

T. Tyrell, S. Reimann, R. Folz, D. Harms, J. Hinrichs and G. Offer

Screening of Brewery Yeast Strains Regarding Organic Acid Profile in Order to Find Low Succinic Acid Producer

The yeast collection of VLB Berlin provides with more than 80 strains a broad range of top and bottom fermenting yeasts for the use in beer and wine production. As organic acids in alcoholic fermentation become more and more of interest regarding health and drinkability [1, 2, 3, 4, 5, 6], a screening was carried out to select strains with a reduced production rate of succinic acid. It was possible to show, that with the right selection of yeast, beer with considerable lower amount of succinic acid can be produced. Additionally this work gives an overview on yeast strain impact on other organic acids. Furthermore scale-up conditions between laboratory and pilot scale fermentations are discussed.

Descriptors: succinic acid, malic acid, organic acid, drinkability, fermentation scale-up, beer

1 Introduction

1.1 Organic acids and drinkability

Drinkability is widely used as one of many attributes available to characterise a beer. Drinkability results from four different effects [7, 8]:

1. Sensory effect is caused by the aroma, taste, temperature, and texture of the beer. In regards to organic acids the balance between sour- and sweetness of a beverage is of high importance.
2. Cognitive effect represents the information, experiences and beliefs of the subject related to the beer properties and its effects on the consumer.
3. Post-ingestive effect includes normal consequences to the human organism caused by the ingestion of beer, e.g., distention of stomach wall, heartburn, rate of gastric emptying, and production of hormones and physicochemical receptors by the intestinal tract.
4. Post-absorptive effect is related to the biochemical mechanisms resulting from metabolites in the blood stream. This occurs after beer metabolites have already been absorbed at the intestinal tract. It includes the effects of alcohol, glucose, purines and amino acids on body functions.

In regards to organic acids and drinkability succinic acid and maleic acid were described as a gastric acid stimulant in a publication of

late 90th [4]. A recent study on the same topic confirmed this and described as well malic acid as a potential stimulant [5, 6]. This is opposite to a study of the early 90th, where malic acid was tested as well, but no impact on gastric acid secretion was observed [3]. This stimulation of gastric acid secretion can be seen as a post-ingestive aspect of drinkability. It can be assumed, that consumers, sensible to heartburn will not continue consumption of a beer, if they can relate negative experiences to it.

For that reason it is of interest for brewers to know more about these C₄-dicarboxylic acids in the brewing process. Unfortunately now existing studies [5, 6] – showing same effect for malic acid – were not known at start of experiment. For that reason line out of experiments was only considering succinic acid. Nevertheless all organic acids were monitored within experiments and therefore some indications on malic acids are shown as well.

The objective of trials was to find out brewing yeast strains with comparatively low succinic acid production in order to be able to produce a beer with low amounts of this C₄-dicarboxylic acid.

1.2 Origin of organic acids

Yeast generates by its amino acid and carbohydrate metabolism a broad range of fermentation by-products. Some of them are able to have a sustainable impact on beer aroma and flavour [9, 10, 11]. The concentration of organic acids in beer is mainly determined by the composition of wort derived from the raw materials and the chosen yeast strain [12, 13, 14, 15, 16, 17]. *Püschner* and *Xang* [14, 17] report, that the organic acid composition of malt is influenced by several parameters: Grain variety, fertilization, year, region, state of maturity, dryness and grain specific micro flora, but as well by malting parameters. The amount of organic acids brought into the product by hops can be neglected [18]. Concentrations of different organic acids as described in literature are listed in table

Authors

Thomas Tyrell, Stefan Reimann, Roland Folz, Diedrich Harms, Johannes Hinrichs, Guido Offer, Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V., Berlin; corresponding autor: tyrell@vlb-berlin.org

Table 1 Concentration of organic acids in beer

Author	Briggs		Klopper		Enebo	Coote/Kirsop	
	bottom ferm.	top ferm.	bottom ferm.	top ferm.	bottom ferm.	bottom ferm.	top ferm.
Organic acids [ppm]							
Acetic acid	10–50	56–136	9–136			12–156	22–107
Citric acid	100–150	173–211	107–186	90	88	71–133	56–158
Fumaric acid				11	15		
Lactic acid	50–300	15–901	10–284	51	31	44–276	48–233
Pyruvic acid	100–200	1–127	19–95	6	11	34–80	10–32
Malic acid	30–50	110–136	24–104	52	42	26–78	14–95
Succinic acid	50–150			67	41	36–166	51–153

1. Püschner described, that a categorization of beer by its organic acid composition alone is not possible [14].

Most of organic acids in beer are derived from fermentation and are originated oxidative directly within the tricarboxylic acid cycle [11, 14]. Another possibility presents the formation of organic acids similar to higher aliphatic alcohols by the Ehrlich-pathway [9, 27].

