R. Folz, R. Hofmann and U. Stahl

Impact of Permeation of O_2 and CO_2 on the Growth Behaviour of *Saccharomyces diastaticus* in Beer

Different bottle types had been tested on their permeation characteristics (including oxygen ingress and carbonation retention) and the microbiological stability of the bottled beer. The microbiological stability was assessed by measuring the turbidity and the concentration of colony forming units (CFU). Measurement of the CO_2 content and the turbidity was done in beer; evaluation of the oxygen ingress had to be done in especially prepared distilled water since beer itself is oxygen-consuming. In case of microbiological growth the colonies were checked with macroscopic and microscopic methods for exclusion of unwanted microorganisms leading to an erroneous positive result.

The results of the study showed the combined influence of oxygen uptake and loss of carbon dioxide on the microbiological stability of beer. With the help of oxygen consuming and passive barrier materials the turbidity in the test beer with and without inoculated microorganisms could be kept at a lower level. Especially the inoculation with *Saccharomyces diastaticus* showed increasing turbidity and concentrations of colony forming units over time. For contamination with *Saccharomyces diastaticus* glass bottles and plastic bottles with combined passive gas and oxygen quenching barrier enhancement showed significantly lower turbidity and concentrations of colony forming units than the other tested bottle formulations.

Descriptors: PET, permeation, microbiological stability, oxygen, turbidity

1 Introduction

The use of plastic packaging is becoming more common in the beverage industry. Due to new developments in the packaging market, the use of plastic materials can be an option to expand the market for even quality sensitive products. At present, there are continuing developments to further improve the barrier characteristics of polymer materials such as PET (multilayer, internal coating, external coating and blends).

Monolayer PET bottles show oxygen uptake rates of 0.5 mg/L per month and higher. Simultanuous to the oxygen uptake a loss of carbon dioxide in a range of approximately 5 % – in regard to a starting carbon dioxide concentration of 5 to 6 g/L – can be expected. Similar data has been proved and published several times in research projects during the last decade [1, 2, 4, 5, 6, 7, 8]. Due to barrier enhancing developments, as the ones mentioned above, the rates for uptake of oxygen could be lowered to a zero-uptake-level over a period of six months and more. Technologies which are able to quench oxygen molecules in the bottle wall were used to achieve a steady state for the oxygen content [4, 9]. With respect to the CO_2 retention an enormous reduction of the losses could be achieved as well. Depending on the type of barrier enhancement which was used, a level of 1 to 3 % absolute loss of carbon dioxide per month is realistic for state of the art barrier enhanced PET bottles [1, 2, 4, 5, 6, 7, 8, 9].

The influence of the packaging material on the microbiological stability of beer came into focus during the unexpected contamination of different PET bottle samples which were tested for their gas permeability. After approximately three months samples with higher CO_2 levels and visible sedimentation occurred. All tests with bottles indicating abnormal conditions had positive results when tested on microbiological contamination. Additionally, the sensorial evaluation of the samples showed off-flavours. These off-flavours could be directly connected to the microbiological contamination.

The following microorganisms were found and in part identified:

Saccharomyces diastaticus

Saccharomyces wild type yeast

Wild type yeast (film-forming)

Micrococcus spec.

The microbiological tests were widened on all types of bottles tested within the project. The tests indicated that all types of bottles were randomly contaminated but only for bottles with low barrier performance an influence on the overall product quality could be observed. The uncontrolled contamination of the

Authors:

Dr.-Ing Roland Folz, Dipl.-Ing. Ruslan Hofmann, Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V., Department Brewing and Beverage Science & Applications; Prof. Dr.-Ing. Ulf Stahl, Berlin University of Technology (TU Berlin), Institute of Biotechnology, Department of Microbiology and Genetics; corresponding author: folz@vlb-berlin.org

Table and figures see Appendix

bottled beer led to visible losses in terms of product quality, yet not all bottles showed a visible influence of the contamination. Since the contamination was not controlled it was not possible to reach a definitive conclusion regarding a possible influence of the barrier quality on the microbial stability but a dependence on the permeation of oxygen and carbon dioxide seemed to be a reason.

To understand the needs of proper barrier characteristics a brief look into the influences of the most important inorganic gases for packaging of beer is necessary. These two important inorganic gases are oxygen on the one hand and carbon dioxide on the other. Generally the absence of oxygen would be perfect for the aspect of beer flavour stability. Besides temperature, light and agitation, oxygen is beer's "arch-enemy" [10]. With the exception of the moment when the yeast is added to the pitching wort ("rousing"), any oxygen uptake should be avoided. A concentration of oxygen in the pitching wort in a range of 8 to 10 mg/L is considered to be ideal for a good fermentation process [3].

Several oxidative and non-oxidative reactions are generally supposed to decrease the beer quality [17, 18]. Research of Kaneda et al. [12] indicated that hydroxyl radicals are the reason for the oxidation reactions since the oxygen molecule itself is stable and not very reactive. Possible pathways for the activation of the oxygen molecules are the Fenton and the Haber-Weiß reactions. The activation of oxygen is catalysed by iron or copper ions [13, 14].

