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Impact of Fermentable and Non-Fermentable Carbohydrates on the Sweetness, Improvement of Palate Fullness and SO_2-Content in Beer

Carbohydrates are involved in many reaction mechanisms during the brewing process, such as formation of Strecker degradation products, Maillard-reaction, oxidative processes, etc. Thus they can have an important influence on the stability and taste of beer. Aim of this study was to investigate influences of fermentable and non-fermentable carbohydrate addition into the brewing process prior fermentation to better understand their influence on the yeast metabolism, osmotic pressure, sweetness, palate fullness and SO_2-formation during fermentation.

Compared to the standard wort without sugar addition, the results demonstrate a general increase in SO_2-formation during fermentation which depends on the carbohydrate. The highest increase in SO_2 results from the fermentable sugars glucose and sucrose followed by the non-fermentable sugar isomaltulose. The addition of polydextrose showed the least increase. The observed increase in the SO_2-formation in correlation to a specific extract addition due to non-fermentable carbohydrates is mainly based on their influence on the osmotic pressure in the pitching wort with the osmotic pressure depending on the molecular size.

When fermentable carbohydrates are used an enhanced yeast metabolism and more active glycolysis need to be considered. These effects lead to an accelerated production rate of pyruvate, acetaldehyde, ethanol and carbon dioxide. Consequently they are responsible for a stronger increase in the SO_2-formation during fermentation. With the right process management, the higher SO_2-content in the final beer caused by carbohydrate addition can improve the oxidative beer and flavour stability. In the sensory analyses a carbohydrate dependent turning point was generally noticeable at which the sweetness started to dominate and the panel rated the influence on the sweetness much stronger in comparison to the palate fullness.

Taking into consideration all data, it can be concluded that increasing the final wort extract up to 2 % and 3 % respectively with non-fermentable carbohydrates, such as isomaltulose* and polydextrose can improve the palate fullness and oxidative stability of beer. A higher sugar addition prior fermentation cannot be advised, because the influence on the sweetness of the beer is enhanced and covers the positive effect on the palate fullness and the typical beer taste.

Descriptors: non-fermentable carbohydrates, sugar addition, palate fullness, oxidative stability, SO2, sulphur dioxide, isomaltulose

1 Introduction

It is well known that carbohydrates are involved in many reaction mechanisms during the production processes of beverages and as such can also have an important influence on their palate fullness and taste.

In the brewing process carbohydrates are especially involved in mechanisms which are responsible for the final beer flavour and stability. In this context it is important to point out in particular the formation of Strecker degradation products, Maillard-reaction and the participation in oxidative processes. Especially during wort boiling reactions of carbohydrates are accelerated. The profile of the fermentable carbohydrates in the pitching wort has also a significant influence on the yeast metabolism and the generation of fermentation by-products.

This counts for example for the sulphur dioxide formation which is formed as an intermediate in the sulphur amino acid biosynthetic pathway [9, 17, 23, 26, 32, 39, 40]. It plays a significant role in masking stale flavour, and in protecting beer from oxidation and slightly from microbial spoilage [7, 8, 12, 14, 16, 27, 30, 38, 47, 52, 54, 55]. There are two factors that promote the positive effect

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of SO_2 on the flavour stability of beer. SO_2 or sulphite (HSO_3-) is a reactive antioxidant, which reduces oxygen and therefore causes a better endogenous antioxidative potential [2, 18, 30, 34, 35, 46, 54, 55, 56]. Furthermore SO_2 is building reversible complexes with carbonyls which cause a masking effect since these carbonyls are mainly ageing flavour related compounds [1, 2, 5, 11, 14, 23, 26, 30, 39, 40, 52].

A multitude of studies have described specific fermentation parameters that affect the sulphite formation by yeast in wine and beer production. Thereby, the wort aeration plays a key role during fermentation. But also the effect of pitching rate, temperature and pressure on the level of sulphur dioxide formation has been investigated by different research groups [6, 15, 34, 36]. The results of these investigations are in line with *Kaneda et al.* [23, 26, 43], who showed that the wort fermentation conditions are influencing the sulphite level and therefore also the flavour stability of the finished beer by inhibiting radical reactions and oxidative processes. Furthermore the sulphite is able to mask staling flavour by formation of sulphite complexes with ageing flavour related carbonyls [1, 2, 13, 34]. In this context *Narziss et al.* [41] and *Foster et al.* [18] recommended that sulphur dioxide content in packaged beer of 8–9/6-7 mg/L generated by yeast is most appropriate for the flavour stability of beer.

Additionally, previous investigations of *Gyllang et al.* [23] have shown that by addition of glucose to the pitching wort the production of sulphite can be increased under comparable fermentation conditions. They proposed that higher osmotic pressure leads to an increased production of glycerol and acetaldehyde, caused by the acceleration of the enzyme synthesis during glycerol production [4, 9, 23, 32]. The resulting glycerol overproduction is known to inhibit alcohol dehydrogenase, which is described to be responsible for the intercellular accumulation of acetaldehyde in fermenting wort [9, 32]. Additionally, an increasing glucose content results in higher pyruvate, acetaldehyde, ethanol, and carbon dioxide formation through glycolysis. In comparison to other carbonyls acetaldehyde and pyruvate are known to generate stronger complexes with sulphite [1, 12].

Gyllang et al. [23] therefore suggested that the described increase of both can bind more intracellular sulphite and divert it from the methionine pathway. Consequently the intracellular sulphite, mainly presented as bisulphite (HSO_3-), which is not able to pass the cell wall, is transferred to carbonyl compounds and via this way it is possible to carry it out of the cell [1, 32, 33, 41]. Furthermore the increased carbon dioxide, present as bicarbonate, leads to a competitive inhibition of the sulphite reductase [32, 37, 42]. In combination with the stronger stability of the sulphite- carbonyl complexes [12, 16, 32] the consumption rate of sulphite by sulphite reductase is inhibited. In consequence, lacking sulphite, methionine and S-adenosylmethionine (SAM) in the cell, an activation of the sulphate-reductase metabolism can be observed, which leads to an increased sulphite production. As long as the acetaldehyde and pyruvate are available, the additionally generated sulphite will be carried out of the cell and causes a higher sulphur dioxide formation during fermentation [16, 25, 27, 32, 42].

In this study, the addition of fermentable carbohydrates in the brewing process served to get better insight in the yeast metabolism and the influencing factors on flavour stability. On the other hand the breweries may also use the addition of non-fermentable carbohydrates to increase the palate fullness of beer. Besides the direct addition of non-fermentable carbohydrates to the final beer, it is a common standard to add sugar at the end of the wort boiling process prior to fermentation, especially in case of low soluble carbohydrates.

Taking into consideration the described knowledge on the influences of fermentable carbohydrates during fermentation and the final impact on the beer flavour and the oxidative stability respectively, this study was conducted to investigate the effect of non-fermentable carbohydrate addition during brewing in direct comparison to fermentable carbohydrates such as glucose and sucrose. Furthermore it aimed to get deeper insight into the influence of isomaltulose* (Palatinose™) and polydextrose on the palate fullness and the general effect of a higher osmotic pressure on the yeast metabolism and the SO2-formation during fermentation.

