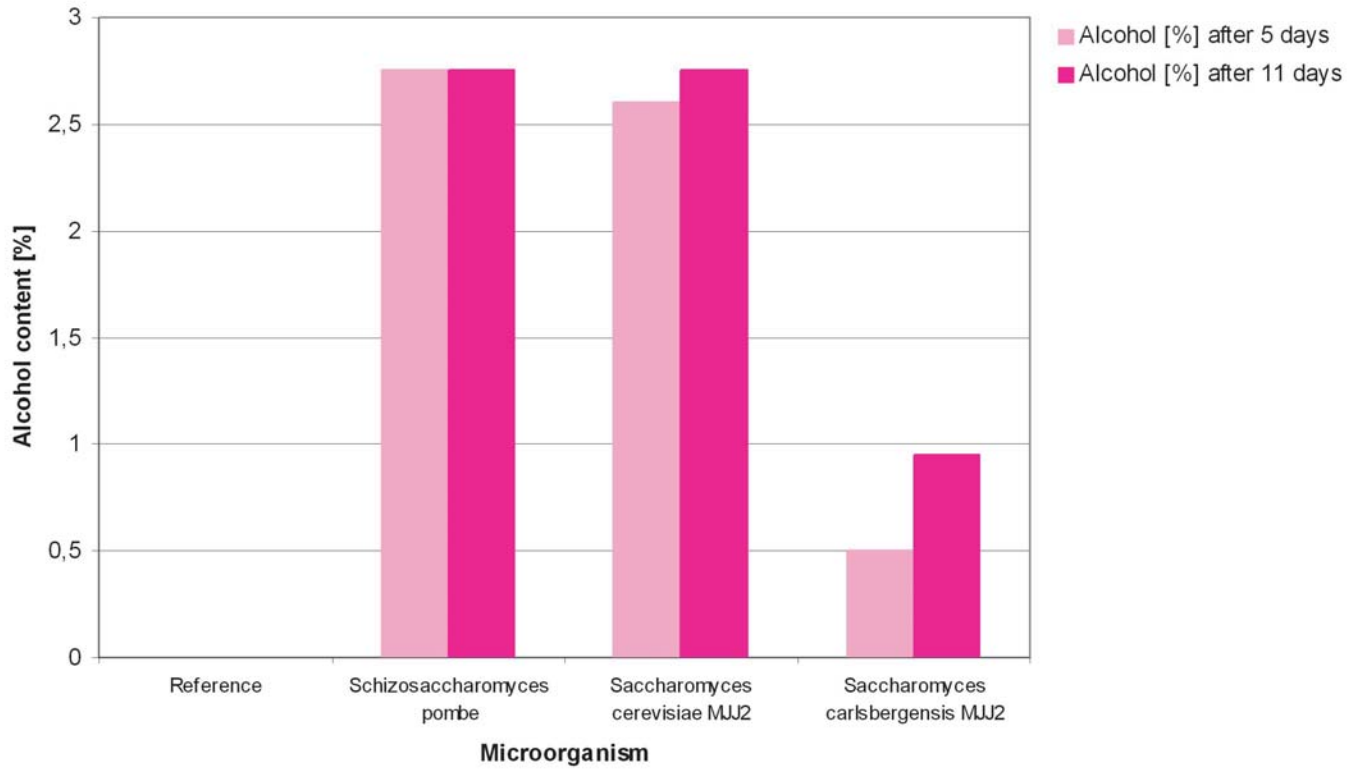

Figure 2 **Alcohol content in model solution after 5 and 11 days of fermentation (appr. 1,5 x 106 cells on 500 ml, 28 °C, aerobic conditions)**