In contrast to oxygen the carbon dioxide should be kept in the packaged beer. Carbon dioxide has no direct influence on taste or flavour of the beer but has a huge impact on the foam quality and the mouth-feel. Therefore, it positively contributes to the essential quality parameters of the beer.

Concerning microbial stability a carbon dioxide content of approximately 5 g/L is toxic for many microorganisms. Oxygen on the other hand is essential for all strictly aerobic microorganisms. Aside from the anaerobic conditions and the content of carbon dioxide, beer has further positive characteristics with regard to microbial stability. Parameters with positive influence on the general microbial stability can be:

- A pH-value of less than 5.0.
- Beer contains alcohol in concentrations of approx. 5 %, depending on the type of beer.
- Hop components have anti-microbial properties.
- The carbon sources in finished beer are limited.

Due to the selective properties of beer – mentioned above – Back [11] suggested distinguishing microorganisms into 5 categories. These categories are:

- Obligatory spoilage microorganisms (category I)
- Potentially damaging microorganisms (category II)
- Indirect beer spoilage microorganisms (category III)
- Indicator microorganisms (category IV) and
- Latent microorganisms (category V)

Microorganisms of category I are able to reproduce in beer and lead to changes in flavour, sedimentations or increased turbidity. Category I includes hetero- and homo-fermentative *Lactobacillus species*, *Pediococcus damnosus*, *Pectinatus*, *Saccharomyces diastaticus*, *Megasphera* or *Brettanomyces*.

Potentially harmful microorganisms are not able to reproduce under standard beer conditions. In case the beer contains less hop components, lower ethanol content or higher sugar contents organisms of category II might be able to reproduce and spoil the product. Organisms to mention are *Saccharomyces* (wild) yeasts and species of lactic acid producing bacteria other than the ones mentioned in category I.

The microorganisms of the categories III-V can spoil the raw materials, intermediate products or indicate microbial spoilage (weak points) without being generally harmful for the finished product.

The categories II-IV include several microorganisms with a certain oxygen demand. In case of a beer which has enough free oxygen available, spoilage with aerobe organisms could be possible. Film-forming yeasts or carbon dioxide tolerant aerobe bacteria (e.g. *Pichia ssp.*, *Acetobacter ssp.*) could be enabled to reproduce in beer.

The fact that barrier enhanced PET bottles can preserve the flavour of beer better than PET monolayer bottles without additional barrier has been previously published [6, 8, 9]. The goal of the research was to observe the influence of carbon dioxide and oxygen permeation on the microbiological stability of beer filled in PET bottles. In the packaging of food the use of oxygen scavengers and the packaging under CO_2 atmosphere are common practices to reduce microbial growth and, therefore, extend the product's shelf life [21, 22, 23, 24]. Beer itself is generally packed under CO_2 atmosphere and absence of oxygen. The question was, if the permeability of PET bottles during a typical shelf life period of six months does contribute in a matter that a significant decrease of microbiological stability in beer – compared to beer in glass bottles – can be detected.

The identification of the parameters influencing the permeation process and the microbiological stability should be fulfilled by testing four different types of PET bottles with varying barrier properties. The tests should be carried out under laboratory conditions and real kinetics. Under controlled conditions (including starting oxygen and carbon dioxide content, pH-value and other product parameters) single parameters were varied to reach a conclusion regarding their influence. A controlled contamination of beer in different PET bottles was the main focus. To evaluate the quality of the different PET bottles their barrier performance was checked with the help of methods for the permeation control.

The results of the mentioned test should lead to a conclusion on the correlation of barrier performance parameters for PET bottles with microbial growth parameters for different types of microorganisms.

2 Materials and methods

Within the research project four different types of transparent PET bottles were tested. All samples were sealed with an aluminium foil using heat induction. As reference brown glass bottles closed with crown corks were used. Bottle volume was 0.5 L for all tested bottle types.

To evaluate the overall quality of the bottles the permeation rate of oxygen and carbon dioxide was checked. The carbonation retention was tested in beer and measured with the help of Hach Lange's Orbisphere Micro Logger 3654. The measurements were carried out monthly over a period of six months. The number of samples per data point was n = 5.

A so called "Glovebox-Method" was used in order to measure the oxygen uptake. The empty sample bottles were prepared with a special sensor spot (Pst6 sensor). Citric acid (8 g) and bicarbonate of sodium (5 g) were added to simulate beer pH (4.3) and carbon dioxide content (approx. 5.2 g/L). Distilled water was used instead of beer to ensure that the sample liquid cannot consume permeated oxygen. 495 g of distilled water were filled into every test bottle. Inside the Glovebox the empty sample bottles, the closures and distilled water were permanently flushed with nitrogen (Stickstoff 5.0 from Air Liquide). With the help of an optical measurement method (Fibox 3 trace oxygen meter; PreSens Precisions Sensing GmbH) the oxygen content inside the Glovebox could be controlled and adjusted. A description of the oxygen measurement method was published by *Huber et al.* [15]. At the desired air-saturation inside the Glovebox (approx. 2 %) the nitrogen flow was lowered to a level of steady-state air-saturation inside the Glovebox. During this steady-state-phase the test bottles for were filled. **Figure 1** shows the correlation of oxygen concentration in the Glovebox at the moment the bottles were filled inside the Glovebox ("steady-state-phase") and the dissolved oxygen concentration measured later in the (water-) filled bottle after closing and shaking into equilibrium. Due to the sensor spot on the inner surface of the bottles, the dissolved oxygen concentration could be measured with the help of PreSens's optical measurement system which was also used to control the oxygen concentration inside the Glovebox. Number of samples was again n = 5. Measurement was carried out over a period of six months.