2 Materials and methods

Glucose monohydrate ≥ 99%, $C_6H_{12}O_6 \cdot H_2O$, M = 198.17 g/mol

AGRANA Fruit Germany GmbH, www.agrana.de; CAS 14431-43-7

Isomaltulose* (Palatinose™) ≥ 98 %, $C_{12}H_{22}O_{11}$, M = 342.30 g/mol

Südzucker AG, www.suedzucker.de; CAS 13718-94-0

Isomaltulose (Palatinose™) is a disaccharide naturally occurring in honey and sugar cane molasses. The functional carbohydrate appearance can be described as pure, white and crystalline. It has a mild sugar-like sweetness with no aftertaste. It is used to replace sucrose. Due to its physiological characteristics it is very suitable for innovative sport- and energy nutrition. However, its physiochemical properties make it suitable for various foods and beverages, such as functional alcohol-free malt-based beverages, dairy products or confectionery. [44, 45, 46, 50]

Polydextrose ≥ 99 %, ($C_6H_{10}O_5$) Südzucker AG, Germany, www.suedzucker.de

Sucrose ≥ 99.7 %, $C_{12}H_{22}O_{11}$, M = 342.30 g/mol

Südzucker AG, Germany, www.suedzucker.de; CAS 57-50-1

2.1 Beer analyses according to MEBAK [37]

These were as follows: extract (2.9.2.3); pH value (2.13); alcohol (2.10.7); colour (2.12.2), osmolarity: SOP TU Berlin, OsmoLAB One/16S

2,2 SO_2-determination using Continuous Flow Analysis (CFA) [34]

The determination of SO_2 was carried out by CFA under an optimized procedure using a Teflon membrane. Sulphur dioxide is released from beer at 95 °C as gas and is dialyzed into a formaldehyde so-

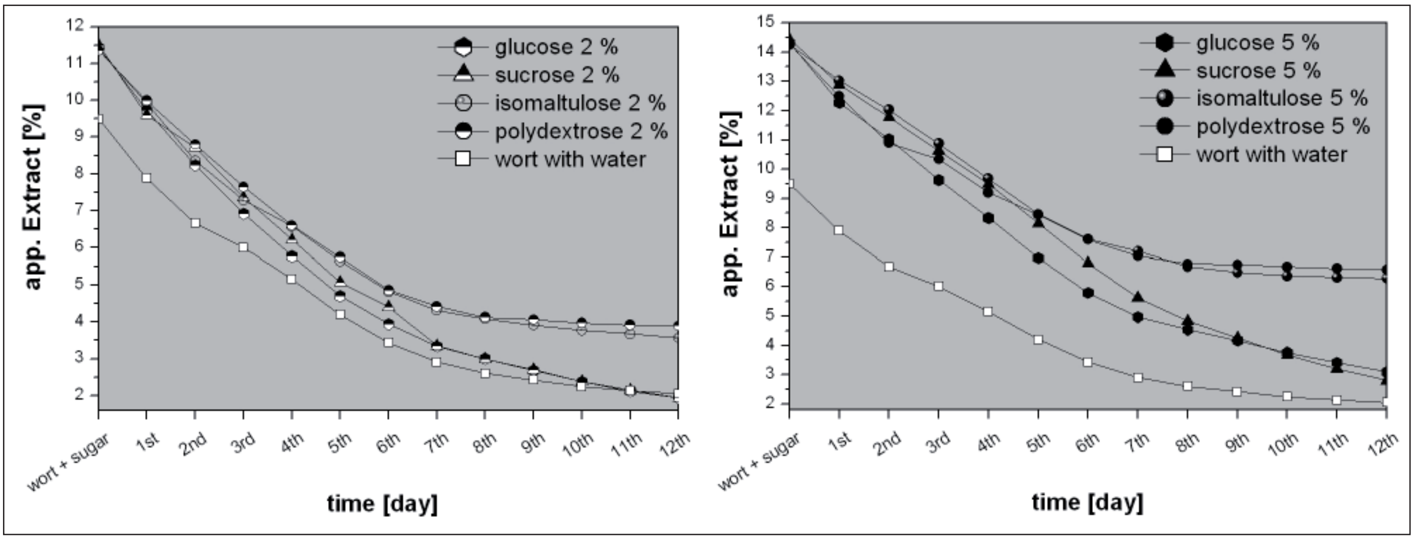

Fig. 1 **Extract degradation with different carbohydrates and concentrations (2 % & 5 %)**

lution. P-rosaniline is added and binds with the SO_2-formaldehyde complex at 45 °C forming a red coloured complex measured by colorimetric at 560 nm – according to MEBAK [37] 2.21.8.3

2.3 Sensory beer analyses according to DLG – MEBAK [37] 2.10.3

The beer was rated using the testing method of the German Agriculture Organization (DLG – Deutsche Landwirtschaftsgesellschaft e.V.). The taste panel with at least ten expert assessors evaluated with focus on palate fullness and sweetness of the beers. The values ranged from 0 to 5 points for each attribute, where 5 was the strongest and 0 the lowest score. After the tasting, the average of the single evaluations was averaged.

2.4 Fermentation pre-trials

The fermentation trials were carried out using standard wort (10 °P original gravity) and adding individual amounts of carbohydrates to increase the final wort extract by 2 % and 5 % prior fermentation. The fermentations were carried out simultaneously and under same conditions (RH-yeast, 11 °C) in 2 L Schott flask. SO_2-production, extract and pH development were monitored.

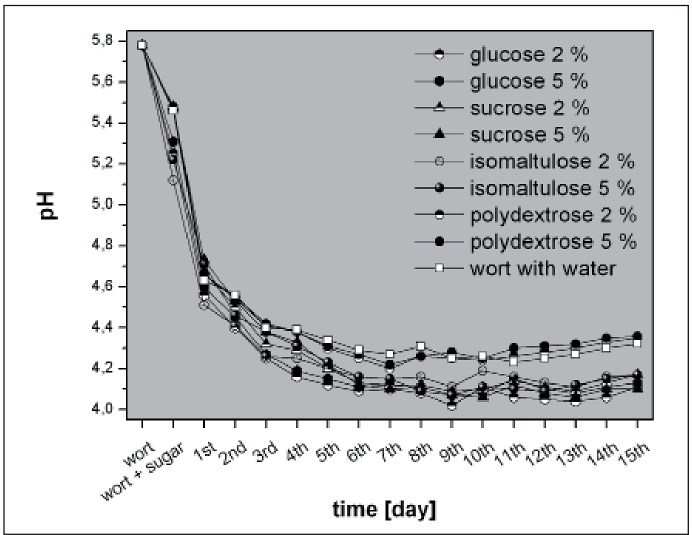

Fig. 2 **pH development with different carbohydrates and concentrations (2 % & 5 %)**

2.5 First fermentation trial

Further investigations were carried out using basic wort (10,5 °P original gravity) and adding individual amounts of carbohydrates to increase the final wort extract by 1 %, 2 %, 3 % and 5 % prior fermentation. Fermentation conditions: RH-yeast, 14°C in 2 L Schott flask. SO_2-production, extract and pH development of every trial was monitored. After filtration a sensory analysis was carried out with focus on palate fullness and sweetness in the final beer.

2.6 Second fermentation trial

For the verification of the first results another fermentation trial was carried out using also standard wort (10,5 °P original gravity) and adding amounts of carbohydrates to increase the final wort extract by 2 % and 5 %. Sugar addition, fermentation and analyses were conducted under analogical conditions to the first fermentation trial

3 Results and discussion

In the fermentation pre-trials, 2 and 5 % of different carbohydrates (glucose, sucrose, isomaltulose*, polydextrose were added to the wort prior fermentation to investigate the influence of non-fermentable and fermentable carbohydrates on the SO_2-formation. The aim of the used fermentation condition with 11 °C, RH-yeast in 2 L Schott flask was to achieve a prolonged fermentation time including the different effects of the carbohydrates on the yeast metabolism. The fermentation process and the progress of extract and pH-reduction during the fermentation period of 12 days is demonstrated in figure 1 (Extract) and 2 (pH).

As expected, the addition of non-fermentable sugars generally led to a higher extract at the end of fermentation, while fermentable sugars were almost completely fermented in both cases (2 and 5 % addition) after 12 days of fermentation (Fig. 1).