Boulton [9] explains the excretion of organic acids under anaerobic conditions by yeast with the following three facts:

- They are not of importance for the yeast regarding the synthesis of cell material.
- Further going metabolic pathways for oxidation of most organic acids do not exist under fermentation conditions.
- To maintain enzyme catalysed metabolism yeast needs an almost neutral intracellular pH-value.

Furthermore a rising amount of dead cells towards the end of fermentation may increase perhaps organic acid concentration in beer due to yeast autolysis [10].

Regarding the excretion of organic acids it has to be mentioned, that it is no continuous process from pitching to diacetyl rest. Investigations of *Coote*, *Kirsop*, *Heerde*, Püschner und *Whiting* [12, 14, 16, 27] state, that individual organic acids are released and taken up at different points of fermentation. For example pyruvate, which is released in the beginning of fermentation, can be reassimilated and metabolised to acetate.

1.3 Flavour

Organic acids show an intrinsic, acidic flavour [12, 16, 17]. Some of them are characterized individually [9, 10, 24, 19, 16, 17]. In general concentrations of relevant organic acids found in beer are below flavour thresholds of 200-400 ppm (Acetic – 200ppm, Citric – 350 ppm, Lactic – 400ppm, Malic – 350ppm, Succinic – 200) [20]. Lower thresholds are found only for organic acids of larger C-chains like Valeric or Caprylic acid or as an exception butyric acid. Even though sensorial thresholds single acids are mostly undergone in beer, synergetic effects with other beer components and for formation of esters with alcohols are given

[14, 21, 22, 23]. This formation of esters is found as well during beer ageing.

1.4 Technological impact on beer quality

The decrease of pH value during fermentation is, beside other effects, strongly related to the release of organic acids by the yeast [9, 10, 12, 24, 14, 16]. Investigations of *Coote* and *Kirsop* [12] have shown, that different organic acids at identical concentrations lead to different beer pH-values. This depends on number of carboxylic acid groups and pKs-values. For biological shelf life of beer organic acids are of high importance as their undissociated molecule can enter microorganisms and effect the intracellular pH-value of these. By lowering intracellular pH-value metabolism of can be disturbed [14, 17].

1.5 Origin of organic acids during fermentation

Püschner et al. [14] describe, that citric, fumaric und formic acid concentration of beer basically depends on concentrations of wort, for that reason they are mainly determined by the raw material. Organic acids like pyruvic, lactic, malic and succinic acid are besides raw material impacts strongly depended on the fermentation process.

The impact of the yeast strain itself is generally accepted. This is the case especially for succinic acid [25, 26, 27]. Additionally to this, it is reported, that yeasts showing a low production of succinic acid have a relatively high output of acetic acid as by-product during fermentation. Japanese publications present fermentation trials with yeast deficient in α -Ketoglutarat-Dehydrogenase activity show a reduced succinic acid release of about 40%. Nevertheless, they report as well slow fermentation speeds and low multiplication rates of such mutants [28, 29, 30]. For that reason a use of yeasts with a complete knock-out of this important metabolism pathway for industrial beer production is less recommendable. Investigations concerning brewers yeasts and their production rate of succinic acid were not known at start of experiment.

2 Material and methods

The aim of this yeast screening was to identify typical brewery yeast strains, that are able to ferment brewers wort by only slight

formation of succinic acid as a fermentation by-product. For that reason a total of 30 different strains were tested in laboratory scale. With a following scale up of 1:10 with selected strains, reproducibility of results was checked. A final comparison of succinic acid concentrations of trial beers with commercial beers helped to place results in relation. Other organic acids were monitored, as well.

2.1 Fermentation recipients

Fermentation recipients: 500 ml Erlenmeyer flask and 2 litre EBC fermentation tubes

2.2 Material

Fermentation substrate: Wort was prepared by dilution of Bavarian Pilsener malt extract (Weyermann). Hop bitterness of 20 bittering units was achieved by addition of Iso- α -acid solution (Barth-Haas).