The test to evaluate the microbiological stability of beer in PET bottles was carried out in original medium. The beer used within these trials was of the same brand. Glass bottles were filled directly in the brewery and PET bottles were filled in the pilot brewery of the VLB Berlin using kegged beer. Analysis of the beer parameters was done using the Beer alcolyzer (Anton Paar GmbH). Furthermore, CO_2 content, colour, pH-value and bitter units were measured using the respective EBC methods (MEBAK Bd. II, Kap. 2.13.2, 2.14 and 2.18.1).

The beer used for the study did not show any difference in beer parameters other than the starting carbon dioxide content which was 5.2 g/L for the glass bottles and slightly lower with 4.8 g/L for PET bottles.

Five different strains of microorganisms were tested for their reproduction over a period of six months. All organisms were reference organisms of the collection of the Institut für Gärungsgewerbe und Biotechnologie e.V. (IfGB). Following microorganisms were used:

Lactobacillus brevis	strain: La-0415 (category I)
Saccharomyces diastaticus	strain: Sa-1108 (category I)
Micrococcus kristinae	strain: Mi-0302 (category II)
Gluconobacter oxydans	strain: Gl-0103 (category IV)
Pichia membranaefaciens	strain: Pi-0518 (category III)

The microorganisms were cultivated in beer to appropriate cell counts (titre solution). The concentrations of the titre solutions were checked using suitable Thoma chambers for bacteria respectively yeasts. The beer-filled bottles were inoculated with suitable volumes of the titre solutions for each of the five microorganisms inside the Glovebox and than re-closed. The anaerobic atmosphere in the glovebox was necessary to keep the oxygen ingress during inoculation as low as possible. Air-saturation inside the Glovebox was < 0.5 %. Reference bottles without microorganisms were opened and re-closed inside the Glovebox without adding any solution.

The artificially contaminated as well as the reference samples were stored over a period of six months at ambient conditions (approx. 21 °C) and dark. Weekly measurements of turbidity (haze) were carried out as well as monthly determinations of the cell count.

The increase in haze respectively turbidity was calculated as follows:

$$\Delta HZ = HZ_{t=x} - HZ_{t=0}$$

$$\text{HZ: Haze } (90°/25°) \qquad \text{(Eq. 1)}$$

The measurement of the turbidity was carried out by using the optical method described in MEBAK Bd. II Kap. 2.15.1.2. The device used for the turbidity measurement was the LabScat 90°/25° from company Sigrist. The evaluation of turbidity is expressed in EBC formazine units. For high turbidity levels with sedimentation the samples had to be shaken to ensure a homogeneous distribution of the particles. Thus, all samples were shaken prior to the measurement. After shaking the samples rested for exactly 60 seconds to avoid an increased turbidity level due to CO_2 bubbles. Turbidity was determined twice until both values were equal. The values for 90° and 25° were recorded. To evaluate the microbial growth the turbidity 25° has been used since the particle size of the microorganisms can be expected to be larger than 0.3 µm. Referring to Pöschl [16] the measurement at an angle of 25° should result in higher values compared to the 90°-values at a medium particle size of more than 0.3 µm.

For the monthly determinations of the cell count the bottles had to be opened and appropriate volumes of beer from the sample bottles were cultivated on media suitable for the respective microorganisms that the beer was inoculated with.

Media for cultivating the different microorganisms were:

1) Wort-Agar (beer wort; extract 11.5 %, pH: 5.5)

- *Saccharomyces diastaticus*
- *Pichia membranaefaciens*

2) Standard-I-Agar (Fa. Merck KGaA; pH: 7.5 ± 0.2 at 25 °C)

- *Micrococcus kristinae*

3) YED-Agar (Fa. Merck KGaA; indicator: bromkresolgreen 0.28 %)

- *Saccharomyces diastaticus*
- *Pichia membranaefaciens*
- *Gluconobacter oxydans*

4) MRS-Agar (MRS Bouillon; CARL ROTH GMBH + Co. KG)

- *Lactobacillus brevis*

Inoculation of the cultivation media was carried out using either membrane filtration or by the Drigalski-method on solid media (MEBAK Bd. III Kap. 10.11.1.1 and MEBAK Bd. III Kap. 10.11.1.2). If the appropriate sample volume exceeded 1 mL the sample was membrane filtrated. If the sample had to be diluted to get processable cell concentrations, dilution was done prior to the membrane filtration in order to receive a suitable volume and concentration of the sample.

Cultivation of the microorganisms *Saccharomyces diastaticus*, *Gluconobacter oxydans*, *Micrococcus kristinae* and *Pichia membranaefaciens* was carried out under aerobic conditions. The samples of *Lactobacillus brevis* were cultivated under anaerobic conditions. The conditions for cultivation were as follows: aerobic samples were cultivated for three days and anaerobic samples for five to seven days. Temperature was held at 28 °C.