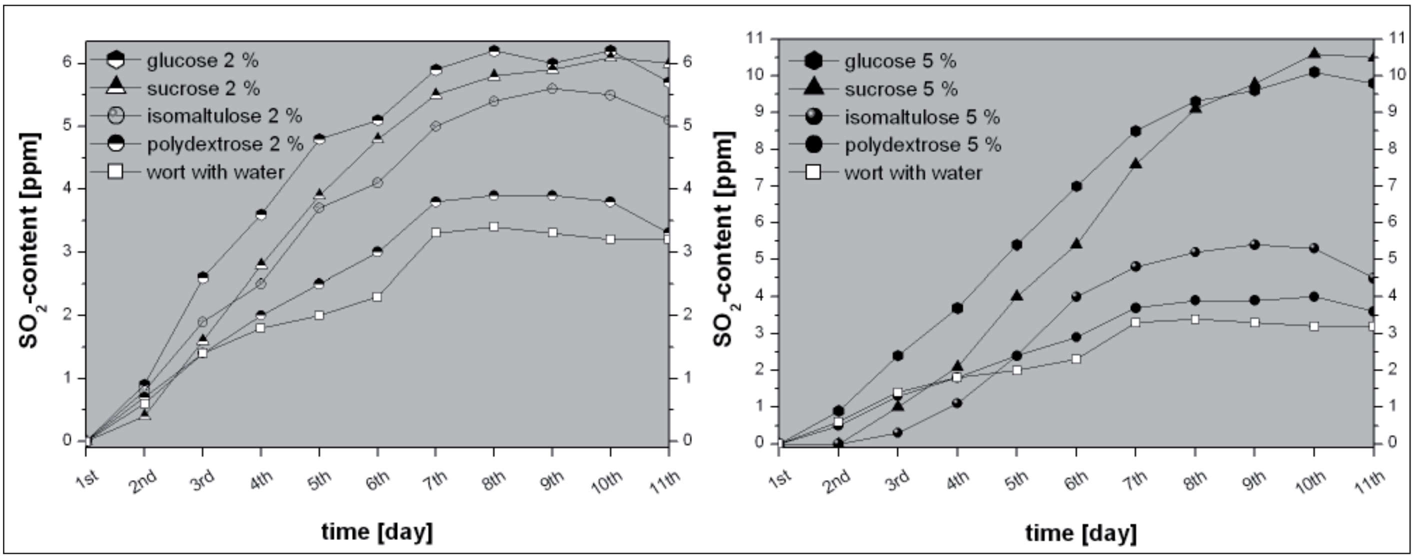

Fig. 3 SO_2 formation during fermentation after addition of different carbohydrates and concentrations (2 % & 5 %)

The evaluation of the pH-decrease as a common parameter to characterize the fermentation process revealed that in comparison to the non-fermentable carbohydrates and the standard wort, the pH decrease during fermentation is slightly accelerated when adding fermentable sugars (Fig. 2).

The development of the SO_2-formation was of particular interest and monitored daily during fermentation due to the described influences of sulphite in masking stale flavour aldehydes and improving the oxidative beer stability. In all cases of carbohydrate addition the highest increase in the SO_2-content resulted by adding fermentable sugars like glucose and sucrose as shown in figure 3.

The increase in sulphite production of the yeast correlates directly with the amount of fermentable carbohydrate addition to the wort. In comparison to the control wort, the 5 % fermentable sugar addition leads to the highest SO_2-content after fermentation followed by 2 % glucose respectively sucrose addition.

Up to 2 % sugar addition also the non-fermentable carbohydrate isomaltulose* leads to a significant increase in the SO_2-formation during fermentation. However, with more than 2% carbohydrate addition, the influence of fermentable sugars on the sulphite production is more significant in comparison to the non-fermentable sugar isomaltulose*. Accordingly just a slight increase in the SO_2-formation can be observed with more than 2 % of isomaltulose* addition. In comparison to the mono- and disaccharides the lowest increase in the SO_2-formation was caused by the addition of polydextrose.

The different response of yeast can be explained by the easier utilization of fermentable carbohydrates in the yeast metabolism and the described influences on the glycolysis and in particular by the different influences of the osmotic pressure in the pitching wort caused by carbohydrate addition.

Taking into consideration all carbohydrates, the monosaccharide glucose causes the highest increase in osmotic pressure at the beginning of fermentation followed by the disaccharides sucrose and isomaltulose*. As a logical consequence of the molecular size, carbohydrates having longer molecular chains cause a less pronounced increase in osmotic pressure. This explains a diminished increase in osmotic pressure when using polydextrose. The varying influences of the different carbohydrates on the osmotic pressure are shown in figure 4.

Further investigations were carried out, using wort with an amount of carbohydrates added to standard wort, which led to an increase of extract in the final wort of 1 %, 2 %, 3 % and 5 %. The characteristic fermentation processes are shown in figure 5, 6, 7 for the cases of 2 and 5 % under consideration of the extract degradation, alcohol and pH-value development during fermentation.

Analogically to the pre-trials, the addition of the non-fermentable sugars led to a higher extract at the end of fermentation, while fermentable sugars were almost completely fermented. In direct correlation to the fermented carbohydrates and the consumption rate of wort extract a stronger increase in the alcohol development in the trials with fermentable carbohydrate addition is observable. As expected non-fermentable carbohydrate addition leads to no significant differences in the alcohol formation compared to the standard wort.

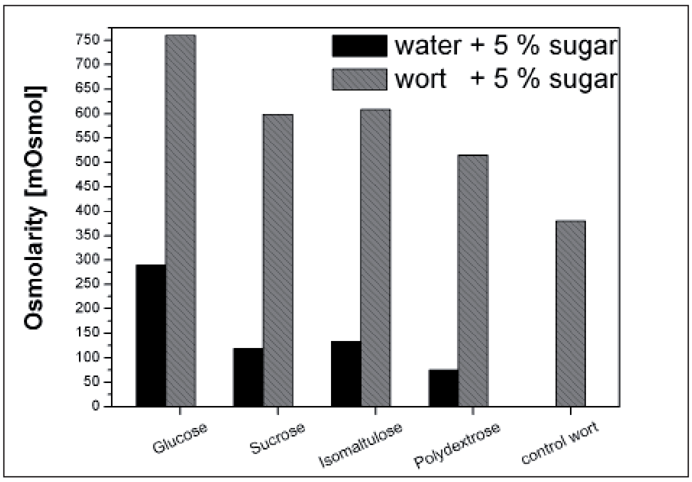

Fig. 4 Osmolarities of different carbohydrates in water solution and increase osmolarity in wort after additon

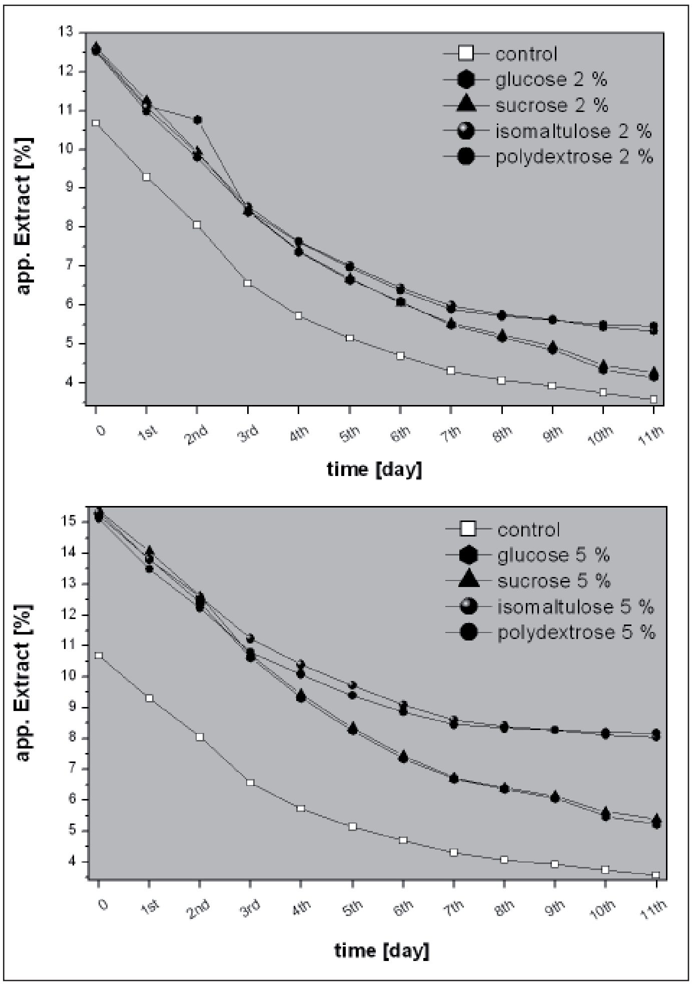

Fig. 5 **Extract degradation with different carbohydrates and concentrations (2 % & 5 %)**