Yeast strains: yeast strains were taken from the VLB yeast bank

2.3 Sample preparation and analyses

For quantification of organic acids, fermented worts were centrifuged and sediment was discharged.

Ion chromatography: DX-600 (Fa. Dionex), with AS50 autosampler, AS50 thermal compartment, GS50 gradient pump and ED50 electrochemical detector

Columns:

- AS11-HC (I.D. 2 mm x L. 250 mm), pre column AG11 (I.D. 2 mm x L. 50 mm),
 - Suppressor ASRS Ultra II (2 mm)
 - Separation of butyric, formic, pyruvic, maleic and citric acid
 - Flow: 0.45 ml/ min, concentration gradient: A: NaOH 100 mM/B: bidestilled water 0.05 μ S/cm, temperature of column: 40 °C, suppressor current: 75 mA
- ICE-AS6 (9 mm x 250 mm)
 - Eluent: 0.4 mM Heptafluorobutyric acid, isocratic
 - Flow: 1.0 ml /min
 - Separation of malic, citric, formic, acetic, lactic, succinic acid
- AS9-HC (2 mm x 250 mm), precolumn AG9-HC (2 mm x 50 mm)
 - Eluent: 9 mM Natriumcarbonate isocratic
 - Flow: 0.25 ml/ min, Suppressor: Suppressor ASRS Ultra II (2mm) Current: 14 mA
 - Separation of oxalic and formic acid

- Tray temperature: 10 °C, injection volume: 10 μ l;
- Temperature of conductivity measurement cell: 30 °C

Enzymatic determination of succinic acid and malic acid: Boehringer Mannheim, Enzytec

Analyses of extract, pH, and yeast cell concentration of wort and beer: MEBAK, Band II, 4th edition, 2.10 (extract), 2.14 (pH), Band III, 2nd edition, 10.4.3 (yeast cell concentration).

2.4 Experimental

2.4.1 Fermentation trials in laboratory scale

200 ml of wort of a concentration of 12°P was filled into Erlenmeyer flasks (500 ml) and autoclaved. After autoclavation flasks were inoculated with yeasts. A double determination was carried out per strain. Fermentation temperature was isothermal at 20 °C. On 3rd and 5th day of fermentation extract was controlled. When final attenuation degree was reached yeast growth was determined by yeast cell count and beers were prepared for analyses. In total 30 strains were tested. For 10 yeasts, with potential of low succinic acid production, trials were repeated.

2.4.2 Fermentation trials in EBC fermentation tubes

Prepared wort was pasteurised prior filling of fermentation tubes and cooled down to 15 °C. In order to guarantee a good mixing of yeast and wort at pitching, tubes were filled first with 1 litre of wort, then yeast was added and another litre of wort was added from the top into the tube. Propagation of yeast was carried out in a prior propagation with the same recipients: Addition of 200 ml culture at "Hochkräusen" to 2 litre of wort. Yeast was then harvested and used for pitching within the trial. Yeast concentration, pH and extract development was checked daily. For this scale the very well known yeast strain RH of VLB (test strain 31) was added to the trials in order to have a comparison with commercial beers.

2.5 Test of succinic acid concentration of commercial beers

More than 60 commercial beers (all malt and with portion of adjuncts, mainly bottom fermenting, some top fermenting) were checked on their succinic acid concentration and compared with trial results. As of some of the commercial beers the used yeast strain was known a correlation with results of trials was possible to realise.

3 Results and discussion

3.1 Results of yeast screening in laboratory scale

During laboratory yeast screening it was observed, that all tested strains (with three exceptions) reached a final apparent extract of ≤ 2.8 (% w/v) after a fermentation time of 5 to 6 days. Regarding pH-value of fermented worts no significant differences had been observed between tested strains. Regarding yeast cell counts most of the strains showed a yeast multiplication rate that correlates well