Determination of the cell count and the purity of the culture were carried out macroscopic and microscopic. Cell concentration was determined by counting the formed colonies (MEBAK Bd. III Kap. 10.11.4.2 respectively 10.11.4.3). Prior to membrane filtration or the Drigalski-method the cell concentration was determined with the help of a suitable Thoma counting chamber (for bacteria or yeast cells; MEBAK Bd. III Kap. 10.11.4.4) using a microscope. The determination of total cell concentration was necessary to calculate a suitable sample volume or dilution for the following inoculation of the media.

3 Results and discussion

Although five different organisms were tested, only the results for *Saccharomyces diastaticus* shall be discussed. The tests with the other four microorganisms indicated similar correlations between permeability and microbial growth but the most significant results could be observed for *Saccharomyces diastaticus*.

Over a period of six months (26 weeks) the ingress of oxygen into the bottle was evaluated. The oxygen ingress was tested for all four PET bottle formulations and is shown in **figure 2**. The bottles were prepared using the Glovebox method as described in the chapter materials and methods. The graphs show the absolute oxygen ingress starting from a level of less than 0.1 ppm oxygen in the fluid after filling/preparation.

The carbonation retention was tested for all four PET bottle formulations and the glass bottles over a period of six months (26 weeks). All sample bottles were filled with beer. The corresponding graphs of the carbonation retention for the five bottle types are shown in **figure 3**. The starting level of carbonation was 5.2 g/L for the glass bottles closed with crown corks and 4.8 g/L for the PET bottles with sealed finishes.

The permeation characteristics showed the expected results. The highest uptake of oxygen and loss of carbon dioxide could be observed for the Monolayer PET bottles without additional barrier (PET 3). When a passive barrier material was added (PET 4) the uptake of oxygen could be slightly lowered and the loss of carbon dioxide was about 40 % less than without passive barrier enhancement. The addition of a selective oxygen barrier (PET 1) kept the oxygen concentration in the test fluid at the starting level but had no influence on the carbon dioxide retention. The best results were achieved when a combination of both barrier technologies was used. With the help of bottles used as PET 2 no oxygen uptake and a decrease of the carbon dioxide loss of approximately 40 % compared to the Monolayer bottle without additional barrier (PET 3) could be achieved.

In **figure 4 and 5** results of the turbidity measurements are shown. The turbidity was measured over a period of 6 months (26 weeks) for all five bottle types. The graphs show the increase of haze at 25° as the mean of two bottles. Generally the graphs for the values at an scattering angle of 25° did not show qualitative differences to the graphs for angle of 90°.

The turbidity due to the beer itself (**Fig. 4**) was measured with reference bottles which have not been artificially contaminated. A control of microbiological growth after completion of the study did not show any evidence for microbial growth in the reference beer.

With the help of the given equation it was possible to filter out the staring haze level of the beer and a possible influence of the bottle material onto the turbidity measurement. So, the increase of turbidity due to reactions of the beer could be observed and the influence of the bottle material regarding its influence on non-biological stability of the beer evaluated. Reasons for the increased turbidity can be oxidation or polymerisation reactions of polyphenols and others [3, 10, 16, 19, 20].

The results show that the Monolayer PET bottles (PET 3) had the highest increase of turbidity. After four to five months the turbidity reached a level of 2 EBC which is known to be the range where turbidity can be detected with the human eye [16]. After five to six months the same level was detected for the bottles with an additional passive barrier (PET 4). In contrast the glass bottles and the PET bottles with oxygen consuming barrier material (PET 1 and

2) did not show a significant increase of the beer‘s turbidity. The increase of the turbidity can be correlated to the increased uptake of oxygen which was detected for the PET bottle formulations 3 and 4. The higher the oxygen uptake the faster was the increase in turbidity and, therefore, the loss of colloidal stability. Since the occurring oxidation reactions cannot be specified here a correlation of a certain oxygen concentration and a visible haze formation (> 2 EBC) is not possible.

Since oxidation reactions are known to produce haze in beer [3, 10, 16, 19, 20] a direct correlation of the oxygen uptake and the increase of turbidity could be expected. The results of the contamination-free beer samples in different packaging materials could clearly show the influence of the oxygen uptake on the haze formation in beer. The bottles with the highest uptake of oxygen (PET 3 and 4) showed the highest turbidity levels and the bottles with effective oxygen barriers (PET 1 and 2) the opposite.

Figure 5 shows the results for the increase of turbidity when the bottled beer was contaminated with *Saccharomyces diastaticus*. The yeast can be categorised as an obligate beer spoilage organism and was expected to grow in all samples due to the fact that the yeast is a so called super-attenuating organism. *Saccharomyces diastaticus* is able to ferment dextrins and the product beer is suitable for the organism to reproduce.