In comparison to non-fermentable sugars and standard wort, the pH decrease during fermentation is slightly accelerated, when adding fermentable sugars as demonstrated in the case of 2 % and 5% carbohydrate addition (Fig. 7).

At any rate of carbohydrate addition the highest increase in the SO_2-content was observed with fermentable sugars sucrose and glucose, followed by the non-fermentable isomaltulose* as shown in figure 8.

Analogically to the pre-trials, sucrose led to the highest SO_2-content at the end of fermentation. It was noticed that from the middle to the end of fermentation the wort with sucrose addition had a higher SO_2-formation than this with glucose addition, but the typical decrease of the sulphite content at the end of fermentation occurs later.

In dependence to a sugar addition of up to 2 % also the non-fermentable sugar isomaltulose* led to a significant increase in the SO_2-formation during fermentation. On the other hand, in direct correlation to the pre-trials with more than 2 % isomaltulose* addition, only a very slight increase of the SO_2-formation could be observed. With a higher dosage of sugar it could be clearly shown that fermentable sugars are influencing the SO_2-formation much more significantly than non-fermentable sugars. Also the increased osmotic pressure due to the non-fermentable sugar addition does not lead to an appreciable further increase in the SO_2-formation.

The results in figure 8 verify the influence of polydextrose on the SO_2-formation. Polydextrose as contains the longest molecular chains, compared to all tested carbohydrates, and the lowest influence on the osmotic pressure, causes the lowest increase in the SO_2-formation in comparison to the other sugars during fermentation. Additionally, in correlation to the non-fermentable isomaltulose* the caused increase in the osmotic pressure with more than 2-3 % polydextrose addition has no further significant influence on the SO_2-formation.

In the sensory evaluation of the final beer samples (Fig. 9) there was a general relationship between palate fullness and sweetness. When adding individual carbohydrates up to a certain level the increase of palate fullness is significantly higher than the increase of sweetness. Dependent to the sugar used a turning point was detectable, at which sweetness dominates and the influence on the sweetness is evaluated much stronger in comparison to the influence of the palate fullness by the taste panel. In case of the fermentable sugars like glucose and sucrose the additional formation of fermentation products like ethanol and the remaining non-fermentable sugars are mainly responsible for an improved palate fullness of the final beer. The increase in sweetness with higher sugar addition is not very distinctive, because of the reduction of the sugar concentration due to fermentation.

The non-fermentable carbohydrate isomaltulose* demonstrates a significant increase in palate fullness with a negligible influence on the sweetness up to 2 % addition. Adding more than 2 % the influence on sweetness is increasing and overlies the influence of the palate fullness. By application of polydextrose this reversal point is shifted up to 3 %, caused by the general lower sweetness of this carbohydrate and therefore the minor effect on the sweetness of the final beer.

Considering the results of the pre-trials and first fermentation, the further fermentation trial was focused on the direct correlation between osmotic pressure and SO_2-formation by yeast. In figure 10 the development of the osmolarity is put into correlation with the SO_2-formation during fermentation by addition of different amounts of fermentable and non-fermentable sugars (2 % and 5 %).

Under the same fermentation conditions as in the first trials and comparable fermentation conditions the results in general show a continuous increase in the osmotic pressure during fermentation due to the formation of fermentation products like Ethanol. Additionally, the general acceleration in SO_2-formation by using a higher original wort extract during fermentation is obvious. As expected a direct correlation of the osmotic pressure and the stronger SO_2-formation is observable. Consequently, the addition of the non-fermentable sugars demonstrates the influence only of the osmotic pressure on the SO_2-content, whereas the fermentable sugars show also the described influence of the enhanced yeast metabolism and more active glycolysis. Accordingly, SO_2-formation caused by osmotic pressure is more pronounced using fermentable sugars than non-fermentable sugars. The effect of osmotic pressure on SO_2-formation, when adding the non fermentable sugar isomaltulose* reaches a maximum at approximately 2 % sugar

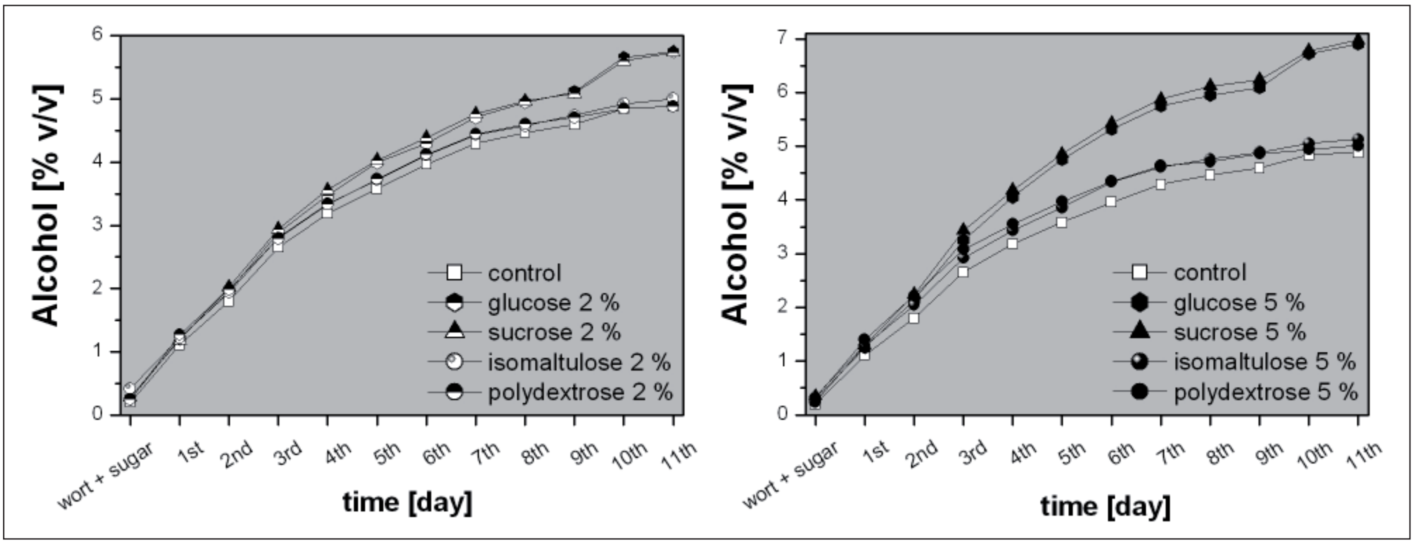

Fig. 6 **Alcohol development with different carbohydrates and concentrations (2% & 5%).**