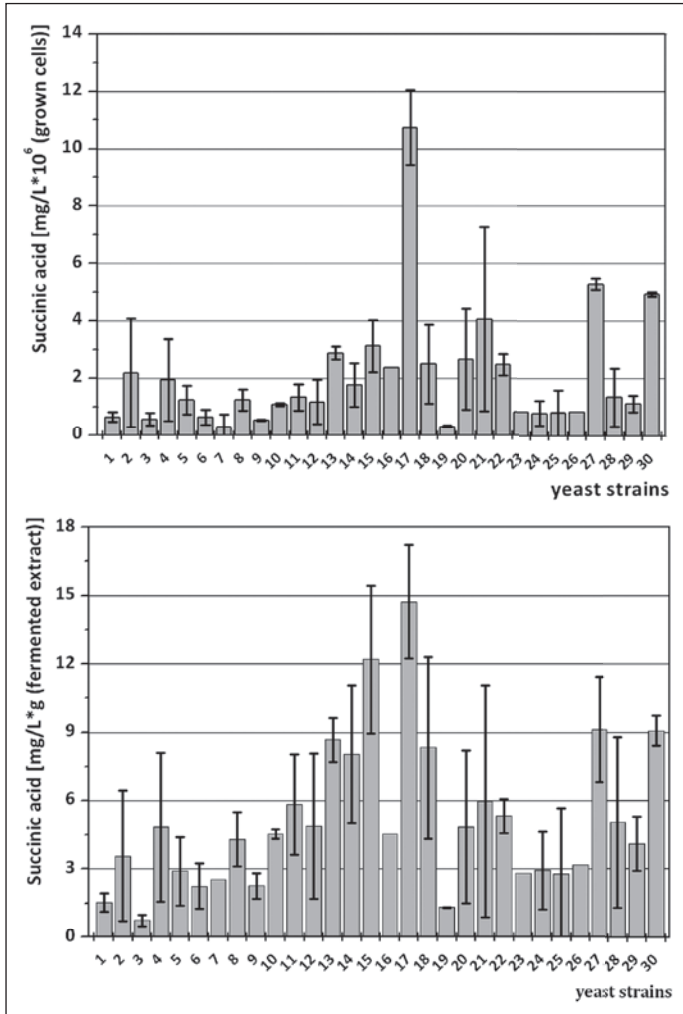


Fig. 1 Strain specific formation of succinic acid during fermentation in relation to yeast cell count (top) and extract decrease (bottom)

with parameters of fermented wort: strong yeast growth and high alcohol, low remaining extract of fermented wort. In comparison to this, five strains show a lower cell count at the end of fermentation, while parameters of fermented wort revealed a comparable high fermentation degree.

The organic acid profile of fermented worts of the yeast screening was set into comparison to the profile of wort (data not shown). From results it was seen that none of the tested strains excreted fumaric or propionic acid into the fermentation media. Regarding butyric, maleic and oxalic acid only slight differences had been observed. For approximately 2/3 of the tested strains citric acid concentration remained unaltered, while for some strains a slight increase was observed. For pyruvic, malic, lactic and succinic acid differences to the original concentration was seen. All four mentioned organic acids show an increase of their concentration during fermentation. The described reassimilation of pyruvic acids and its metabolization to acetic acid was not confirmed by trial set-up. Maximum values such as 300 ppm for pyruvic acid (strain 10), 100 ppm for Malic acid (strain 25), 400 ppm for lactic acid (strain 17) and even 800 for succinic acid (strain 17) give an indication how high the impact of yeast can be. Following the idea to find a low succinic acid producer a repetition of trials with a few

