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# Culture medium optimization for osmotolerant yeasts by use of a parallel fermenter system and rapid microbiological testing

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ABSTRACT

In the present study, a culture medium for qualitative detection of osmotolerant yeasts, named OM, was developed. For the development, culture media with different concentrations of glucose, fructose, potassium chloride and glycerin were analyzed in a Biolumix™ test incubator. Selectivity for osmotolerant yeasts was guaranteed by a water activity ($a_w$)-value of 0.91. The best results regarding fast growth of *Zygosaccharomyces rouxii* (WH 1002) were achieved in a culture medium consisting of 45% glucose, 5% fructose and 0.5% yeast extract and in a medium with 30% glucose, 10% glycerin, 5% potassium chloride and 0.5% yeast extract. Substances to stimulate yeast fermentation rates were analyzed in a RAMOS® parallel fermenter system, enabling online measurement of the carbon dioxide transfer rate (CTR) in shaking flasks. Significant increases of the CTR was achieved by adding especially 0.1–0.2% ammonium salts ($(NH_4)_2HPO_4$, $(NH_4)_2SO_4$ or $NH_4NO_3$), 0.5% meat peptone and 1% malt extract. Detection times and the CTR of 23 food-borne yeast strains of the genera *Zygosaccharomyces*, *Torulaspora*, *Schizosaccharomyces*, *Candida* and *Wickerhamomyces* were analyzed in OM bouillon in comparison to the selective culture media YEG50, MYG50 and DG18 in the parallel fermenter system. The OM culture medium enabled the detection of $10^2$ CFU/g within a time period of 2–3 days, depending on the analyzed yeast species. Compared with YEG50 and MYG50 the detection times could be reduced. As an example, *W. anomalus* (WH 1021) was detected after 124 h in YEG50, 95.5 h in MYG50 and 55 h in OM bouillon. Compared to YEG50 the maximum $CO_2$ transfer rates for *Z. rouxii* (WH 1001), *T. delbrueckii* (DSM 70526), *S. pombe* (DSM 70576) and *W. anomalus* (WH 1016) increased by a factor ≥2.6. Furthermore, enrichment cultures of inoculated high-sugar products in OM culture medium were analyzed in the Biolumix™ system. The results proved that detection times of 3 days for *Z. rouxii* and *T. delbrueckii* can be realized by using OM in combination with the automated test system even if low initial counts ($10^1$ CFU/g) are present in the products. In conclusion, the presented data suggest that the OM culture medium is appropriate for the enrichment of osmotolerant yeasts from high-sugar food products.


## 1. Introduction

Osmotolerant yeasts are able to grow in sugar concentrations up to 40–70% (Lodder, 1970) and frequently cause spoilage in sugar syrup and sugar-rich matrices like chocolate fillings, fruit juice concentrates, molasses, dried fruit, marzipan and honey (Deák, 2008; Fleet, 1992; Tokuoka, 1993; Marvig et al., 2014; Wang et al., 2015b). Products such as dressings, ketchup, mayonnaise, soy sauce and condensed milk are also considered susceptible to a contamination with osmotolerant yeasts (Deák, 2008; Xu et al., 2014). One main problem of a contamination with osmotolerant yeasts is due to the slow, significant $CO_2$ formation over long storage times. Gas formation leads to product spoilage, for instance, cracking of marzipan or chocolate pralines and packaging swellings (Jermini et al., 1987; Tokuoka et al., 1985; Martorell et al., 2007). Temperature changes may result in condensation of water vapor on product surfaces which accelerates the growth of osmotolerant yeasts (Tokuoka, 1993; Rojo et al., 2014). Moreover, the formation of metabolic compounds such as alcohol, acetic acid or acetaldehyde may cause off-flavors (Fleet, 1992; Wang et al., 2015a). Gas formation and sensory modifications of food entail complaints and product recalls which implicate considerable economic and image losses for the enterprises concerned. As lots of foods are being processed, preserved and stored or transported over long distances nowadays, the importance of osmotolerant spoilage yeasts is increasing (Martorell et al., 2007).

Yeasts of the genus *Zygosaccharomyces* are most frequently isolated from sugar-rich raw materials and products and are often associated with food spoilage (James and Stratford, 2003; Jermini et al., 1987; Martorell et al., 2005; Vermeulen et al., 2012). Furthermore, species of the genera *Schizosaccharomyces*, *Torulaspora*, *Candida*, *Pichia*,

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*Wickerhamomyces*, *Kluyveromyces*, *Hanseniaspora* and *Debaryomyces* can also be detected in these matrices (Fleet, 1992; Marvig et al., 2014; Combina et al., 2007; Deák and Beuchat, 1992). Many strains of these species tolerate high amounts of ethanol, acetic acid and preserving agents such as benzoic acid, sorbic acid or sulfur dioxide (Dittrich and Grossmann, 2005; El Halouat and Debevere, 1996; Stratford et al., 2013; Rojo et al., 2015).

Culture media such as YGC, YPD, TGYC (Samson et al., 1992), DG18 (Hocking and Pitt, 1980) or DRBC (King et al., 1979) are frequently used for isolation, cultivation and enumeration of osmotolerant yeasts. DG18 medium applied in ISO/FDIS 21527-2 (2008), is characterized by an $a_w$-value of 0.95 which is realized by addition of glycerin. Selectivity towards fast growing fungi and bacteria is achieved by adding dichloran and chloramphenicol. Comparative studies illustrated that DG18 agar cannot always be recommended for analyzing yeasts from dry matrices with low $a_w$-values (Beuchat et al., 2001; Deák et al., 2001). Many company and service laboratories carry out their tests in broth cultures on a qualitative basis (presence-absence tests) by proving gas formation. Apart from the above mentioned culture media standard culture media such as glucose broth or wort broth are often applied for this purpose. All these culture media have in common that the $a_w$-values are not significantly reduced, i.e., when used in quality control osmotolerant yeasts are not specifically detected.

Another strategy is the use of culture media which attain their selectivity for osmotolerant yeasts by low $a_w$-values. Jermini et al. (1987) and Pitt and Hocking (1985) published culture media with $a_w$-values between 0.89 and 0.91 by high glucose concentrations. Detection times in these culture media vary between 5 and 10 days (Jermini et al., 1987; Beuchat, 1993). Although a high selectivity for osmotolerant yeasts is achieved, detection in these culture media may be impeded due to long growth periods and low fermentation rates depending on the yeast species present in the analyzed foods. In general, differences in performance among media are attributed to the diversity of yeasts likely to be present in test foods and differences in nutrients, pH, and water activity requirements for resuscitation of stressed cells and colony development (Beuchat and Mann, 2016). From our perspective, there is a need for a standardized method for qualitative testing of osmotolerant yeasts in high-sugar foods and raw materials which produces comparable results in microbiological quality control laboratories.