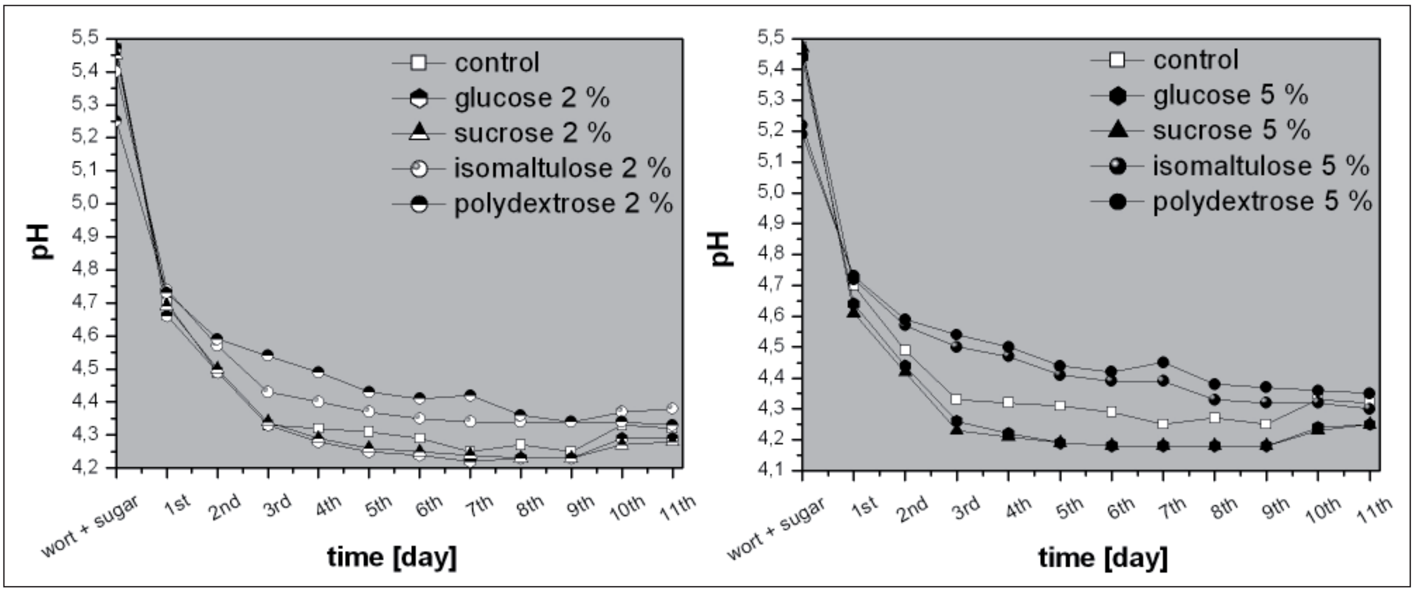

Fig. 7 **pH development with different carbohydrates and concentrations (2 % & 5 %)**

addition. A further increase doesn't lead to a significant rise in the SO_2-formation anymore.

The sensory analysis of the final beer as shown in figure 10 verifies the results of the previous investigations. It becomes clear that the palate fullness dominates the taste up to 2 % of sugar addition. With higher sugar addition however, the sweetness dominates the beer flavour, whereas the influence on the palate fullness is covered.

4 General discussion

Considering all results it must be pointed out that the addition of non-fermentable sugars prior to fermentation and their influence on the SO_2-formation during fermentation is mainly based on the increase of the osmotic pressure, according to Gyllang et al. [23] resulting in an overproduction of glycerol and acetaldehyde [5, 9]. Consequently, the intracellular sulphite which is not able to pass the cell wall is transformed to be carried out of the cell as carbonyl complexes with acetaldehyde [42, 51]. To compensate the lack of sulphite in the methionine pathway an activation of the sulphate-reductase metabolism can be observed, which leads to an increased sulphite production [32, 52].

The demonstrated results on the impact of isomaltulose* or polydextrose addition on the SO_2-formation during fermentation indicate that the enhanced sulphite formation caused by the increase of osmotic pressure on the described pathway is limited. This can be based on the fact that the known stimulation of the enzyme synthesis involved in the glycerol production [5, 9, 23, 29, 43] caused by the increased osmotic pressure, due to the non-fermentable sugar addition is limited. Consequently, with a given number of yeast cells and glycolysis activity, the described glycerol overproduction [23, 29, 43] and the increase in available acetaldehyde are well limited. Thus the intracellular sulphite can be transformed into carbonyl complexes to be carried out of the cell wall.

In direct comparison to the used non-fermentable carbohydrate isomaltulose*, polydextrose due to its molecular size causes a less pronounced increase in osmotic pressure resulting in less increase

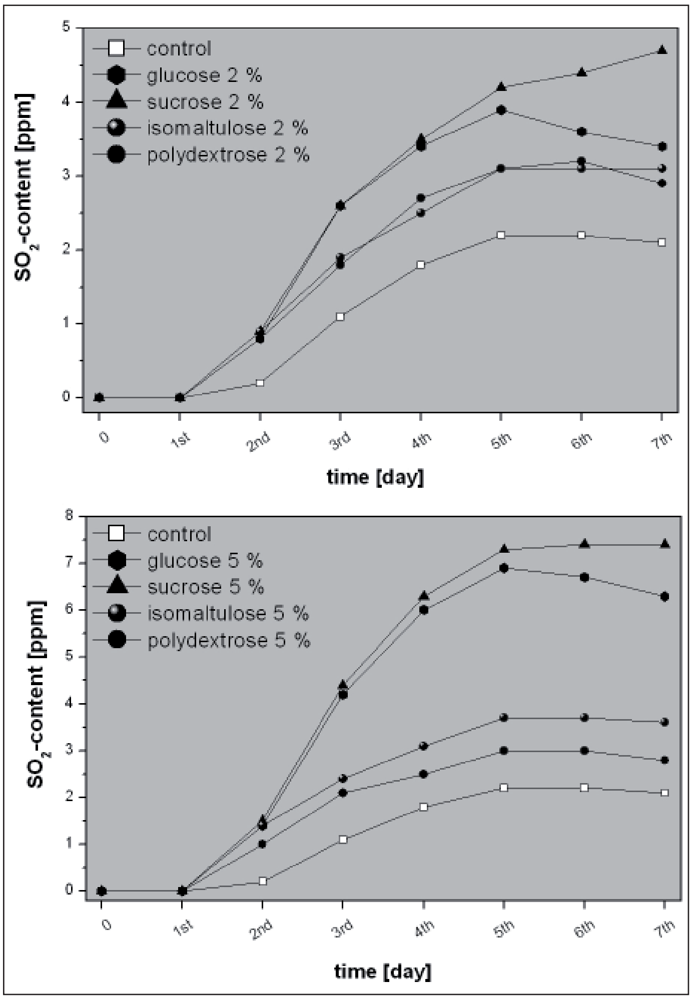

Fig. 8 SO_2 formation during fermentation after addition of different carbohydrates and concentrations (2 % & 5 %)

of SO_2 during fermentation. A higher polydextrose dosage would be necessary to achieve the same effect on the osmotic pressure with respective consequences for the sensory profile. As the results show the turning point where sweetness dominates the influence on the palate fullness is at 2 % addition in case of isomaltulose* and shifted up to 3 % using polydextrose. This is due to the general lower sweetness of polydextrose and the resulting minor effect on the sweetness of the final beer.

On the other hand the addition of fermentable carbohydrates prior to fermentation results in an enhanced yeast metabolism and more active glycolysis, thus leading to an accelerated production rate of pyruvate, acetaldehyde, ethanol and carbon dioxide as shown by [23, 32, 42].

The resulting activation of the glycolysis is reflected in a lower pH value, extract degradation and the development of the alcohol content as common parameter to characterize the fermentation process. The results revealed evidently that in comparison to the non-fermentable carbohydrates and the used standard wort the pH decrease during fermentation is slightly accelerated and the alcohol content is increased, when adding fermentable carbohydrates. The combination of all parameters showed that due to the enhanced yeast metabolism in comparison to the non-fermentable carbohydrates more fermentation by-products are generated.