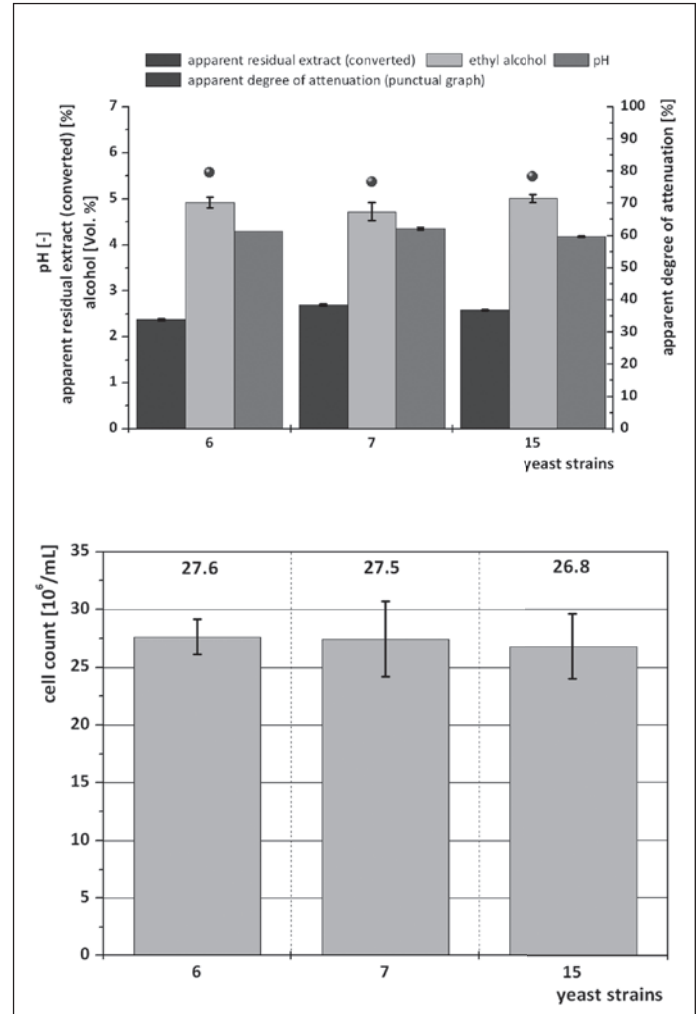


Fig. 2 Fermentation characteristics of chosen test strains (6, 7 and 15) for further going trials in EBC fermentation tubes

yeasts of very high (strains 15, 17) some of intermediate (strains 13 and 14) and some of comparatively low succinic acid production (strains 1, 3, 6, 7, 9, 19) was carried out. Results of the first screening set-up was confirmed.

In order to get qualitative information on yeasts a quantitative relation between produced succinic acid, extract decrease and cell count increase was chosen to evaluate their characteristics (Fig. 1). From this figure it can be seen, that strong differences between yeast strains can be observed.

3.2 Results of fermentation trials in EBC fermentation tubes

For scale-up of fermentation trials in EBC fermentation tubes yeast selection was set exclusively on succinic acid out-put. The aim was to proof low succinic acid production of selected strains. In order to assure a good comparability of the trials, observed growth characteristics were as well taken into account. Finally strains 6 and 7, as low succinic acid yeast, were chosen for a comparison with strain 15, as high succinic acid yeast. Within figure 2 and figure 3 results of the yeast screening are shown in detail and in a comparative way. Additionally to this a very well known yeast strain

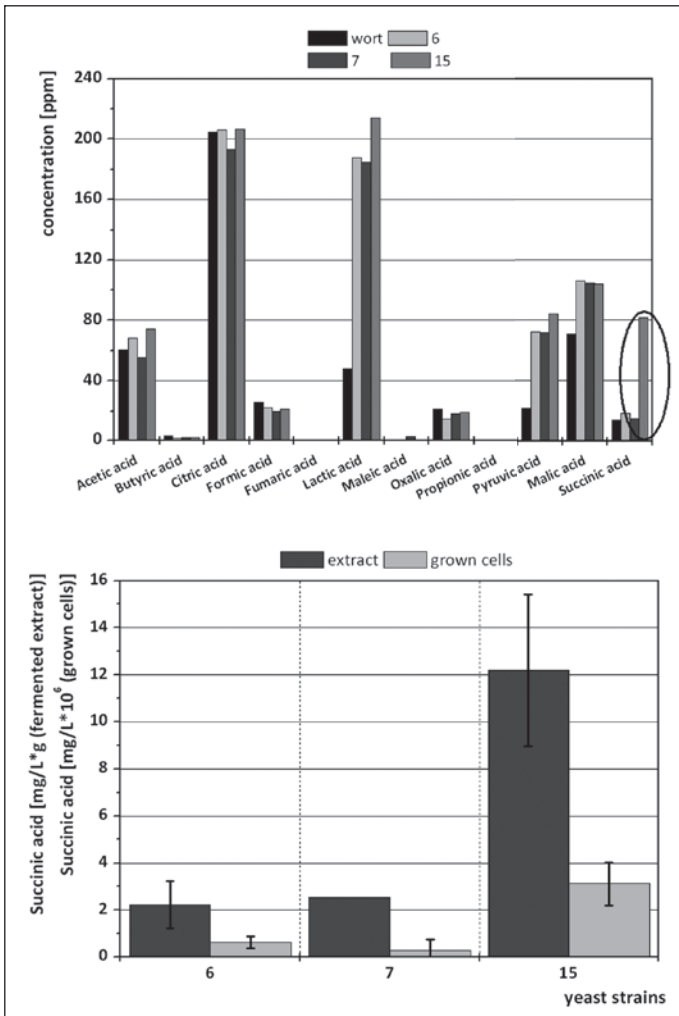


Fig. 3 Organic acid and especially succinic acid characteristics in substrate after fermentation with test strains (6, 7 and 15) in laboratory scale. These strains were elected for further going trials in EBC fermentation tubes

of VLB named Rh (strain 31) was chosen to serve as a reference. This strain was not tested within prior screening. All trials run in double determination.