The objective of this study was the development of a culture medium for qualitative detection of osmotolerant yeasts in high-sugar foods with special attention to rapid growth and high fermentation rates. The medium was supposed to detect microbial counts from $10^1$–$10^2$ colony forming units (CFU)/mL as well as enabling a selective and rapid detection in <5 days. An automated test incubator was applied for initial experiments concerning fundamental issues of culture medium composition in regard of sugars and osmotically active substances. The influence of individual substances and concentrations on the metabolic activity of osmotolerant yeasts was observed in a RAMOS® parallel fermenter system (HiTec Zang, Herzogenrath, Germany). The focus was particularly on the analysis of the carbon dioxide transfer rate (CTR) during yeast growth. Respiratory rates such as the CTR and the oxygen transfer rate (OTR) are universal parameters to represent the physiological state of a biologic culture (Anderlei et al., 2004). Characteristic measuring curves can be used to depict substrate limitation, diauxic growth or other biologic phenomena (Anderlei and Büchs, 2001).

Based on the data generated through these experiments a culture medium for osmotolerant yeasts, designated OM, was compiled. Yeast growth and fermentation rates in OM culture medium were directly compared to those in YEG50, MYG50 and DG18 under defined conditions. Until now, no data have been available concerning the comparative analysis of yeast fermentation rates in selective culture media with reduced $a_w$-values. Experimental inoculation tests for the detection of osmotolerant yeasts in high-sugar foods were carried out with simultaneous $CO_2$ measurements by use of the parallel fermenter system and the automated test incubator.

## 2. Materials and methods

### 2.1. Osmotolerant yeast strains

Strains from culture collections and yeast isolates which were originally isolated from high-sugar raw materials and products were applied (Table 1). Strains from culture collections were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany), the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands) and the culture collection of the Institute of Microbiology and Wine Research, University of Mainz, Germany. Additional osmotolerant yeasts were isolated from different high-sugar foods and raw materials by enrichment of 10 g product in 90 mL YEG50 bouillon (Jermini et al., 1987). The enrichment samples were incubated for 7 days at 30 °C and streaked out on YEG50 agar for several times to ensure the isolation of pure cultures. The yeast isolates were identified by amplification of the internal transcribed spacer (ITS) region using Primer ITS 1 and ITS 4 (White et al., 1990) and subsequent sequencing (Eurofins Genomics, Ebersberg, Germany). To verify the identification results, the yeast strains were additionally examined by DNA fingerprinting via SAPD-PCR using primer A-Not (Pfannebecker and Fröhlich, 2008). For strain maintenance aliquots were prepared in Cryoinstant (VWR, Darmstadt, Germany) and stored at −152 °C. The yeast strains were cultivated by adding one cryo pearl to 10 mL of the culture medium to be tested with subsequent incubation for 3–5 days at 30 °C. For testing in the Biolumix™-system (I&L Biosystems) and the RAMOS® parallel fermenter system (HiTec Zang, Herzogenrath, Germany), precultures were produced by transferring cultures twice in 10 mL of the culture medium to be tested and incubated under the above-mentioned conditions.

### 2.2. Use of an automated test incubator for culture medium optimization

The Biolumix system comprises an incubator with analyzer, control software and special test vials filled with culture medium. The test vials consist of an incubation and detection area with an embedded optical carbon dioxide ($CO_2$) sensor at the bottom of the test vial. $CO_2$ which occurs due to metabolic processes diffuses into the sensor, reacts

**Table 1**
Osmotolerant yeast strains used in this study.

| Strain | Species | Source |
|---|---|---|
| CBS 4512 | *Zygosaccharomyces rouxii* | Marzipan |
| WH 1001 | *Zygosaccharomyces rouxii* | Fructose syrup |
| WH 1002 | *Zygosaccharomyces rouxii* | Marzipan |
| WH 1003 | *Zygosaccharomyces rouxii* | Treacle |
| WH 1004 | *Zygosaccharomyces rouxii* | Invert sugar syrup |
| WH 1005 | *Zygosaccharomyces rouxii* | Date |
| WH 1018 | *Zygosaccharomyces rouxii* | Treacle |
| DSMZ 70492 | *Zygosaccharomyces bailii* | Apple juice |
| CBS 1091 | *Zygosaccharomyces mellis* | Honey |
| DSMZ 70526 | *Torulaspora delbrueckii* | Marzipan |
| WH 1013 | *Torulaspora delbrueckii* | Chocolate truffle |
| DSMZ 70576 | *Schizosaccharomyces pombe* | Grape must |
| CBS 1042 | *Schizosaccharomyces pombe* | Grape must |
| IMW 81 | *Schizosaccharomyces pombe* | Wine |
| IMW 330 | *Schizosaccharomyces pombe* | Wine |
| IMW 339 | *Schizosaccharomyces pombe* | Wine |
| WH 1016 | *Wickerhamomyces anomalus* | Chocolate marshmallow |
| WH 1021 | *Wickerhamomyces anomalus* | Praline |
| WH 1026 | *Wickerhamomyces anomalus* | Invert sugar syrup |
| WH 1010 | *Candida parapsilosis* | Chocolate truffle |
| WH 1015 | *Candida lusitaniae* | Nougat filling |
| WH 1023 | *Candida magnoliae* | Honey |
| WH 1025 | *Candida* sp. | Invert sugar syrup |

DSMZ = German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. IMW = culture collection of the Institute of Microbiology and Wine Research, University of Mainz, Germany.

with substances in the sensor and leads to color changes of the sensor. During incubation these optical modifications are detected every 6 min by LEDs in the analyzer.

Some *Zygosaccharomyces* species were described as fructophilic (Leandro et al., 2011; Pina et al., 2004). To examine a possible positive influence of fructose on the growth of these yeasts, batches were prepared in which a part of the glucose contained in a reference medium (50% (w/w) D-glucose, 0.5% (w/w) yeast extract; pH 5.6, Jermini et al., 1987) was replaced by D-fructose (Merck, Darmstadt, Germany). A culture medium consisting of 45% (w/w) D-glucose, 5% (w/w) D-fructose and 0.5% (w/w) yeast extract (medium 1) and a culture medium of 47.5% (w/w) D-glucose, 2.5% (w/w) D-fructose and 0.5% (w/w) yeast extract (medium 2) were prepared. 8 mL of the sterilized culture media were filled into each of the empty Biolumix test vials with $CO_2$ sensors. Each of the different culture media was inoculated with $2.1 \cdot 10^3$ CFU/mL of the yeast *Z. rouxii* (WH 1002). To assure the selectivity for osmotolerant yeasts it was intended to achieve an $a_w$-value of $0.90 \pm 0.01$ for the culture medium to be developed. The examinations should demonstrate the effect on the growth of *Z. rouxii* by replacing a part of glucose of the reference medium by the osmotically active substances potassium chloride (KCl) and glycerin. Culture media consisting of 0.5% (w/v) yeast extract, 5% (w/v) KCl and different concentrations of D-glucose and glycerin (Carl Roth, Karlsruhe, Germany) were prepared and sterilized by autoclaving. The following combinations of D-glucose and glycerin were added: 24% (w/v) + 15% (v/v) (medium 3), 30% + 10% (v/v) (medium 4), 36% + 7.5% (v/v) (medium 5). The pH values were adjusted to 5.6 prior to autoclaving. The $a_w$-values of these culture media, measured with AquaLab 4TE DUO (Decagon Devices, Pullman, USA), were constant and each attained a value of 0.90. Each culture medium was inoculated with $5.7 \cdot 10^2$ CFU/mL of the yeast *Z. rouxii* (WH 1002). All test assays were incubated at 30 °C for up to 6 days in the Biolumix system.