Additionally to the described effect of the increased osmotic pressure more active glycolysis during fermentation is responsible for the significant influences of fermentable sugars on the sulphite formation [16, 25, 27, 32, 42].

The accelerated production rate and higher availability of intracellular pyruvate and acetaldehyde via glycolysis lead to an improved transformation of sulphite into relatively stable sulphite carbonyl complexes, which can pass the cell wall [16, 25, 27, 32, 42]. The resulting lack of sulphite in the methionine pathway due to transport out of the cell in combination with competitive inhibition of the sulphite reductase [37, 42] due to the accelerated production rate of bicarbonate, seems to inhibit the consumption rate of sulphite by sulphite reductase. The resulting lack of sulphite, methionine and S-adenosylmethionine (SAM) in the cell in comparison to the non-fermentable carbohydrate addition may be responsible for a further activation of the sulphate-reductase metabolism and sulphite production. As long as the acetaldehyde and pyruvate are available, due to the enhanced yeast metabolism any excessively formed, sulphite will be carried out the cell thus being responsible for the higher SO_2-formation during fermentation using fermentable sugars [27, 42]. As demonstrated by the results in figure 3, 8, 10.

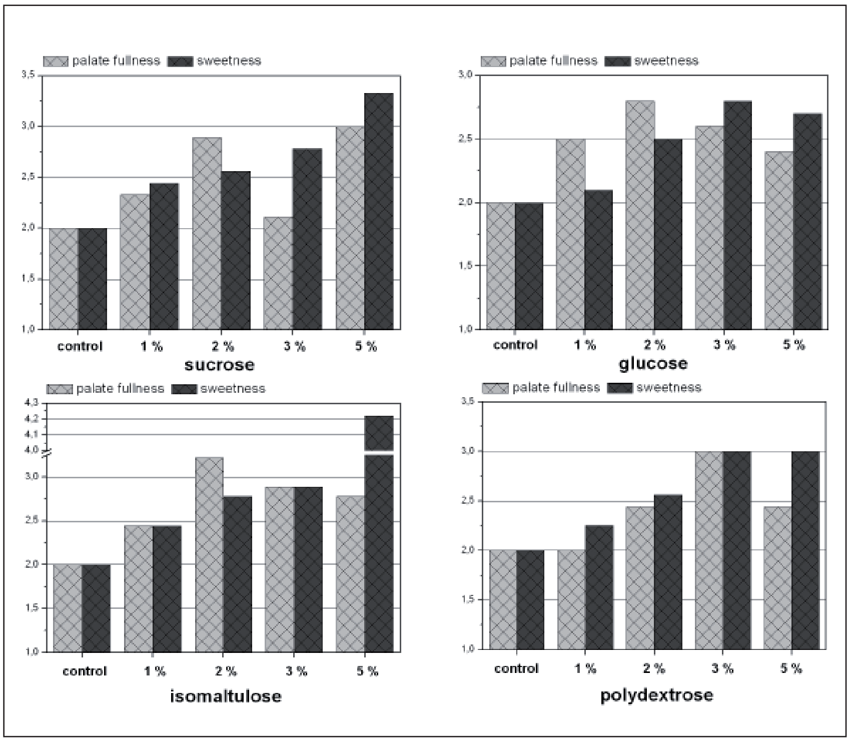

Fig. 9 Sensory analysis with focus on palate fullness and sweetness in the final beer with carbohydrate addition prior fermentation – trial 1

The higher osmotic pressure at the beginning of the fermentation, when using the monosaccharide glucose in order to increase the wort extract, does not necessarily lead to a higher SO_2-formation in comparison to the disaccharide sucrose. This can be explained by the fact that glucose can generally be

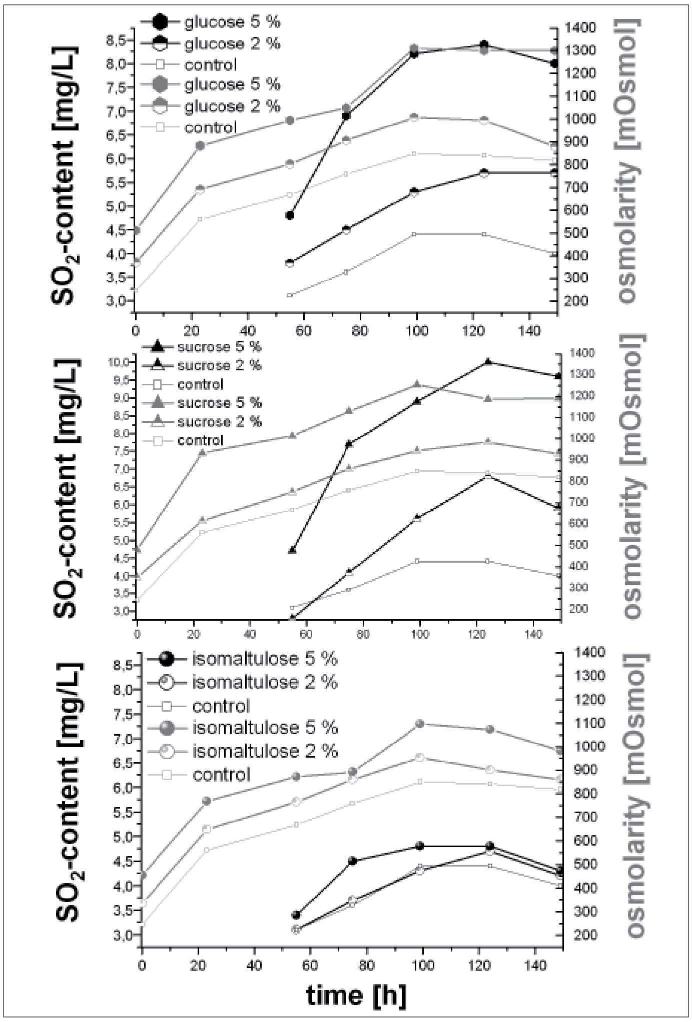

Fig.10 **Correlation between osmotic pressure and SO_2-generation by yeast during fermentation (14 °C) after addition of different carbohydrates (glucose, sucrose, isomaltulose) and concentrations prior Fermentation**

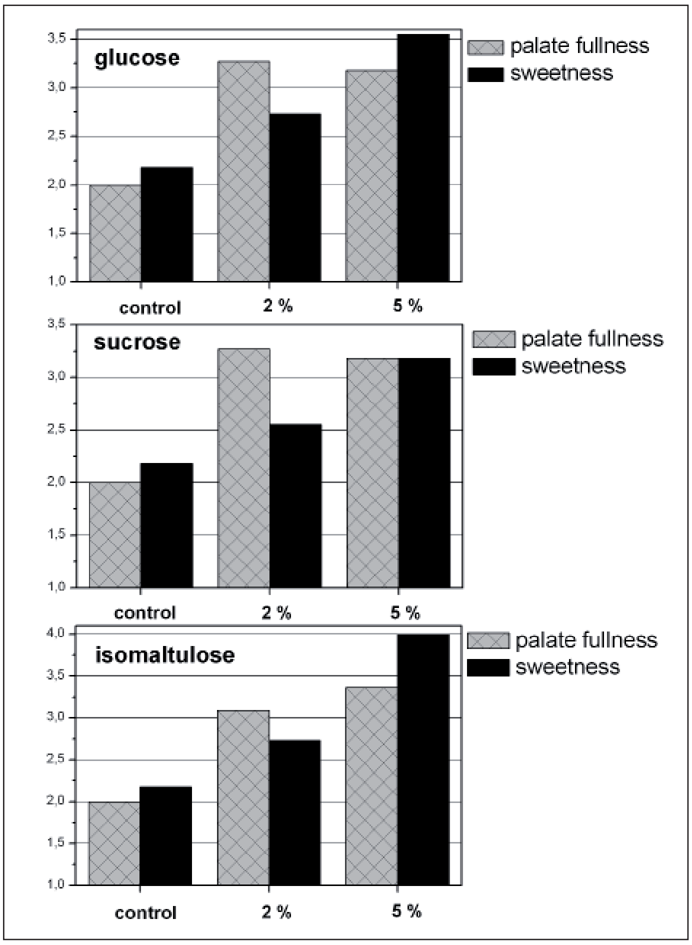

Fig.11 **Sensory analysis with focus on palate fullness and sweetness in the final beer with carbohydrate addition prior fermentation – trial 2**