In figure 4 fermentation characteristics such as extract, pH-value and yeast cell count development are shown. It can be seen that all 4 strains showed a very similar fermentation profile. Only strain 7 shows a slightly higher pH-value throughout fermentation until the final beer.

With figure 5 organic acid concentrations of wort and beer are described. Differences of organic acid concentration between resulting beers of corresponding yeast strains were observed for succinic acid as well for citric, lactic, malic and for maleic acid. These organic acids showed a rise in concentration. For butyric, formic, oxalic and pyruvic acid comparable decreasing concentration development from wort to beer for all strains was observed. A comparable increase to similar concentrations was observed for acetic acid.

Comparing behaviour of strains between laboratory and EBC tube fermentation (Fig. 2 and Fig. 5) it can be seen, that strong

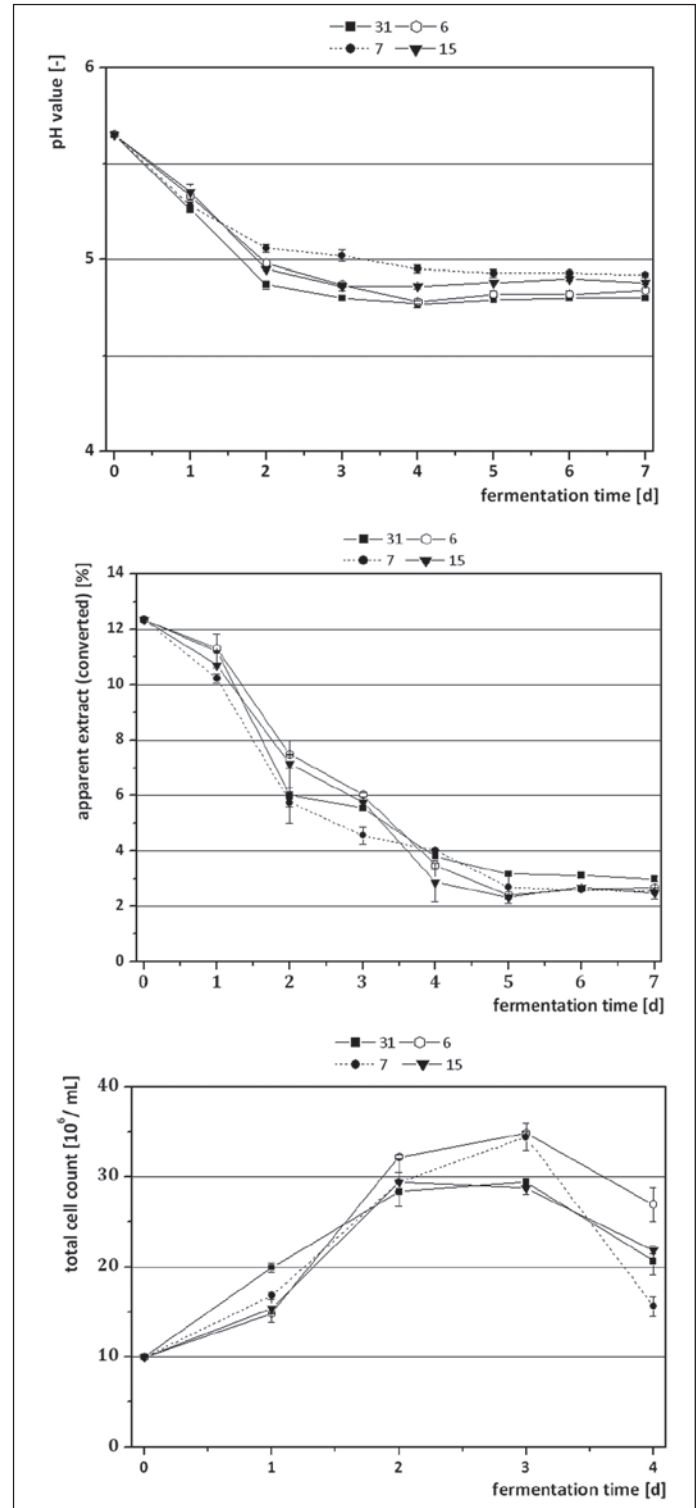


Fig. 4 Trials in EBC fermentation test tubes: extract, pH and yeast cell count development of test strains 6, 7, 15 and 31

differences between fermentation scales can be observed. This is the case especially for acetic, formic, oxalic and pyruvic acid. It was assumed, that higher oxygen intake, higher fermentation temperature and lower hydrostatic pressure of lab scale fermentation have an impact on this.