### 2.3. Substances for culture medium optimization in the parallel fermenter system

A basic culture medium consisting of 50% (w/w) D-glucose (Merck) and 0.5% (w/w) yeast extract (Merck) was sterilized by autoclaving. The produced medium showed a pH value of 5.6 and an $a_w$-value of 0.91. For optimization of the basic culture medium substances described as stimulating growth and fermentation rates of osmotolerant yeasts were deliberately chosen. These were ammonium salts ($NH_4NO_3$, $NH_4SO_4$, $(NH_4)_2HPO_4$), peptones (from meat and casein), amino acids (L-cysteine, L-proline), malt extract, thiamine, glutathione, trehalose, $MgSO_4$ and $ZnCl_2$. The appropriate concentrations were chosen on the basis of literature data (Ok and Hashinaga, 1997; Jermini et al., 1987; Fiedler, 1995; Vermeulen et al., 2012). All substances and concentrations investigated in this study are listed in Table 3 (Section 3.2). The optimization assays were carried out with *Z. rouxii* (WH 1001) with initial counts of $1{,}0 \cdot 10^2$ CFU/mL in 25 mL fermentation batches in the RAMOS parallel fermenter system (Section 2.4). The media supplements were added to the basic medium prior to autoclaving. The heat sensitive thiamine hydrochloride was sterile filtered by a 0.2 μm polyethersulfone syringe filter (VWR) and added to the cooled sterilized medium.

### 2.4. Examination of carbon dioxide transfer rate in the parallel fermenter

To determine the metabolic activity of the yeasts, tests were carried out in the RAMOS parallel fermenter system under standardized cultivation conditions. The system permits parallel online monitoring of eight fermentation batches under quantitative measuring of the $CO_2$ formation. $CO_2$ is measured in the RAMOS system indirectly via oxygen and pressure sensors in special 250 mL fermentation flasks (Anderlei et al., 2004; Seletzky et al., 2007). The RAMOS software allows to indicate parameters such as the oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR), oxygen partial pressure and differential pressure. To measure the oxygen partial pressure in the gas compartment of the flasks a measuring cycle consisting of a flushing and measuring phase is continuously repeated in the system. The persistent metabolic activity of the yeasts leads to a modification of the oxygen and carbon dioxide partial pressure in the gas compartment of the measuring flasks.

In this study flushing phases of 20 min and measuring phases of 10 min were applied in the system resulting in measuring cycles totaling up to 30 min. The fermentation batches were incubated without oxygen supply (flow flushing phase parameter = 0 mL/min). 25.5 mL of the culture medium were filled into a RAMOS fermentation flask, respectively. The batches were inoculated with 100 μL of the diluted preculture (Section 2.1). Microbial counts were determined at the beginning and at the end of the assays. At the beginning of the measurements, 100 μL and 500 μL were plated on YEG50 agar (Jermini et al., 1987), and incubated at 30 °C for 3–5 days. The assays in the parallel fermenter system were carried out at an incubation temperature of 30 °C while shaking gently (60 rpm). Growth and gas formation were examined over a maximum period of 6 days. Prior to each measurement in the parallel fermenter system an oxygen calibration test and a leak test were performed, respectively. To evaluate the measured curves, the times at which the CTR reached ≥4 mmol/L · h (in hours) were applied which marked the beginning of the fermentation. At this measurement point, yeast growth could also be detected visually on the basis of turbidity and foam formation. Furthermore, the values of the maximum $CO_2$ transfer rates ($CTR_{max.}$) of different batches were compared. By calculation of the respiratory quotient (RQ), which is the ratio between produced $CO_2$ and consumed $O_2$ (CTR/OTR), it was also possible to determine the metabolic state of the cultures. A RQ value of ≤1 indicates a fully aerobic metabolism, and values >1 are consistent with fermentative metabolism (Anderlei et al., 2004).

### 2.5. Optimized culture medium for osmotolerant yeasts

Subsequent to the initial assays regarding optimal sugar combinations and osmotically active substances in the Biolumix system and the assays on individual substances in the RAMOS parallel fermenter, culture media were compiled on the basis of the test results. The following three culture media were prepared: one culture medium consisting of 285 g glucose, 15 g fructose, 5 g yeast extract, 100 mL glycerin, 50 g KCl, 1 g $MgSO_4$, 2 g $(NH_4)_2HPO_4$, 1.3 mg thiamine and 800 mL distilled water (medium A), as well as a culture medium consisting of 285 g glucose, 15 g fructose, 5 g yeast extract, 5 g meat peptone, 100 mL glycerin, 50 g KCl, 1 g $MgSO_4$, 2 g $(NH_4)_2HPO_4$, 1.3 mg thiamine and 800 mL distilled water (medium B) and the final culture medium, designated OM (Table 2). For media production the indicated quantities of both sugar types were weighed and dissolved with 300 mL of distilled water and autoclaved. The remaining substances (excepted thiamine) were dissolved with 500 mL distilled water and autoclaved separately. After autoclaving both solutions were conflated under sterile conditions. Thiamine was added to the cooled culture medium as a sterile-filtered solution in a volume of 2 mL. The preparation of the OM culture medium as described above lead to a pH-value of 5.7 after autoclaving. Investigations for the detection of the optimal pH-value of the OM culture medium were carried out by lowering the pH with 1 M HCl in decrements of 0.2 to pH 4.0 and subsequent testing of *Z. rouxii* (WH 1001) and *T. delbrueckii* (WH 1013) in the parallel fermenter system. Lowering the pH-value of the culture medium had no significant impact in regard to a faster growth of the examined yeast species. Therefore, no adjustment of the pH-value was made for the OM culture medium.

### 2.6. Comparison of culture media for osmotolerant yeasts in the parallel fermenter system

The OM culture medium was compared to three culture media described for osmotolerant yeasts. These were YEG50 (Jermini et al.,

**Table 2**
Composition of analyzed culture media for osmotolerant yeasts.

| Medium | Ingredients | Concentration | $a_w$-Value | pH value | Reference |
|---|---|---|---|---|---|
| YEG50 | D-glucose | 500 g | 0.91 | 4.5 | Jermini et al., 1987 |
| | Yeast extract | 5 g | | | |
| | Deionized water | 495 g | | | |
| MYG50 | D-glucose | 500 g | 0.89 | 5.6 | Pitt and Hocking, 1985 |
| | Malt extract | 10 g | | | |
| | Yeast extract | 2.5 g | | | |
| | Deionized water | 500 mL | | | |
| DG18 | D-glucose | 1% | 0.95 | 5.6 | Hocking and Pitt, 1980 (ISO/FDIS 21527-2) |
| | Peptone | 0.5% | | | |
| | Dichloran | 0.002 g | | | |
| | Chloramphenicol | 0.1 g | | | |
| | $KH_2PO_4$ | 0.1% | | | |
| | $MgSO_4 \cdot 7\ H_2O$ | 0.05% | | | |
| | Glycerin | 18% | | | |
| | Deionized water | 1.000 mL | | | |
| OM | D-glucose | 285 g | 0.91 | 5.7 | This study and patent application: Pfannebecker and Becker, 2015 |
| | D-fructose | 15 g | | | |
| | Yeast extract | 5 g | | | |
| | Malt extract | 10 g | | | |
| | Meat peptone | 5 g | | | |
| | Glycerin | 100 mL | | | |
| | KCl | 50 g | | | |
| | $MgSO_4 \cdot 7\ H_2O$ | 1 g | | | |
| | $(NH_4)_2HPO_4$ | 2 g | | | |
| | $(NH_4)_2SO_4$ | 1 g | | | |
| | Deionized water | 800 mL | | | |
| | Thiamine | 1.3 mg | | | |

1987), MYG50 (Pitt and Hocking, 1985) and DG18 (Hocking and Pitt, 1980) of the international standard procedure for the enumeration of yeasts and moulds in products with water activity less than or equal to 0.95 (ISO 21527-2). The culture media were characterized by $a_w$-values of ≤0.91 (Table 2) with the exception of DG18 ($a_w = 0.95$).