The results show different growth behaviour for *Saccharomyces diastaticus* depending on the bottle material. The highest turbidity increase was measured for the Monolayer PET bottles without additional barrier (PET 3). After five weeks these bottles showed a visible turbidity (> 2 EBC) and reached a maximum value of approximately 150 EBC after ten to twelve weeks. After eight weeks the bottles of PET 4 (with additional passive barrier material) had a visible haze formation and after 14 weeks PET-4-bottles reached their maximum turbidity of about 140 EBC. The bottles of PET 1 (oxygen selective barrier) showed visible haze after seven weeks. The increase of turbidity was slower compared to PET 3 and 4 (no selective oxygen barrier) and also the maximum turbidity level of 60 EBC, reached after 15 weeks, was significantly lower than for PET 3 and 4 (without selective oxygen barrier). The PET bottles containing oxygen consuming components and an additional passive barrier (PET 2) had visible haze formation after 14 weeks. By the end of the study they reached a maximum turbidity level of 10 EBC. The lowest turbidity increase was detected for the glass bottles. The haze formation was visible after 19 weeks and was at 5 EBC after 23 weeks of storage.

The starting concentration of cells in the artificially contaminated bottles was calculated to approx. 6 CFU/mL for *Saccharomyces diastaticus* by adding 0.1 mL of an appropriate dilution of a titre solution with known cell count (determined with a Thoma chamber for yeasts). The results for the tests on growth of microorganisms were positive for all tested bottle types as can be seen in **figure 6**. Differences between the different bottle types could be observed for the growth rates and the overall cell concentration in the samples.

The fastest growth and highest cell count could be observed for PET bottles with high oxygen uptake (PET 3 and 4). The slowest growth of *Saccharomyces diastaticus* was detected in the bottles containing oxygen consuming and passive barrier enhancements (no measurable oxygen uptake – PET 2). In-between were the PET bottles with only oxygen consuming barrier material but no passive barrier enhancement (PET 1). During the first two months the glass bottles did not show any increase in the colony forming units (CFU) but after the test period of five month the concentrations were on the same level as the PET-2-bottles with very good oxygen barrier and acceptable carbon dioxide retention.

The results show a direct influence of especially the oxygen barrier on the growth of microorganisms. In the case of PET bottles with passive barrier and additionally oxygen consuming characteristics (PET 2 – low oxygen uptake and good carbonation retention) the microbiological stability of beer was similar to the microbiological stability of beer in glass bottles. Compared to the PET bottles without additional barrier the time until the microbial contamination could be detected with the human eye (turbidity of approximately 2 EBC) was 2.5 to 3 times longer. A possible influence of the carbon dioxide retention on the growth of *Saccharomyces diastaticus* could not be observed directly. Still the early start of haze formation in PET 1 (low oxygen uptake but accelerated CO_2 losses compared to the bottles with only passive barrier – PET 4) and the zero-growth at the beginning for the glass bottles are indicators for an inhibition of the microbial growth due to higher carbon dioxide concentrations in the beer.

The results of the study show that oxygen and carbon dioxide permeation are not only important parameters for the flavour and colloidal stability of beer. For a long term microbial stability the use of sufficient barrier materials is of great importance. Aside from the absence of oxygen, good carbonation retention has to be present to preserve the natural growth-inhibiting characteristics of beer. Regarding the tested barrier PET bottles the best results could be achieved for the combination of oxygen consuming barrier material with added passive barrier characteristics (PET 2). The growth rates and the increase of turbidity were similar to the reference glass bottles for PET 2.

A good compromise between effective packaging material and useful methods for minimising microbiological risks during production and filling can lead to minimised reclamation of products. Of course good packaging cannot replace efficient cleaning and disinfection but the right choice of packaging material can help to ensure a longer microbial stability of the product.

4 Conclusions

Plastic packaging is a growing market in the beverage industry and is suitable even for oxygen sensitive products. The goal of the research work was to correlate possible influencing factors for the permeation of inorganic gases with the microbiological stability of beer. The need for sufficient barrier material for the packaging of beer can therefore be concluded from the results of the microbiological tests.

The different bottle types had been tested on their permeation characteristics (including oxygen ingress and carbonation retention) and the microbiological stability of the bottled beer. The microbiological stability was assessed by measuring the turbidity and the concentration of colony forming units (CFU). Therefore the filled beer was artificially contaminated with the microorganisms *Saccharomyces diastaticus*, *Micrococcus kristinae*, *Gluconobacter oxydans*, *Pichia membranaefaciens* and *Lactobacillus brevis*. The volume of the bottles was the same for plastic and glass (0.5 L). Measurements of the CO_2 content and the turbidity were done in beer; evaluation of the oxygen ingress had to be done in especially prepared distilled water since beer itself is oxygen-consuming. The glass bottles had been closed with standard crown corks; the PET bottles were sealed with aluminium foils to avoid additional permeation via the closure. In case of microbiological growth the colonies were checked with macroscopic and microscopic methods for exclusion of unwanted microorganisms leading to an erroneous positive result.