The concentration development of single acids was as well monitored during fermentation. Figure 6 to figure 14 show the corresponding data. Depending on time of uptake and excretion

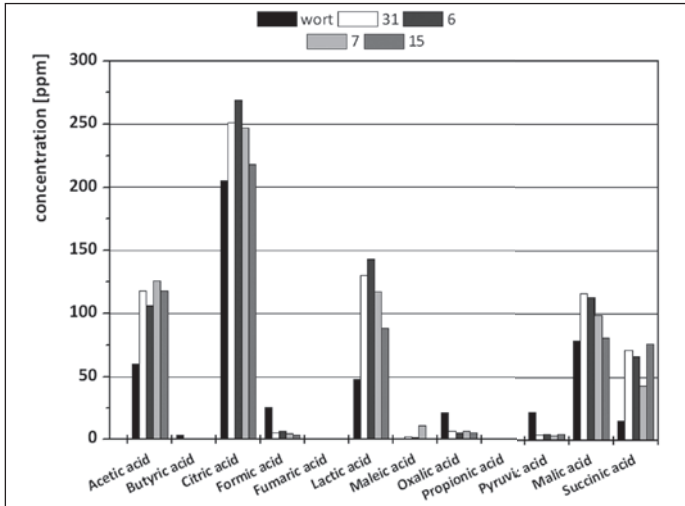


Fig. 5 Trials in EBC fermentation test tubes: overview of organic acid concentration of wort and beers from test strains 6, 7, 15, 31

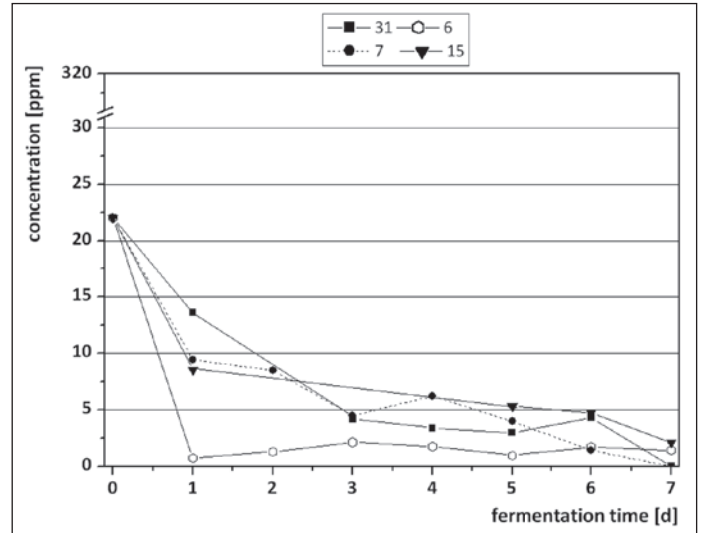


Fig. 6 Pyruvic acid development during fermentation in EBC fermentation tubes with different yeast strains

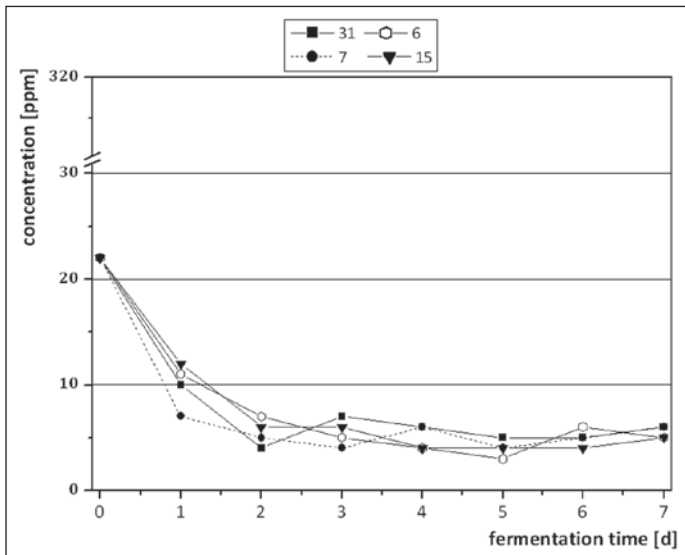


Fig. 7 Oxalic acid development during fermentation in EBC fermentation tubes with different yeast strains