The yeast strains in the culture medium to be examined (YEG50, MYG50, DG18, OM) were cultivated as indicated in Section 2.1. The 3–5 days old precultures were diluted in decadic order in the respective culture medium and used for inoculation of the test batch with microbial counts of $10^2$ CFU/mL. The preparation of the fermentation flasks as well as the measurements in the parallel fermenter system were carried out as described in Section 2.4. To directly compare the tested culture media, the assay results were evaluated on the basis of the CTR measurements. Particular attention was attributed to the times to CTR ≥ 4 mmol/L·h and the maximum $CO_2$-transfer rates ($CTR_{max.}$).

### 2.7. Experimental inoculation of yeasts in high-sugar products

Eight high-sugar products including marzipan paste ($a_w$ 0.74), nougat filling ($a_w$ 0.26), granulated honey ($a_w$ 0.30), sugar syrup (34% sucrose, 66% invert sugar; $a_w$ 0.75), persipan paste ($a_w$ 0.77), poppy seed filling ($a_w$ 0.92), strawberry jam ($a_w$ 0.87) and treacle ($a_w$ 0.68) were used for the preparation of inoculation experiments with osmotolerant yeasts. Before the start of the inoculation experiments, all products were tested on the presence of microorganisms. These tests were carried out by qualitative analyses of 10 g product in 90 mL OM-bouillon. The samples were incubated for 7 days at 30 °C. Afterwards 500 μL of each enrichment culture was plated on OM-agar and incubated for additional 7 days.

For the inoculation experiments, the products were prepared with low ($10^1$ CFU/g) and high ($10^2$ CFU/g) counts of the yeasts *Z. rouxii* (WH 1001) and *T. delbrueckii* (WH 1013), respectively. Therefore 1 mL of a preculture was centrifuged at 2500 rpm and washed twice in the same volume with phosphate buffered saline (PBS) (Carl Roth). Decadic dilutions were made of the washed cell suspension in PBS. 10 g of each product was weighted in a stomacher filter bag (Interscience, Saint-Nom-la-Bretêche, France) and inoculated with 100 μL of a cell suspension and homogenized in a stomacher (AES Chemunex, Bruchsal, Germany). The inoculated samples were mixed with 90 g OM culture medium and homogenized for 30 s. For testing in the RAMOS parallel fermenter system, 25 mL of the mixture were filled in sterilized fermentation flasks and for testing in the Biolumix system, 8 mL of the mixture were filled in empty Biolumix test vials, respectively. The incubation temperatures and test parameters were chosen as described in Sections 2.2 and 2.4. Microbial counts were determined at the beginning and at the end of the assays on OM supplemented with 1.5% agar. All inoculation experiments were carried out by double testing of each assay.

## 3. Results and discussion

### 3.1. Results of automated testing in the Biolumix system

When testing different sugar combinations of D-glucose and D-fructose the fastest growth of *Z. rouxii* (WH 1002) was observed in a medium consisting of 45% glucose, 5% fructose and 0.5% yeast extract. The detection times in the Biolumix system were 20.9 h (medium 1), 23.1 h (medium 2) and 27.5 h (reference medium) (Fig. 1a). A fructose amount of 5% besides 45% glucose had a growth stimulating effect in comparison to the reference medium (50% glucose, 0.5% yeast extract). Although some *Z. rouxii* strains are described as fructophilic (Leandro et al., 2011, 2014) a growth stimulation in connection with higher fructose concentrations could not be observed with our test strain during these experiments. At fructose concentrations above 20% combined with glucose to total sugar amounts of 50%, respectively, the growth of *Z. rouxii* was significantly reduced, resulting in detection times ≥35 h in the Biolumix system (data not shown).

To guarantee selectivity for osmotolerant yeasts an $a_w$-value of $0.90 \pm 0.01$ was aimed at. This value was chosen on the basis of literature data (Tokuoka and Ishitani, 1991; Grant, 2004). While spoilage yeasts still grow at an $a_w$-value of 0.90, lower values are less appropriate because they inhibit the growth of some yeasts (e.g. different *Candida* species) and only a few single, extremely tolerant yeasts are able to reproduce (Fiedler, 1995; Restaino et al., 1983). $A_w$-values <0.85 are very selective and mainly lead to the identification of one yeast species, *Z. rouxii* (Fleet, 1992). An additional reason for the selection of an $a_w$-value around 0.90 was the prevention of shock effects when transferring osmotolerant yeasts from foods with high sugar content into culture media with increased water availability. This could result in longer adaptation phases (Hernandez and Beuchat, 1995). Also due to this reason the water activity of the culture medium for osmotolerant yeasts should not be too high (Jermini et al., 1987; Cava and Hernandez, 1994).

To achieve an $a_w$-value around 0.90 the influence of different glucose and glycerin concentrations on the growth of *Z. rouxii* was examined in this study in culture media combined with 5% (w/v) KCl (= 0.67 M) and 0.5% (w/v) yeast extract (media 3–5). The assays demonstrated that the best results regarding fast growth of *Z. rouxii* were attained by a combination of 30% glucose, 10% (v/v) glycerin, 5% KCl and 0.5% yeast extract (medium 4). The detection times were 29.7 h (medium 3), 25.5 h (medium 4), 37.7 h (medium 5) and 37.7 h (reference medium) (Fig. 1b).

According to literature, especially sugars, glycerin and salts such as KCl and NaCl are used to reduce $a_w$-values in yeast culture media (Hernandez and Beuchat, 1995; Pribylova et al., 2007; El Halouat and Debevere, 1996; Membré et al., 1999; Munitis et al., 1976; Restaino et al., 1983). The increased accumulation of glycerin serving as a compatible solute or osmoregulator by osmotolerant yeasts under high osmotic pressures has been reported (Reed et al., 1987; André et al., 1988). In general, osmotolerant yeasts are able to synthesize glycerin (Tofalo et al., 2009) and some yeasts have active glycerin uptake pumps (Hohmann, 2002). Munitis et al. (1976) examined osmotically active substances like sugar (glucose, fructose, sucrose, sorbose), glycerin, salts (NaCl, KCl) and polyethylene glycol to reduce the $a_w$-values in a culture medium for *Z. bisporus*. In these assays fast growth was realized