The results of the study showed the combined influence of oxygen uptake and loss of carbon dioxide on the microbiological stability of beer. With the help of oxygen consuming and passive barrier materials the loss of carbon dioxide could be reduced by 40 % and no oxygen uptake could be measured. Both led to a significantly lower turbidity in the test beer with and without inoculated microorganisms. Especially the inoculation with *Saccharomyces diastaticus* showed increasing turbidity and concentrations of colony forming units over time. For contamination with *Saccharomyces diastaticus* glass bottles and plastic bottles with combined passive gas and oxygen quenching barrier enhancement showed significantly lower turbidity and concentrations of colony forming units than the other tested bottle formulations. Thus, the use of plastic bottles with insufficient barrier properties leads to a more rapid loss of microbiological stability and consequently to a decrease of the product's quality. A high oxygen uptake might also give microorganisms which are not considered as typical beer spoiling organisms, e.g. aerobic bacteria, the ability to grow. To identify the higher risk potentials for beer, packaged in low-barrier bottles, further studies will be necessary. It has to be identified in which way the microorganisms and the beer ingredients compete for the available oxygen molecules. Furthermore, a classification of the (potentially) beer spoiling organisms due to their oxygen demand could be useful in this case. At present a set up of suggested limits for the oxygen uptake cannot be made since the kinetics of the oxygen consumption in the (contaminated) beer could not be observed. Therefore, a direct correlation of a certain uptake of oxygen with the accelerated growth behaviour can yet not be concluded.

5 References

1 Methner, F.-J.; Peters, U. and Mock, K.: Vergleich verschiedener PET-Flaschen für Bier, BRAUWELT, **141** (2001), pp. 1101-1107.

2 Mette, M: Stoffaustausch – Ein Beitrag zur Gasdurchlässigkeit permeabler Getränkeflaschen unter dem Aspekt der Haltbarkeit des Füllgutes – Teil 2, BRAUWELT, **140** (2000), pp. 202-208.

3 Narziß, L.: Abriß der Bierbrauerei, 6. Aufl., Ferdinand Enke Verlag, Stuttgart, 1995; p. 181.

4 Orzinski, M.; Embs, F. W.; Schneider, J.; Weber, I. and Fritsch, K.: Monolayer-Barriere-Blend-PETFlaschen – Untersuchung zur Eignung für die Bierabfüllung, Brauindustrie, **91** (2006), no. 11, pp. 50-57.

5 Orzinski, M.; Weber, I. and Schneider, J.: New Requirements of the Measurements of Permeation through Plastic Bottles and Closures, MBAA Tech. Quart., **42** (2005), pp. 346-351.

6 Orzinski, M.: Untersuchung der Permeation von anorganischen Gasen und organischen Verbindungen durch barriereverbesserte Kunststoffflaschen und ihre messtechnische Erfassung, Diss., TU Berlin, 2007.

7 Orzinski, M.; Schneider, J. and Weber, I.: Hohe Genauigkeit und gute Reproduzierbarkeit, Permeationsmessungen an der VLB Berlin, Brauindustrie **89** (2004), no. 11, pp. 66-71.

8 Folz, R.; Hofmann, R.; Pahl, R. and Weber, I.: Bier in PET – eine Qualitätsdiskussion, BRAUWELT **149** (2009), no. 7, pp. 166-169.

9 Folz, R. and Hofmann, R.: Are Plastic Bottles Ready to Replace Glass as a Beer Package?, MBAA Tech. Quart., doi:10.1094/TQ-47-3-0720-01.

10 Kunze, W.: Technologie Brauer und Mälzer, 9. Aufl., Verlag der VLB Berlin, 2007.

11 Back, W.: Farbatlas und Handbuch der Getränkemikrobiologie. Teil I, Hans Carl Verlag, Nürnberg, 1994.

12 Kaneda, H.; Kobayashi, N.; Takashio; M., Tamaki; T. and Shinotsuka K.: Beer Staling Mechanism; MBAA Tech. Quart. **36** (1999), pp. 41-47.

13 Kunz, T.; Stephan, A.; Methner, F.-J.; Kappl, R. and Hüttermann, J.: Grundlegendes zur Elektronenspinresonanz-Spektroskopie (ESR) und Untersuchungen zum Zusammenhang zwischen oxidativer Bierstabilität und dem SO_2-Gehalt, Monatsschrift für Brauwissenschaft **55** (2002), no. 7/8, pp. 140-153.

14 Uchida, M. and Ono, M.: Improvement for Oxidation Flavor Stability of Beer – Role of OH-Radical in Beer Oxidation, J. Am. Soc. Brew. Chem. **54** (1996), no. 4, pp. 198-204.

15 Huber, C.; Nguyen, T.-A.; Krause, C.; Humele, H. and Stangelmayer, A.: Oxygen Ingress Measurement into PET-bottles using Optical-Chemical Sensor Technology, Monatsschrift für Brauwissenschaft **59** (2006), no. 11/12, pp. 5-15.

16 Pöschl, M. R.: Die kolloidale Stabilität untergäriger Biere – Einflussmöglichkeiten und Vorhersagbarkeit, Dissertation; TU München, 2008

17 Drost, B.W.; van den Berg, R.; Freijee, F.J.M.; van der Velde, E.G. and Hollemans, M.: Flavor Stability, J. Am. Soc. Brew. Chem. **48** (1990), pp. 124-131.

18 Lustig, S.: Das Verhalten flüchtiger Aromastoffe bei der Lagerung von Flaschenbier und deren technologische Beeinflussung beim Brauprozess, Diss., TU München, 1994.

19 Kusche, M.: Kolloidale Trübungen in untergärigen Bieren – Entstehung, Vorhersage und Stabilisierungsmaßnahmen, Diss., TU München, 2005.

20 Kusche, M. and Geiger, E.: Kinetics of haze formation in beer – turbidity and flavan-3-ols, BrewingScience, **60** (2007), no. 1/2, pp. 38-47.