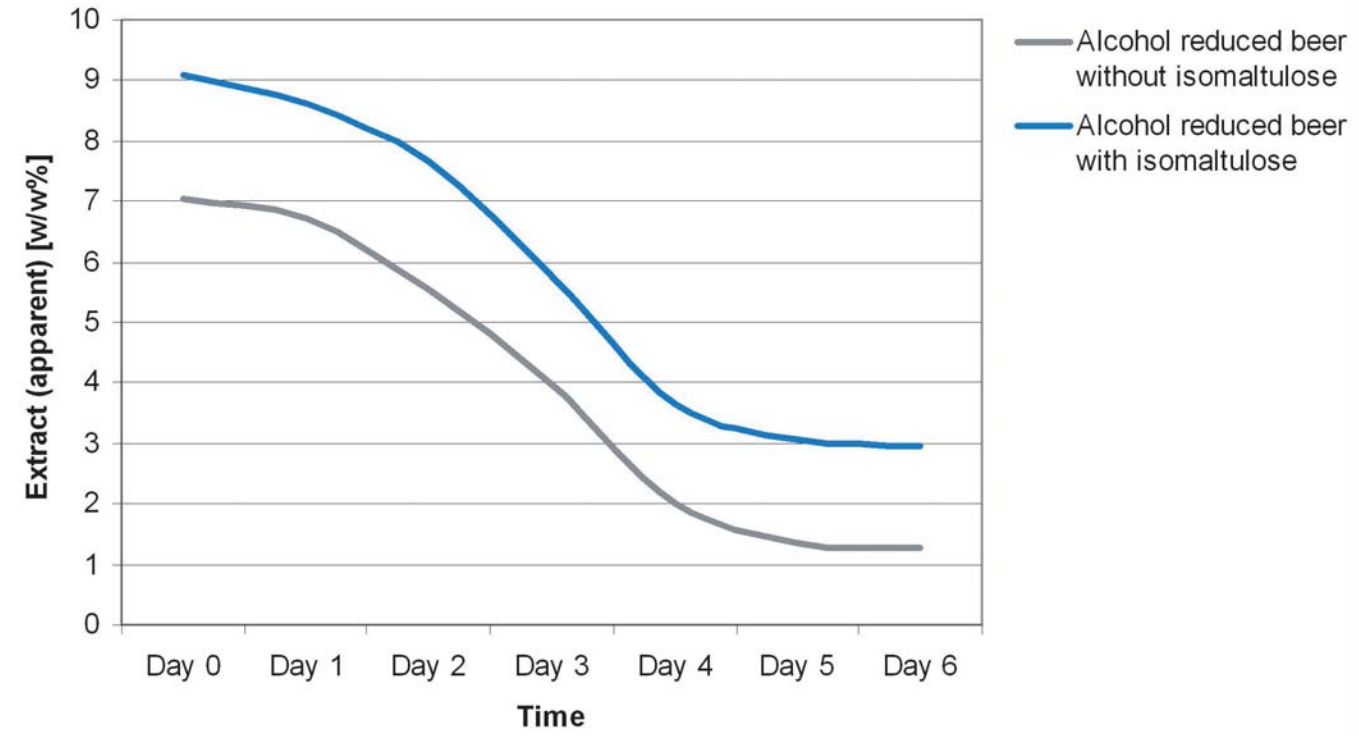

Figure 3 **Fermentation of wort with and without isomaltulose at 12 °C, open fermentation**