used much easier within the yeast metabolism than sucrose. As a result the difference in the osmotic pressure is rapidly equalized at the beginning of fermentation resulting in a comparable influence on the SO_2-formation and a comparable or rather slightly higher SO_2-content in the final beer, using the disaccharide sucrose. Additionally, analogical trials have also shown that the addition of fructose leads to a slightly higher SO_2-formation at the end of fermentation in comparison to glucose. Whereas the SO_2-development at the beginning of fermentation is slightly lower. It is concluded that due to the delayed start of the SO_2-formation in the case of sucrose and the generated fructose from sucrose a slightly higher SO_2-content is achieved at the end of the fermentation.

5 Conclusion

The results of the study demonstrate impressively that the increase of the osmotic pressure by addition of fermentable as well as non-fermentable carbohydrates is responsible for a higher formation of SO_2 during fermentation.

The shown additional effect of the higher osmotic pressure on the sulphite production should also be an advantage for high gravity fermentation. With the appropriate process management, the higher SO_2 content in the final beer can improve the oxidative beer and flavor stability. Taking into consideration all data, it can be concluded that increasing the final wort extract up to 2 % and 3 % respectively due to non-fermentable carbohydrates such as isomaltulose* or polydextrose is a useful tool to improve the palate fullness and oxidative stability of beer. A higher sugar addition prior fermentation is inadvisable, because the influence on the sweetness of the beer is enhanced and covers the positive effect on the palate fullness and the typical beer taste.

Using isomaltulose (Palatinose™) this sugar will not contribute to a higher alcohol content or reduced microbiological stability. Additionally, Palatinose™ is fully digestible and provides the same amount of energy (4 kcal/g), but it is much more slowly digested and absorbed. Being fully digestible yet slowly released, it provides glucose to muscles and the brain over a longer period of time with the essential energy of glucose – physiological and nutritional benefits that cannot be achieved with any other non-fermentable carbohydrate [24, 44–46]. The described influences of the osmotic pressure on the SO_2-formation during fermentation in combination with the competitive inhibition of the sulphite reductase [49, 53] due to carbon dioxide (bicarbonate) should also play an important role on the observable additional SO_2-formation during pressure fermentation.

Acknowledgment

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Footnote

* Isomaltulose (Palatinose™) is a product of BENEO GmbH, a member of Südzucker Group.

6. References

1. Adachi, T.; Nonogi, H,; Fuke, T.; Ikuzawa, M.; Fujita, K.; Izumi, T.; Hamano, T.; Mitsuhashi, Y.; Matsuki, Y.; Suzuki, H.; Toyoda, M.; Ito, Y. and Iwaida, M.: On the Combination of Sulphite with Food Ingredients (Aldehydes, Ketones and Sugars), Z. Lebensm. Unters. Forsch. 168, pp. 200 - 205, 1979.
2. Andersen, M. L.; Outtrupk, H. and Skibsted, H.: Potential Antioxidants in Beer Assessed by ESR Spin Trapping, J. Agr. Food Chem. **48**, 8, pp. 3106 - 3111, 2000.
3. Andersen, M. L. and Skibsted, L. H.: Electron Resonance Spin Trapping Identifaction of Radicals during Aerobic Forced Aging of Beer, J. Agric. Food Chem. **46**, pp. 1972 - 1975, 1998.
4. Barker, R. L.; Gracey, D. E. F.; Irwing, A. J.; Pipasts, P. and Leiska, E. (1983): Liberation of staling aldehydes during storage of beer. In: J. Inst. Brew., **89**, pp. 411 - 415.
5. Blomberg, A. and Adler, L. (1989): Roles of glycerol and glycerol-3-phosphate dehydrogenase (NAD+) in acquired osmotolerance of Saccheromyces cerevisiae. In: Journal of Bacteriology, **171**, pp. 1087 - 1092.
6. Bößendörfer, G.: Ein Beitrag zur Geschmacksstabilität des Bieres. In: BRAUWELT, 27 - 28, 2001, pp. 1042 - 1047.
7. Brenner, M. W. and Stern, H.; Technical Quarterly MBAA **7**, p. 150, 1970.
8. Brenner, M. W. and Stern, H.; Birra e Malto **20**, p. 48, 1973.
9. Brewer, J. D. and Fenton, M. S.: The formation of sulphur dioxide during fermentation. In: Proc. Conv. Inst. Brew. Aust. N.Z. Sect. **16** 1980, pp. 155 - 164.
10. Brown, A. D. and Edgley M. In: Genetic Engineering of Osmoregulation, pp. 155 - 164.
11. Bushnell, S. E.; Guinard, J.-X. and Bamforth, C. W.: Effects of Sulfur Dioxide and Polyvinylpolypyrrolidone on the Flavor Stability of Beer as Measured by Sensory and Chemical Analysis, J. Am. Soc. Brew. Chem. **61**, (3), pp. 133 - 141, 2004.
12. Carr, J. D. (1976): The Toxicity of Sulphur Dioxide Towards Certain Lactic Acid Bacteria from Fermented Apple Juice. In: Journal of Applied Bactreriology, **40**, p. 201.
13. Chapon, L.; Chapon, S. and Djeuga, N.(1986): Free Sulfite in Beers – Kinetic Studies. In: ASBC Journal 40 No.**1**,1986, pp. 31 - 39.
14. De Schutter, D. P.; Saison, D.; Delvaux, F.; Derdelinckx, G. and Delvaux, F. R.: The Chemistry of Aging Beer, Beer in Health and Disease Prevention Volume **1**, 2008.
15. Drost, B. W.; van den Berg, R.; Freijee, F. J. M.; van der Velde, E. G. and Hollemans, M. (1990): Flavor stability. In: J. Am. Soc. Brew. Chem., **48**, pp. 124 - 131.
16. Dufour, B. (1989): Alternation of SO_2-production during fermentation. In: Proc. Eur. Brew, Cong. Conv, **22**, pp. 331 - 338.
17. Dufour, J.-P.: Influence of industrial brewing and fermentation working conditions on beer SO_2 level and flavour stability, EBC Congress, pp. 209 - 216, 1991.
18. Forster, R. T.; Samp, E.-J.; Giarratano, C. E.; Fletcher, S.; Miller, M. and Quilliam, W.: Electron Paramagnetic Resonance Studies Comparing Wort Boiling Temperatures and Various Levels of Sulfur Dioxide in Packaged Beer, MBAA Technical Quarterly vol. **42**, no. 3, pp. 209 - 213, 2005.
19. Foster, R. T.; Samp, E. J.; Patino, H. and Barr, D. P.: Electronic Paramagnetic Resonance (EPR) Profiling for Potential Flavor Stability Improvements in Beer, In: MBAA Technical Quarterly **38** (4), pp. 247 - 250, 2001.
20. Forster, C.: Die antioxidative Aktivität von Bier – eine neue Methode zur Verbesserung der Geschmacksstabilität, Mitteilungen österreichisches Getränkeinstitut, 11/12, S. 132-139, 1998.
21. Forster, C.; Schweiger, J.; Narziß, L.; Back, W.; Ushida, M.; Ono, M. and Yanagi, K.: Untersuchungen zur Geschmacksstabilität von Bier mittels Elektronenspinresonanz-Spektroskopie freier Radikale. In: Monatsschrift für Brauwissenschaft **52**, (5/6), pp. 86 - 93, 1999.
22. Guido, L. F.; How do sulfites help to control beer ageing?, Paper based on a lecture given at the Chair J. De Clerck XI, Louvain-la-Neuve, September 8th 2004.
23. Gyllang, H. (1989): Regulation of SO_2 formation during fermentation. In: Proc. Eur. Brew. Conv. Congr., pp. 347 - 354.
24. Holub, I.; Gostner, A.; Theis, S.; Nosek, L.; Kudlich, T.; Melcher, R. and Scheppach, W.: Novel findings on the metabolic effects of the low glycaemic carbohydrate isomaltulose (Palatinose™). British Journal of Nutrition 2010. First view articles, published online by Cambridge University Press 09 March 2010
25. Hysert,, D. W. and Morrison, N. M.: Sulfate Metabolism during Fermentation, In: ASBC Journal **34** (1), pp. 25 - 31, 1975.
26. Ilett, D. R. (1995): Aspects of the Analysis, Role, and Fate of Sulphur Dioxide in Beer – A Review. In: MBAA Quarterly **32** (4) pp. 212 - 221.
27. Joslyn, M. A. and Braverman, J. B. S. (1954): The Chemistry and Technology of the Pretreatment and Preservation of Fruit and Vegetable Products With Sulphur Dioxide and Sulphites. In: Advances in Food Research (5), p. 97.
28. Kaneda, H. (1991): Role of fermentation conditions on flavour stability of beer. In: J. Ferment. Bioeng, **72**, pp. 26 - 30.
29. Kaneda, H. (1992): Effect of pitching yeast an wort preparation on flavor stability of beer. In: J. Ferment. Bioeng, **73**, pp. 456 - 460.
30. Kaneda, H.; Osawa, T.; Kawakishi, S.; Munekata, M. and Koshino, S.: Contribution of carbonyl-bisulfite adduct to beer stability,J. Agr. Food Chem. **42**, pp. 2428 - 2432, 1994.
31. Kaneda, H,; Masachika, T.; Osawa, T.; Kawakishi, S. and Tamaki, T.: Behavior of sulfites during fermentation and storage of beer, J. Am. Soc. Brew: Chem. **54**, pp. 115 - 120, 1996
32. Korch, C.; Mountain, H. A., Gyllang, H., Winge, M. and Brehmer, P.: A mechanism for sulfite production in beer and how to increase sulfite levels by revombinant genetics, EBC Congress Lisbon, pp. 201 - 208, 1995.
33. Kunz, T. : Grundlegendes zur Elektronenspinresonanz-Spektroskopie (ESR) und Untersuchungen zum Zusammenhang zwischen oxidativer Bierstabilität und dem SO_2-Gehalt. In: Monatsschrift für Brauwissenschaft **55** (2002), no. 7/8, pp. 140-152.
34. Kunz, T.: Optimized analysis methods for the determination of SO_2 in beer and malt. In: BRAUWELT International, **27 (2009)**, no. 4, pp. 216 - 220.