21 Cutter, C.N.: Microbial Control by Packaging: A Review, Critical Reviews in Food Science and Nutrition 42 (2002), no. 2, pp. 151-161.

22 López-Rubio, A.; Almenar, E.; Hernandez-Muñoz, P.; Lagarón, J.M.; Catalá, R. and Gavara, R.: Overview of Active Polymer-Based Packaging Technologies for Food Applications, Food Reviews International **20** (2004), no. 4, 2004, pp. 357-387.

23 Coma, V.: Bioactive packaging technologies for extended shelf life of meat-based products, Meat Science **78** (2008), no. 1/2, pp. 90-103.

24 Ahvenainen, R.: Novel food packaging techniques, Woodhead Publishing Limited, Cambridge, 2003.

Received 16 May 2011, accepted 27 June, 2011

Appendix

Table 1 List of bottles used for the research project

Number	Abbreviation	Material	Barrier type
1	PET 1	PET Monolayer blend	Oxygen capturing
2	PET 2	PET Monolayer blend	Oxygen capturing and passive barrier
3	PET 3	PET Monolayer	No additional barrier
4	PET 4	PET Monolayer blend	Passive barrier
5	Glass	Glass	No additional barrier

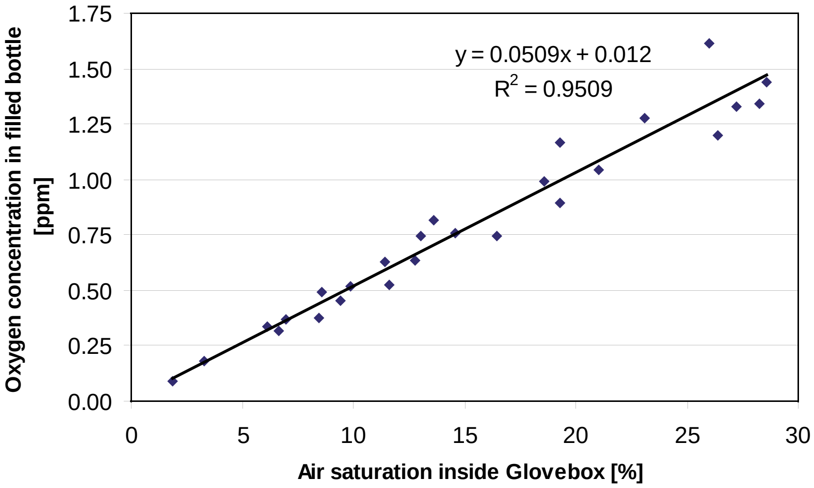

Fig. 1 Correlation of the air saturation in the Glovebox and the oxygen concentration in the filled bottle

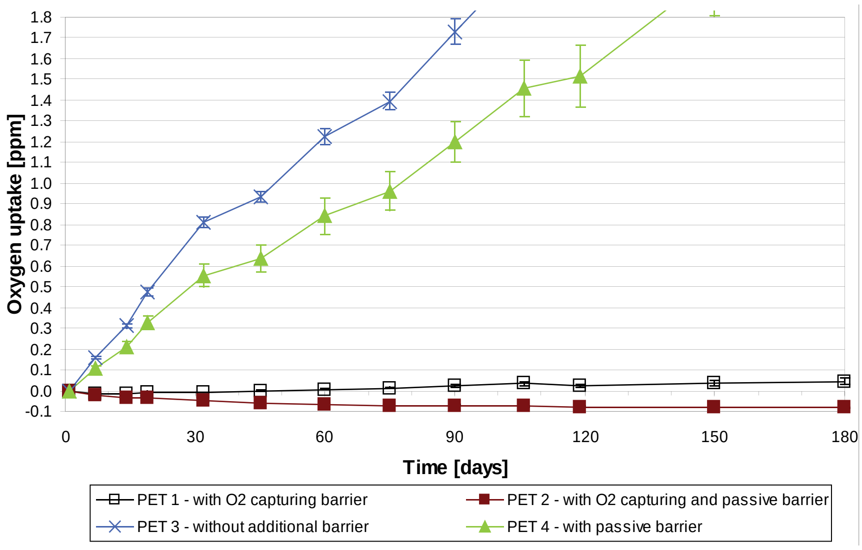

Fig. 2 Oxygen uptake for the tested PET bottle formulations with standard deviation for n = 5

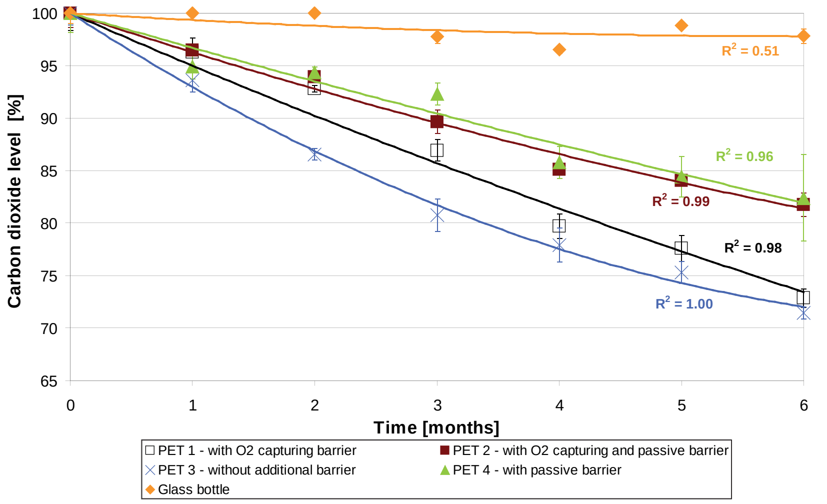

Fig. 3 Loss of carbon dioxide for the tested bottles with standard deviation for n = 5