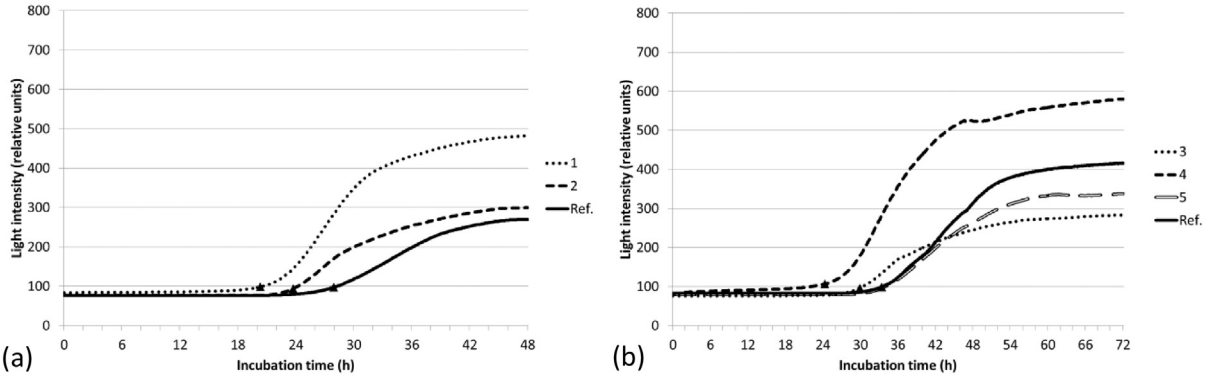


**Fig. 1.** Growth of *Z. rouxii* (WH 1002) in compiled culture media in the Biolumix system. Examination of different combinations of glucose, fructose and yeast extract (Inoculum: $2.1 \cdot 10^3$ CFU/mL) (Panel a) and combinations of glucose, KCl, glycerin and yeast extract (Inoculum: $5.7 \cdot 10^2$ CFU/mL) (Panel b). The composition of the culture media tested are as follows: 1: 45% glucose, 5% fructose, 0.5% yeast extract; 2: 47.5% glucose, 2.5% fructose, 0.5% yeast extract; 3: 24% glucose, 15% glycerin, 5% KCl, 0.5% yeast extract; 4: 30% glucose, 10% glycerin, 5% KCl, 0.5% yeast extract; 5: 36% glucose, 7.5% glycerin, 5% KCl, 0.5% yeast extract; Ref.: 50% glucose, 0.5% yeast extract. Triangles show the points of positive detection in the system.

by adding KCl (0.5 M–1 M) and glucose (20%–40%) to a basic medium consisting of 0.4% (w/v) yeast extract and 0.5% (w/v) peptone. Regarding optimal glucose and KCl concentrations for rapid growth of *Zygosaccharomyces* species, our results are comparable to the results of Munitis et al. (1976). Inorganic salts like KCl play an important role in the adaptation to environments of high osmotic pressures (Brown, 1976). It has been known for a long time that these low-molecular substances serve as a protectant for enzymes and structural proteins against inactivation and denaturation (Brown, 1978). An $a_w$-value of 0.91 for the OM culture medium was chosen. At this value, the growth of *Z. rouxii* (WH 1001) was faster compared to the growth in published culture media based on glucose with equal $a_w$-values (e. g. YEG50). Furthermore, at an $a_w$-value of 0.91, the culture medium was sufficiently selective for preventing the growth of bacteria and fast growing molds (Grant, 2004). No use was made of dichloran, that not only inhibits fungi from spreading but also can suppress growth and fermentation activity of yeasts (Michaels et al., 1977; Henson, 1981).

### 3.2. Culture medium optimization by use of the parallel fermenter system

The analysis of individual substances stimulating growth and fermentation rates of *Z. rouxii* (WH 1001) in the parallel fermenter system showed that malt extract, at a concentration of 10 g/L, lead to a significant increase of the $CTR_{max.}$ of 9.1 mmol/L·h in comparison to the basic culture medium (Table 2). Similar effects have been found for the addition of meat peptone (5 g/L) and casein peptone (5 g/L) which resulted in the rise of the $CTR_{max.}$ of 5.7 mmol/L·h or 3.9 mmol/L·h. Also, the addition of the ammonium salts $(NH_4)_2HPO_4$ (2 g/L), $(NH_4)_2SO_4$ (1 g/L) and $NH_4NO_3$ (1.5 g/L) led to a significant increase of the $CTR_{max.}$. These findings are conform to the results of Bely et al. (1990) who stated that a good correlation was found between the maximum $CO_2$ production rate and the assimilable nitrogen content during wine fermentation. Nitrogen is typically the limiting nutrient during fermentation. The addition of ammoniacal nitrogen increases the fermentation rate and reduces the fermentation time (Colombie et al., 2007). Particularly in the stationary phase of the yeast growth, the availability of ammonium positively affects cell proliferation and fermentation (Mendes-Ferreira et al., 2004; Schnierda et al., 2014).

The addition of individual amino acids (L-cysteine, L-proline) in the concentrations indicated in Table 3 showed only a slight increase of the $CTR_{max.}$ up to 2.2 mmol/L·h. Proline is believed to protect yeast cells from various stresses during fermentation processes (García-Martínez et al., 2013) and it was shown that the accumulation of L-proline led to enhanced stress tolerance (Takagi, 2008). For the same reason L-glutathione and L-cysteine were chosen as possible protective metabolites against oxidative stress during yeast fermentation (Zechmann et al., 2011; Gales et al., 2008; Kaur et al., 2009). Also, the investigations with L-glutathione in the specified concentrations resulted in a decrease of the $CTR_{max.}$ compared to the basic medium. Under osmotic stress the synthesis and uptake of trehalose was reported for *Saccharomyces cerevisiae*. The substance acts as a compatible solute or as a carbohydrate reserve material (Hounsa et al., 1998). Our investigations, whether D-trehalose at the concentrations indicated in Table 3 has a positive effect on growth of *Z. rouxii*, showed no reduction in time to $CTR_{max.}$ compared to the culture medium without trehalose.

The probably most limiting steps of alcoholic fermentation are the decarboxylation of pyruvate to acetaldehyde and its subsequent reduction to ethanol catalyzed by the pyruvate decarboxylase and the alcohol dehydrogenase. The addition of thiamine to fermentation batches can

**Table 3**
Comparison of maximum $CO_2$ transfer rate ($CTR_{max.}$) and time to $CTR_{max.}$ of *Zygosaccharomyces rouxii* (WH 1001) at a cell count of $10^2$ CFU/mL in a basic culture medium (50% D-glucose, 0.5% yeast extract) supplemented with single substances in different concentrations in the RAMOS parallel fermenter system.