35. Kunz, T.; Methner, F.-J.; Kappl, R. and Hüttermann, J. (2005): Method for determining the endogenous antioxidative potential of beverages by means of EPR spectroscopy. Patentanmeldung U30121, TU-Berlin/Universität des Saarlandes, IPAL. DE 10 2005 043 113 A1, US Patent 20080248580.
36. Maw, G. A., Wall. Lab. Comm., 28 (95), 49 (1965).
37. MEBAK. Brautechnische Analysemethoden, Band Würze, Bier, Biermischgetränke. 4th ed. Methodensammlung der Mitteleuropäischen Brautechnischen Analysekommision. Mitteleuropäische Brautechnische Analysekommision. 4. Aufl. Freising-Weihenstephan: Mitteleuropäische Brautechnische Analysekommision.
38. Methner, F.-J.; Kunz, T. and Schön, T.: Application of Optimized Methods to Determine Endogenous Antioxidative Potential of Beer and Other Beverages, EBC Congress 2007.
39. Modderman, J. P.: Focus on Sulfites in Food, J. Assoc. Off. Anal. Chem. Vol.69, 1, pp. 1 - 3. 1985.
40. Narziß, L.; Miedaner, H.; Lustig, S. and Kubrich, L.: Einfluß von biereigenem SO_2 auf die Alterung des Bieres. In: BRAUWELT **135** (1995), no. 49, pp. 2576 - 2606.
41. Narziß, L. (1993): Technological approach to improve flavor stability. In: Tech. Q. Master Brew. Assoc. Am., **30**, pp. 48 - 53.
42. Narziß, L.; Reicheneder, E. and Nothaft, H.: BRAUWELT International 1 (1982), pp. 502 - 509.
43. Nodiov, H. (1985): Formation of sulphur diocide during beer fermentation. In: Proc. Eur. Brew. Cong. Conv., 20, pp. 291 - 298.
44. Pahl, R.; Dörr, T.; Radowski, A. and Hausmanns, S.: Isomaltulose: A new, non-fermentable sugar in beer and beer specialities, BRAUWELT International 28 (2010), 2, pp. 88 - 91.
45. Pahl, R. and Methner, F.-J.: The Applicability of Isomaltulose (PalatinoseTM) in Beer and Beer Specialities and its remarkable Results, Brewing Science **61** (2008), no. 3/4, pp. 49 - 55.
46. Pahl, R.; Methner, F.-J.; Schneider, J.; Kowalczyk, J.; Hausmanns, S. and Radowski, A.: New study on isomaltulose shows remarkable results, BRAUWELT International, **26** (2008), no. 3, pp. 172 - 175.
47. Piendl, A.; Speckner, J.; Draxinger, A. and Wilnhammer, J. (1980): Schwefeldioxid im Bier. Bedeutung, Vorkommen und Beeinflussung. BRAUWELT **120** (1980), pp. 1746 - 1762.
48. Ryder, D. S. (1989): A question of flavor stability or instability. In: Proc. Inst. Brew. Central Southern African Sect. Conv., pp. 366 - 391.
49. Ryder, D. S. (1990): EBC Biochemistry Group Bulletin, 1990, p. 219
50. Sentko, A. and Willibald-Ettle, I. (2007): Isomaltulose . In: Ingredients Handbook Sweeteners, pp. 179 - 186.
51. Stratford, M. and Rose, A. H.: Journal of General Microbiology 1896, **132**, pp. 1 - 6.
52. Thalacker, R. and Kaltwasser: Zur lebensmittelrechtlichen Beurteilung des Schwefeldioxidgehalts von Bier, In: Mschr., Brauerei **34** (1981), pp. 115 - 118, 1981.
53. Umemoto, S. (1994): Effect of doubling fermentation method on sulphite content in beer. In: J. Ferment. Bioeng. (78), pp. 333 - 335.
54. Uchida, M. and Ono, M.: Determination of Hydrogen Peroxide in Beer and Its Role in Beer Oxidation, J. Am. Soc. Brew. Chem. **57**, 4, pp. 145 - 150, 1999.
55. Uchida, M.; Suga, S. and Ono, M.: Improvement for oxidative flavour stability of beer – rapid prediction method for beer flavour stability by electron spin resonance spectroscopy,J. Am. Soc. Brew. Chem.54, 4, pp. 198 - 204, 1996.
56. Wackerbauer, K. and Hardt, R.: Radikalreaktionen und die Geschmacksstabilität des Bieres, BRAUWELT **136** (1996), no. 40/41, pp. 1880 - 1888.

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