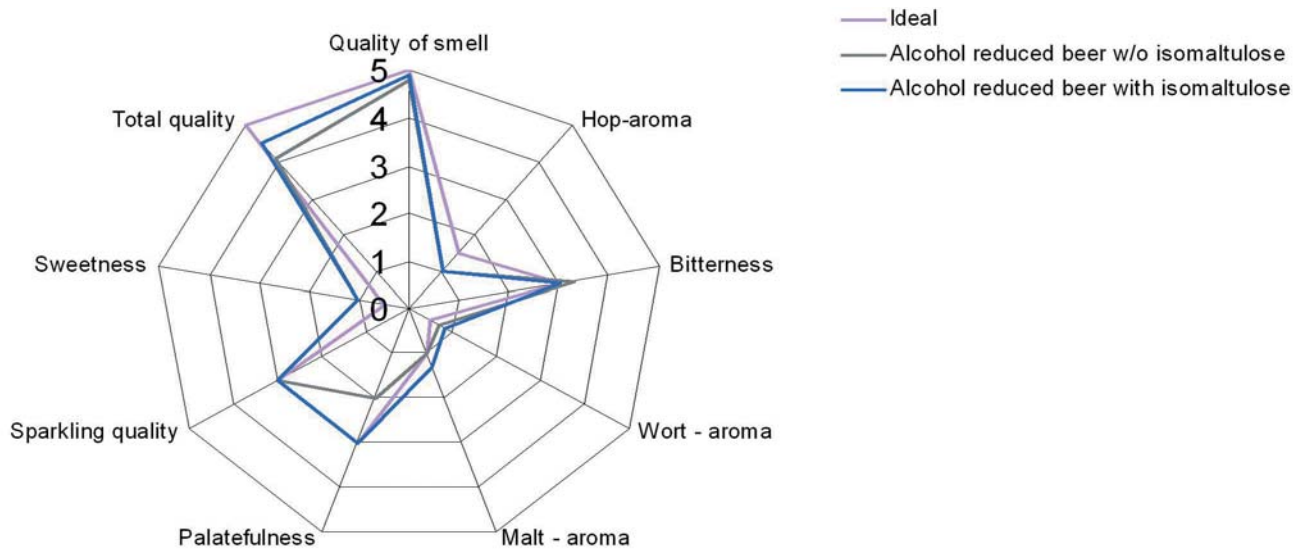

Figure 4 Comparative tasting of alcohol reduced beer with and without isomaltulose (10 testers)