| Supplement | Supplier | Concentration of supplement (g/L) | Interval to $CTR_{max.}$ (h)[a] | Intensity of $CTR_{max.}$ (mmol/L h)[a] |
|---|---|---|---|---|
| Thiamine hydrochloride | Merck, Darmstadt, Germany | 0.0013 | −1.5 | −1.6 |
| Malt extract | Merck | 10 | +2.0 | +9.1 |
| Meat peptone | Oxoid, Wesel, Germany | 2 | +2.5 | +2.4 |
| | | 5 | +3.5 | +5.7 |
| Casein peptone | Merck | 5 | +3.0 | +3.9 |
| $NH_4NO_3$ | Merck | 1.5 | +3.5 | +3.3 |
| | | 2 | +4.5 | +2.6 |
| $(NH_4)_2HPO_4$ | Merck | 1.5 | +1.5 | −0.5 |
| | | 2 | +4.5 | +3.6 |
| $(NH_4)_2SO_4$ | Sigma-Aldrich, Taufkirchen, Germany | 1 | −2.0 | +2.4 |
| L-cysteine | Merck | 1 | +4.5 | +2.2 |
| L-proline | Merck | 1 | +1.0 | +1.9 |
| | | 2 | +1.0 | +1.2 |
| | | 5 | +1.0 | +1.7 |
| L-glutathione (reduced) | AppliChem, Darmstadt, Germany | 0.1 | +2.0 | −0.1 |
| | | 0.25 | +1.0 | −10.5 |
| | | 0.5 | +3.5 | −0.3 |
| D-Trehalose · 2 $H_2O$ | AppliChem | 1 | ±0 | +0.5 |
| | | 2 | ±0 | −0.9 |
| | | 5 | +1.0 | −0.5 |
| $ZnZl_2$ | Sigma-Aldrich | 0.012 | +4.0 | −0.5 |
| | | 0.025 | +20.5 | −17.3 |
| $MgSO_4 \cdot 7\ H_2O$ | Merck | 1 | ±0 | −0.3 |

[a] In relation to the basic culture medium without supplement.

therefore accelerate yeast fermentation rates as it acts as a cofactor of the pyruvate decarboxylase in the form of thiamine pyrophosphate (Dittrich and Grossmann, 2005). In our study, the addition of thiamine hydrochloride in a concentration of 1.3 mg/L to the culture medium led to a shortened interval to $CTR_{max.}$ of 1.5 h compared to the basic culture medium. In yeasts, the alcohol dehydrogenase consists of four catalytically active zinc-binding subunits (Raj et al., 2014). For this reason $Zn^{2+}$ was investigated in form of $ZnCl_2$ as a culture medium additive, indicating a possible positive effect on the fermentation rate of *Z. rouxii*. $ZnCl_2$ was examined in two concentrations of 12 mg/L and 25 mg/L but led in both cases to a decrease of the fermentation rate of *Z. rouxii*. Apparently, the yeast was already sufficiently supplied with $Zn^{2+}$. Concentrations ≥25 mg/L $ZnCl_2$ had a strong inhibitory effect on the growth of *Z. rouxii*. Due to the possibility to detect least differences in the progression and intensity of $CO_2$ transfer rate, the RAMOS parallel fermenter system was well suited to examine the influence of individual culture media supplements.

The investigations of individual culture media supplements were predominantly carried out with *Z. rouxii* (WH 1001). Other yeast species such as *T. delbrueckii* (WH 1013), *Z. bailii* (DSMZ 70492) and *C. parapsilosis* (WH 1010) were also examined for this purpose and showed similar responses in relation to growth and fermentation activity (data not shown). While the addition of individual substances, such as $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, meat peptone and malt extract to basic culture medium led to a moderate increase of the fermentation rates (Table 3), a significant increase could be achieved through combination of these substances (Fig. 2).

### 3.3. Comparison of OM culture medium with published culture media for osmotolerant yeasts in the parallel fermenter system

The respiratory quotients (CTR/OTR) were calculated in every fermentation flask by the RAMOS parallel fermenter software. Respiratory quotients >1 in all assays showed that the oxygen consumption was smaller than the carbon dioxide production. These results demonstrated that even if remaining oxygen was present in the gas compartment of the fermentation flasks at the beginning of experiments, the yeast metabolism was based on fermentation. Independently of the tested culture medium, yeast counts reached mean values of $5.0 \cdot 10^7$ CFU/mL at the end of experiments in the fermentation flasks.

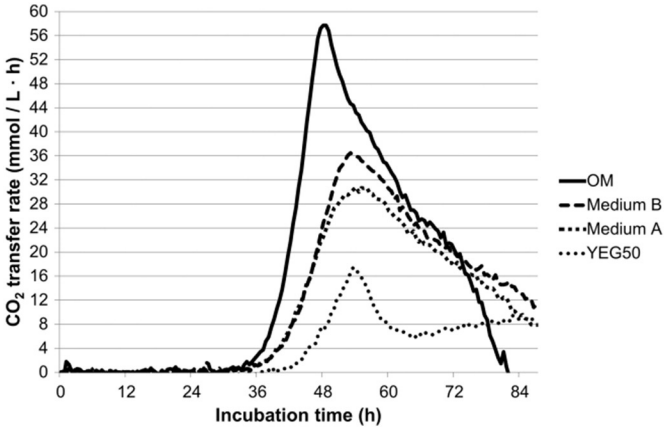


**Fig. 2.** Development of the $CO_2$ transfer rates of *Zygosaccharomyces rouxii* (WH 1001) in YEG50, medium A (285 g glucose, 15 g fructose, 5 g yeast extract, 100 mL glycerin, 50 g KCl, 1 g $MgSO_4$, 2 g $(NH_4)_2HPO_4$, 1.3 mg thiamine, 800 mL distilled water), medium B (285 g glucose, 15 g fructose, 5 g yeast extract, 5 g meat peptone, 100 mL glycerin, 50 g KCl, 1 g $MgSO_4$, 2 g $(NH_4)_2HPO_4$, 1.3 mg thiamine, 800 mL distilled water) and OM culture medium in the RAMOS parallel fermenter system. Inoculum $1.1 \cdot 10^2$ CFU/mL. Determination in duplicates. The corresponding values of maximum $CO_2$ transfer rate ($CTR_{max.}$) in mmol/L·h are 17.5 (YEG50), 30.8 (medium A), 36.5 (medium B) and 57.7 (OM).

Depending on the tested yeast species and strains, the investigations in the parallel fermenter system showed differences regarding the times to the beginning of fermentation (CTR ≥ 4 mmol/L·h) in the culture media YEG50, MYG50, DG18 and OM. Because yeast growth could be visually detected in the fermentation flasks at CTR-values ≥4 mmol/L·h, the times to these measuring points can also be considered as detection times for qualitative testing. For example for *Z. rouxii* (WH 1001) the beginning of fermentation was reached 8 h earlier in OM and the $CTR_{max.}$ was increased by a factor of 3.3 compared to YEG50 (Fig. 2). *T. delbrueckii* (DSMZ 70526) showed a 47 h faster beginning of the fermentation in OM than in YEG50 and a 2.6-fold higher $CTR_{max.}$ was reported for this strain (Fig. 3a). *S. pombe* (DSMZ 70576) was detected in OM in a reduced time of 29 h and showed a 3.4-fold increase of the $CTR_{max.}$ in relation to YEG50 (Fig. 3b).

The growth of *Z. rouxii* strains was fastest in all culture media tested compared to other *Zygosaccharomyces* species or other yeast species examined. This fact can be explained by the ability of this species to adapt to environments with extreme low water activities (Fleet, 1992). For *C. lusitaniae* (WH 1015) no growth could be observed in YEG50 and MYG50 bouillon. Within all 23 yeast strains tested, the detection times varied from 31.5 h to 82 h in OM bouillon depending on the species and strains at initial cell counts of $10^2$ CFU/mL (Fig. 4a). In contrast to that, the detection times in the two comparable culture media with $a_w$-values around 0.9 varied between 38.5 h to 128 h (YEG50 bouillon) and 34 h to 95.5 h (MYG50 bouillon). The yeast growth could be enhanced for most of the test strains by using OM bouillon instead of YEG50 and MYG50 which was reflected by shortened detection times. The results show, that detection times of 2–3 days for the majority of the tested osmotolerant yeast species with initial counts of $10^2$ CFU/g can be realized in OM bouillon.