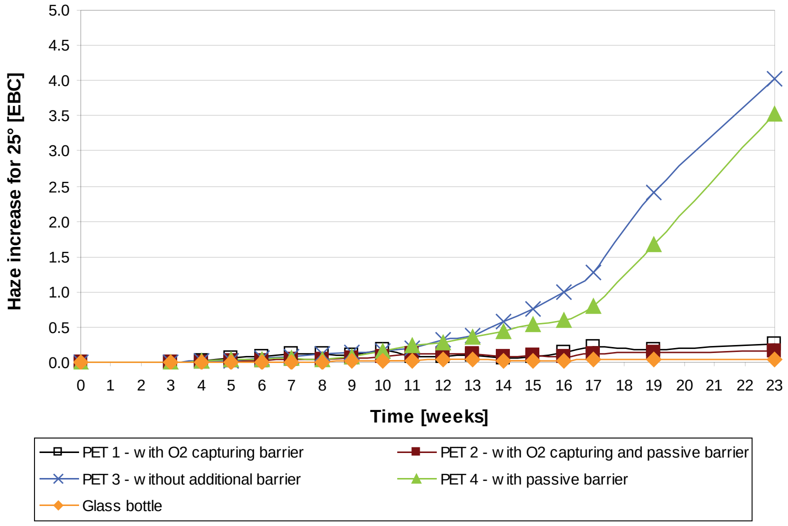

Fig. 4 Increase of turbidity (haze) in beer without microorganisms for the tested bottles

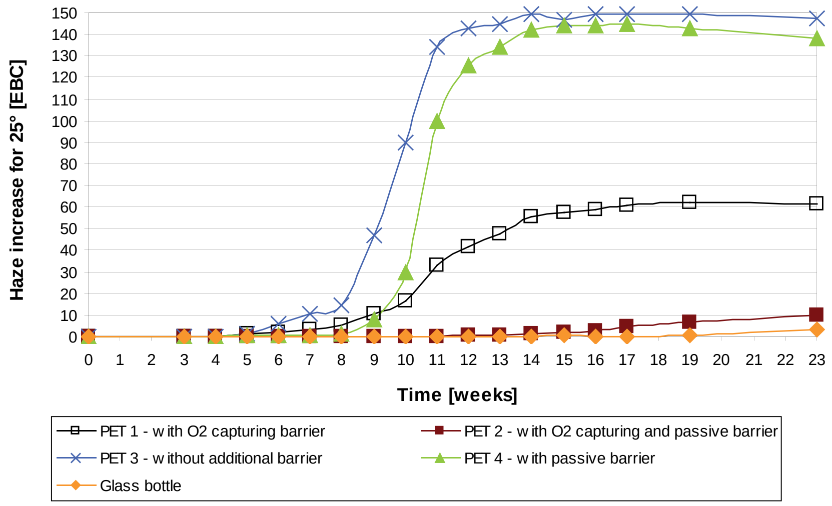

Fig. 5 Increase of turbidity in beer with *Saccharomyces diastaticus* for the tested bottles

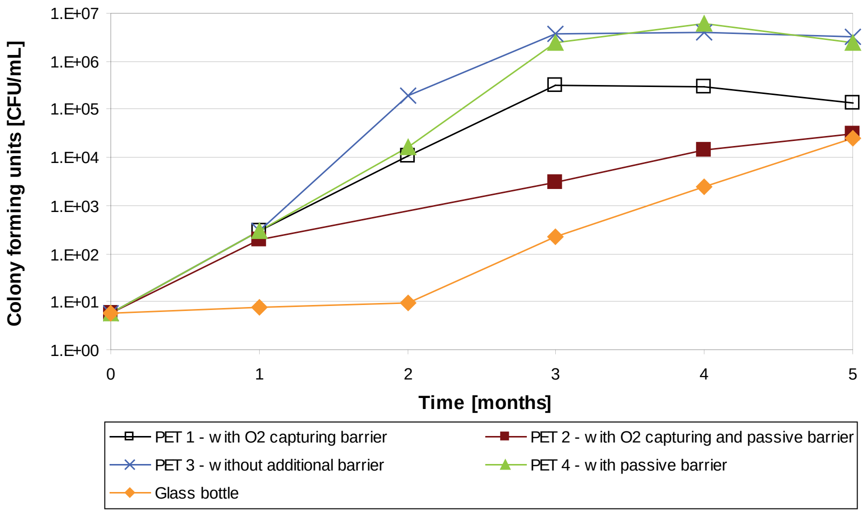

Fig. 6 Concentration of colony forming units (CFU) in beer with *Saccharomyces diastaticus* for the tested bottles