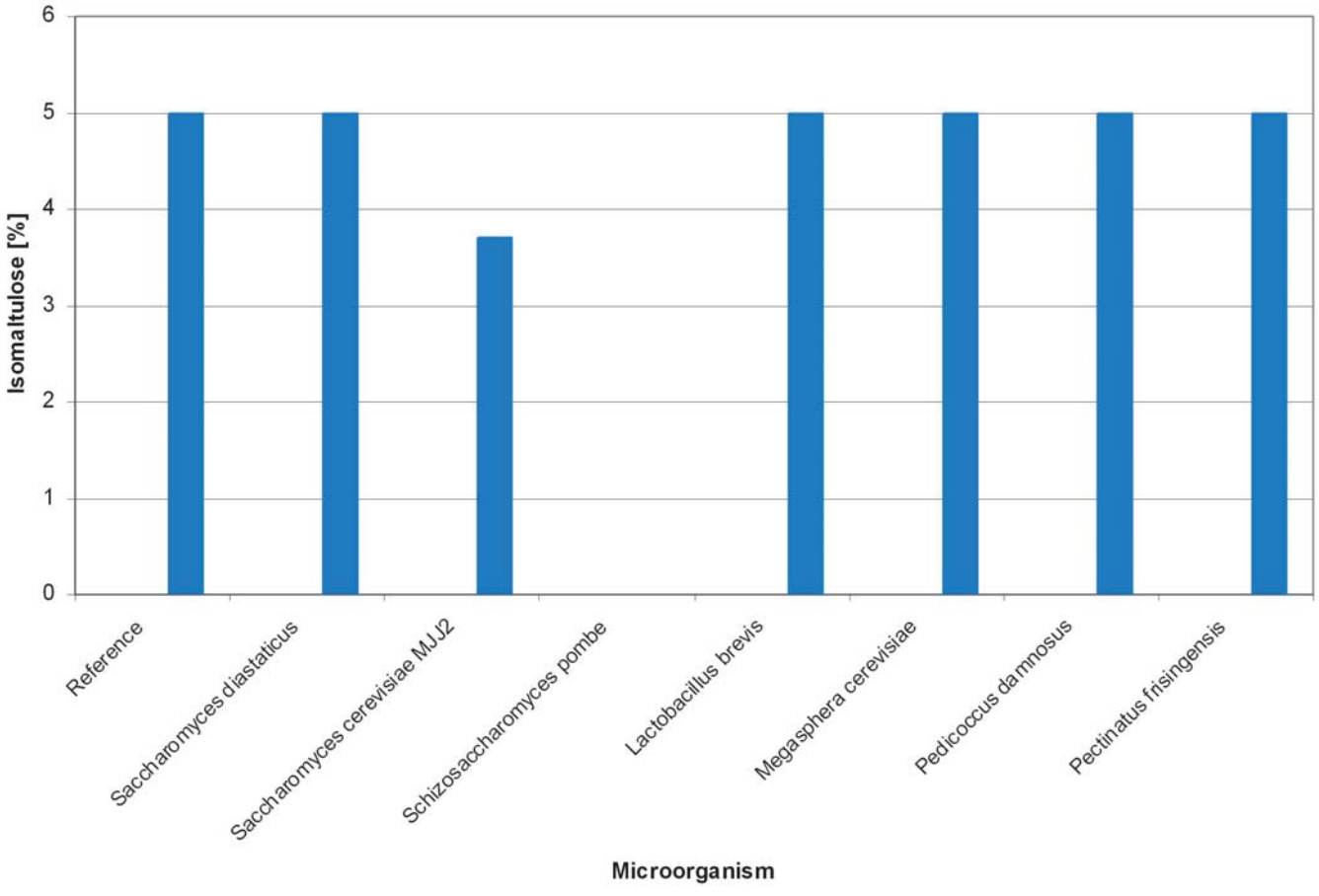

Figure 5 Isomaltulose fermentation by beer spoilage microorganisms and two isomaltulose-fermenting yeasts in model solution under anaerobic conditions, 28 °C, 7d, test tube scale

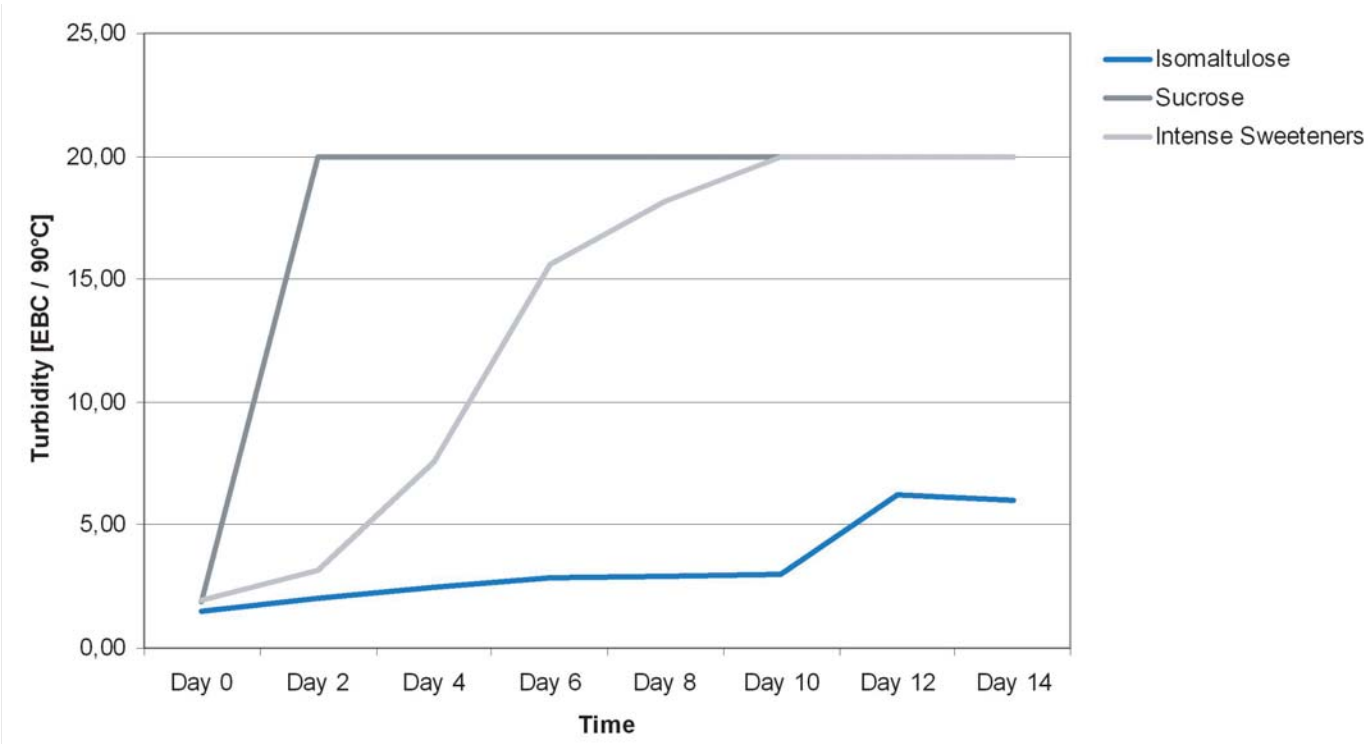

Figure 6 **Occurring turbidity in standard pilsner type beer based beer mix drinks after incubation with** *Saccharomyces diastaticus* **(appr. 1,5 x 106 cells on 500 ml, 28 °C, 14d, max. turbidity: 20 EBC at 90°)**