The less selective culture medium DG18 bouillon, featuring an $a_w$-value of 0.95, led to a more rapid yeast growth compared to the culture media with $a_w$-values around 0.90 (see supplementary material). *Z. mellis* (CBS 1091) and *C. magnoliae* (WH 1023) did not grow in DG18 bouillon. In fact, the yeast fermentation rates in DG18 were the lowest of all examined culture media. For example the mean value of the $CTR_{max.}$ for the tested *Zygosaccharomyces* species was 5.9-fold higher in OM bouillon compared to DG18 bouillon and the $CTR_{max.}$ for the tested *S. pombe* strains was increased by a factor of 8.9. Overall considerable increases of the $CTR_{max.}$ were achieved in the developed culture medium (Fig. 4b).

Besides the lower total amount of fermentable glucose in DG18 in relation to the 3 other culture media tested, one reason for the low fermentation rates in this culture medium could be the negative influence of dichloran on yeast fermentation (Michaels et al., 1977; Henson, 1981). In our opinion this culture medium is less appropriate for a selective qualitative test for osmotolerant yeasts, even though the growth of osmotolerant yeasts on DG18 agar resulted in defined yeast colonies. Regarding this statement, we are in accordance with Beuchat et al. (2001) who showed that DG18 cannot be recommended as general purpose medium for recovering yeasts from a desiccated condition. Table S1 of the supplementary material gives more detailed information about detection times and maximum fermentation rates for every single strain in the tested culture media.

### 3.4. Inoculation experiments in the parallel fermenter system

Using the RAMOS® parallel fermenter in combination with OM culture medium for inoculation experiments with osmotolerant yeasts enabled the observation of the influence of the food matrix on the yeast growth and fermentation rates. The enrichment samples, performed before these tests, showed that one product, treacle, was naturally contaminated by osmotolerant yeasts. Further cultural analyses of the product revealed the presence of osmotolerant yeasts in low counts (2 CFU/g). Analysis of the ITS-sequencing results and comparative SAPD-PCR fingerprinting of the yeast isolate proved the presence of a

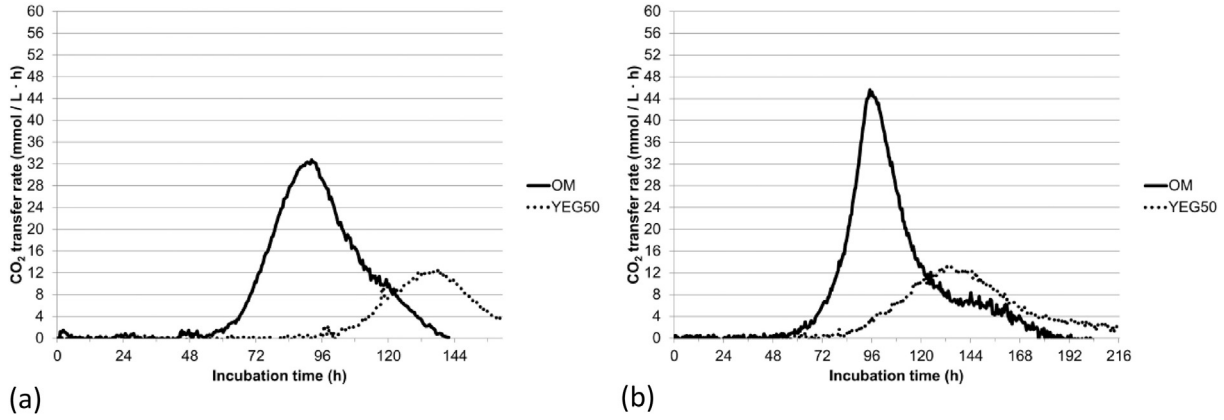


**Fig. 3.** $CO_2$ transfer rate of *Torulaspora delbrueckii* (DSMZ 70526, Inoculum $1.0 \cdot 10^2$ CFU/mL) (Panel a) and *Schizosaccharomyces pombe* (DSMZ 70576, Inoculum $9.7 \cdot 10^2$ CFU/mL) (Panel b) in OM bouillon and YEG50 bouillon analyzed in the RAMOS parallel fermenter system.

*Z. rouxii* strain (WH 1018). The foods were inoculated with high ($10^2$) and low ($10^0 - 10^1$) counts of *Z. rouxii* (WH 1001) and *T. delbrueckii* (WH 1013), respectively (Section 2.7). *Z. rouxii* and *T. delbrueckii* were chosen for these experiments because they represent two of the most widespread spoilage yeasts in sugar-rich foods and drinks. The naturally contaminated treacle was analyzed without inoculation in the parallel fermenter system. The inoculation experiments with *Z. rouxii* in sugar-rich products showed detection times (times to CTR ≥ 4 mmol/L·h) of 32.5 h–38 h (high level contamination) and 46.3 h–63 h (low level contamination) by using OM culture medium, whereas the detection times in the corresponding control assays without food matrix were 34.5 h ± 0.9 h for high level contamination and 48.3 h ± 3.1 h for low level contamination. All tested foods, except granulated honey, had only little influence on detection times compared with the control assays. For the detection of low counts of *Z. rouxii* in granulated honey, detection times were prolonged up to 9.5 h. The detection of *Z. rouxii* (WH 1018) in the naturally contaminated treacle took 63 h due to the extremely low yeast counts present in this product. In most of the enrichment cultures with *Z. rouxii*, higher maximum fermentation rates ($CTR_{max.}$) were reached in contrast to the control assays without sugar-rich foods. The $CTR_{max.}$ varied between 44.1 and 53.6 mol/L·h in these tests compared to 44.8 mol/L·h (±1.3) in the control assays. Highest fermentation rates were reached in the assays with marzipan paste.

The inoculation experiments with *T. delbrueckii* (WH 1013) lead to detection times of 62 h–93 h (high level contamination) and 76 h–97 h (low level contamination). The corresponding detection times in the negative controls without food matrix were 70.5 h (±0.4 h) for high level contamination and 71 h (±1.3 h) for low level contamination. The detection of *T. delbrueckii* in granulated honey was prolonged significantly for up to 26.5 h. The $CTR_{max.}$ varied between 19.4 and 24.6 mol/L·h (high level contamination) and 30.8 and 35.5 mol/L·h in (low level contamination) in these tests compared to 26.0 mol/L·h (±0.6) and 33.8 mol/L·h (±0.6) in the control assays, respectively. Overall fermentation rates in the enrichment cultures with *T. delbrueckii* were lower compared to the experiments with *Z. rouxii*.

Regardless of the examined yeast species (*Z. rouxii* or *T. delbrueckii*) and food, the yeast counts reached mean values of $1.3 \cdot 10^7$ CFU/mL at the end of the experiments when inoculating with high initial yeast counts ($10^2$ CFU/g product). Originating from lower initial yeast counts ($10^0$–$10^1$ CFU/g), higher yeast counts with mean values of $1.4 \cdot 10^8$ CFU/mL were found. Appropriately to this observation, low initial yeast counts led to higher carbon dioxide transfer rates ($CTR_{max.}$) in the enrichment cultures with *T. delbrueckii* in all test assays compared to the tests with higher initial yeast counts. This illustrates that even contaminations with few yeast cells are taking a big risk for food spoilage e. g. when raw materials or foods are processed. The experimental inoculation of *Z. rouxii* (WH 1001) in high-sugar foods showed, that detection times of 3 days are possible for a qualitative detection in OM bouillon even if low microbial counts ($10^1$ CFU/g) occur in high-sugar foods. For the detection of low counts ($10^0$ CFU/g) of *T. delbrueckii* (WH 1013) in OM bouillon up to 4 days were required.

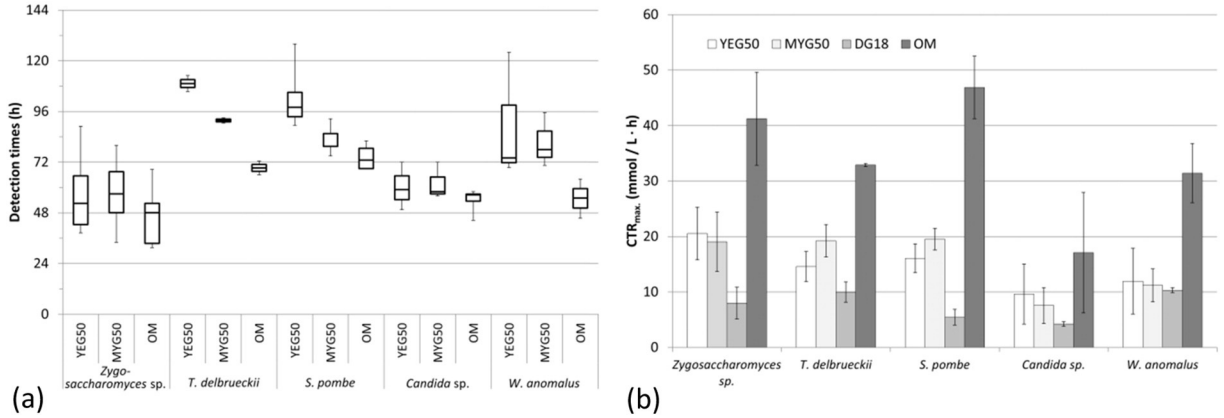


**Fig. 4.** Box plot showing the detection times (time to CTR ≥ 4 mmol/L·h) (Panel a) and comparison of the maximum $CO_2$ transfer rates ($CTR_{max.}$) (Panel b) of 9 *Zygosaccharomyces* strains, 4 *Candida* strains, 2 strains of the species *Torulaspora delbrueckii*, 5 *Schizosaccharomyces pombe* strains and 3 *Wickerhamomyces anomalus* strains in YEG50, MYG50 and OM bouillon analyzed at initial yeast counts of $10^2$ CFU/mL in the RAMOS parallel fermenter system in this study. The corresponding strains are listed in Table 1.

### 3.5. Inoculation experiments in the Biolumix system

The idea for applying the OM culture medium in an automated test incubator was to use the enhanced fermentation rates of yeasts in this medium for a sensitive and rapid detection. For experimental inoculation of sugar-rich foods, samples contaminated with $10^1$ to $10^2$ CFU/g were prepared (Section 2.7). Colony counting of the inoculated food suspension out of the Biolumix test vials on OM agar revealed that the initial yeast counts varied between $1.0 \cdot 10^1$ CFU/mL and $3.5 \cdot 10^2$ CFU/mL. Without food matrix, the detection times for *Z. rouxii* (CBS 4512 and WH 1001) were 34.6–36.5 h. The type of food had only less influence on the detection of *Z. rouxii* at low yeast counts, resulting in means of 51.9 h (Fig. 5). The testing of *T. delbrueckii* (DSMZ 70526 and WH 1013) in OM culture medium without product matrix lead to detection times of 35–37.7 h. Overall, the detection times for *T. delbrueckii* in sugar-rich foods were more variable depending on the product matrix compared to the inoculation tests with *Z. rouxii*, varying between 33.9 h for the detection in persipan paste to 69.8 h for the detection of 10 CFU/g in a poppy seed filling (Fig. 5).

The use of an automated test incubator in combination with the developed OM bouillon led to a more rapid detection of *Z. rouxii* and *T. delbrueckii* in high-sugar foods compared to existing cultural test methods using culture media with appropriate $a_w$-values. In these studies 5 days to 7 days were required for the detection of osmotolerant yeast species at initially low counts (10 CFU/g or mL) in enrichment cultures of high-sugar products (Jermini et al., 1987; Beuchat, 1993). In general, one benefit of using culture media in combination with automated test incubators is to give up time-consuming presence or absence tests over a period of several days.

## 4. Conclusions

A culture medium for qualitative testing of osmotolerant yeasts was developed by use of a parallel fermenter system and rapid microbiological testing. The OM culture medium led to shortened detection times and significantly increased carbon dioxide transfer rates for yeasts of the genera *Zygosaccharomyces*, *Torulaspora*, *Schizosaccharomyces*, *Wickerhamomyces* and *Candida* compared to existing culture media for osmotolerant yeasts with equal $a_w$-values. By increasing the yeast fermentation rates in OM culture medium, the development of a more rapid and reliable test assay for osmotolerant yeasts was possible especially in combination with an automated microbiological test incubator.

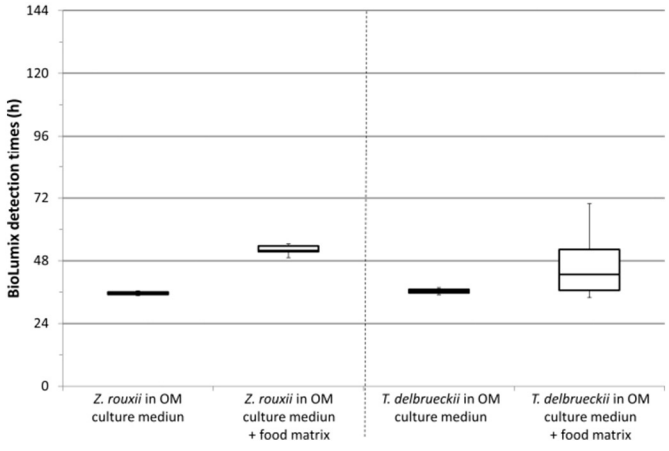


**Fig. 5.** Box plot showing the detection times for *Zygosaccharomyces rouxii* (WH 1001) and *Torulaspora delbrueckii* (WH 1013) in the Biolumix system at initial yeast counts between $10^1$ and $10^2$ CFU/mL in OM culture medium and in OM culture medium with high-sugar food matrices in a ratio of 1:10 respectively. The data are based on the testing of the following foods: marzipan paste, nougat filling, granulated honey, sugar syrup, persipan paste, poppy seed filling and strawberry jam. Determination in duplicates.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mimet.2016.08.021.

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