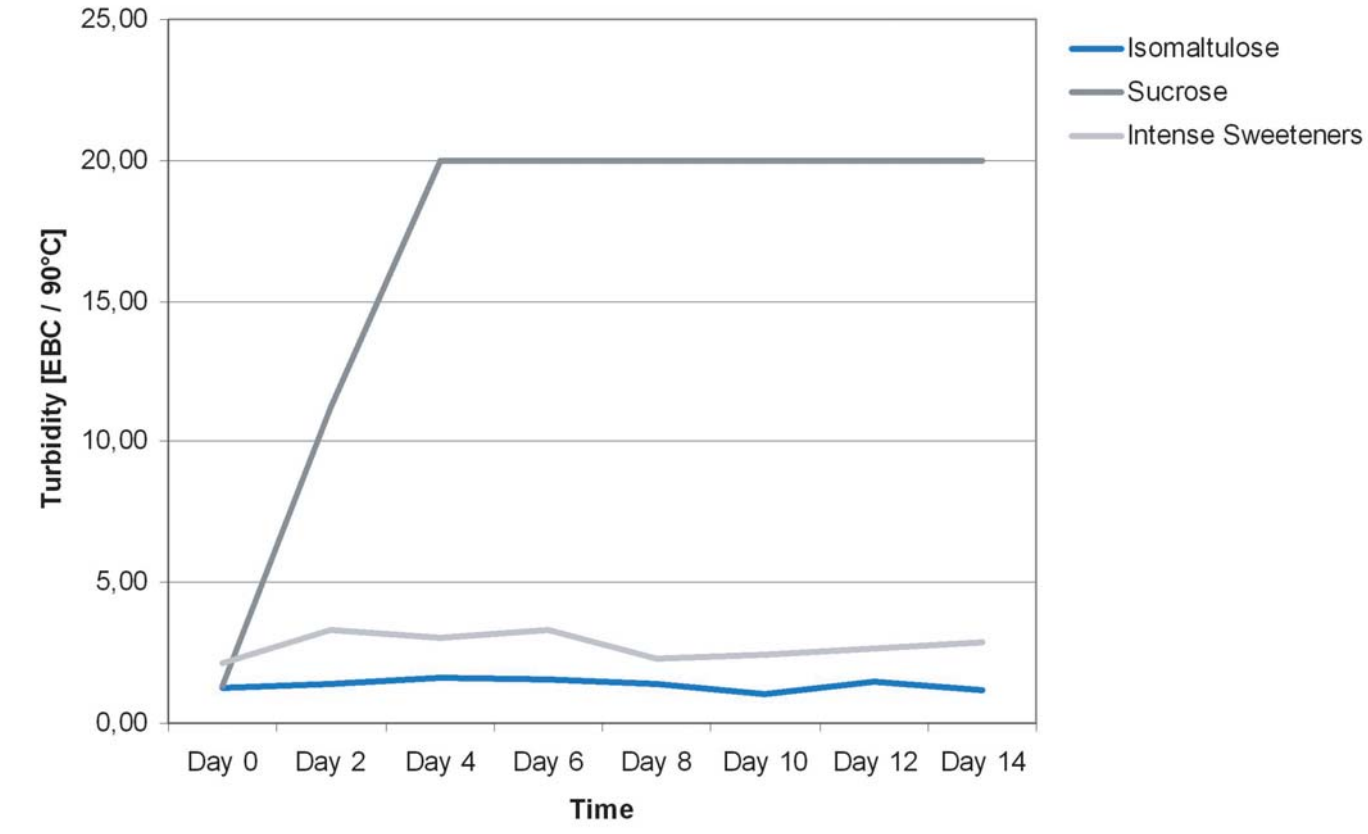

Figure 7 **Occurring turbidity in lite pilsner type beer based beer mix drinks after incubation with** *Saccharomyces diastaticus* **(appr. 1,5 x 106 cells on 500 ml, 28 °C, 14d, max. turbidity: 20 EBC at 90°)**