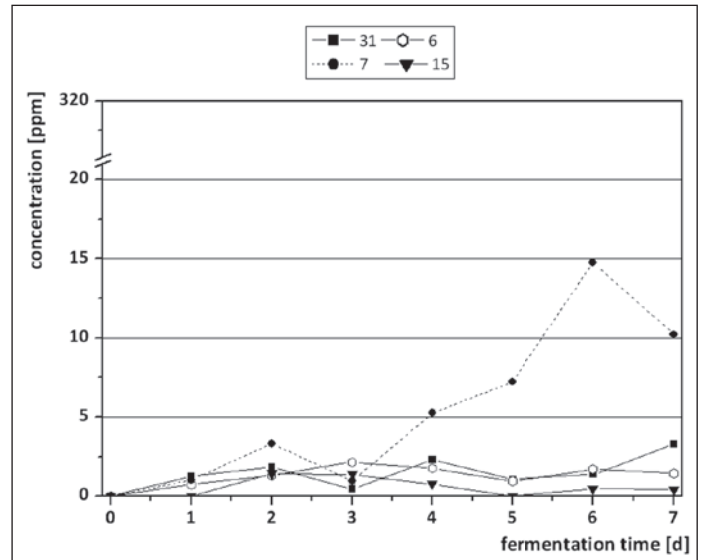


Fig. 8 Maleic acid development during fermentation in EBC fermentation tubes with different yeast strains

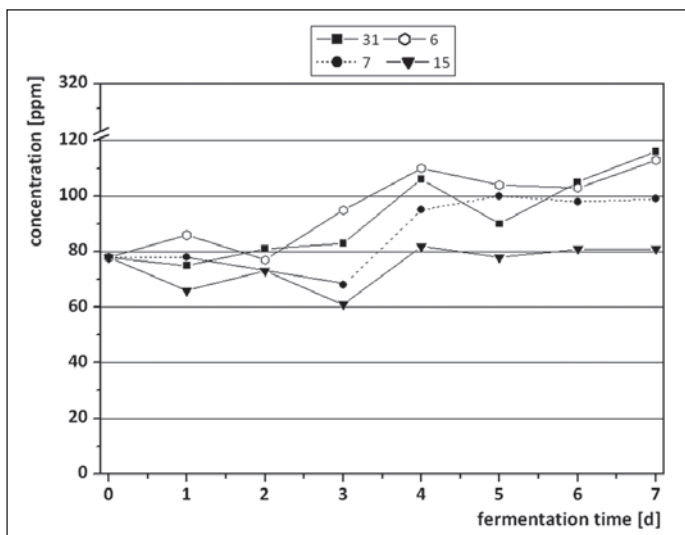


Fig. 9 Malic acid development during fermentation in EBC fermentation tubes with different yeast strains

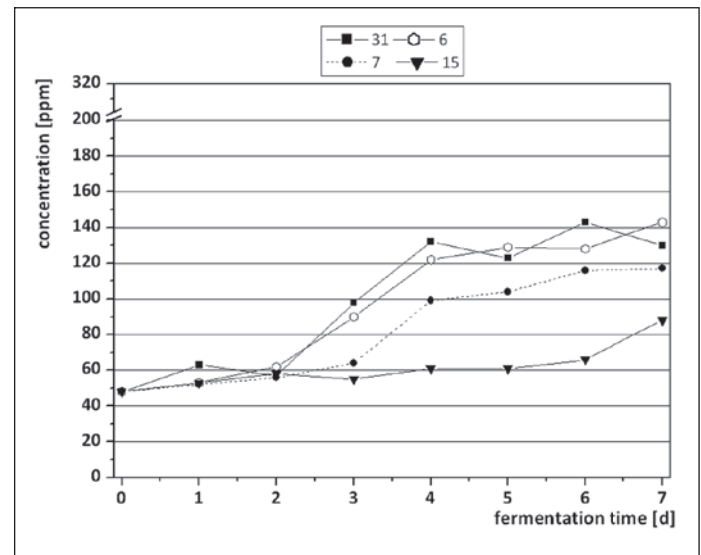


Fig. 10 Lactic acid development during fermentation in EBC fermentation tubes with different yeast strains

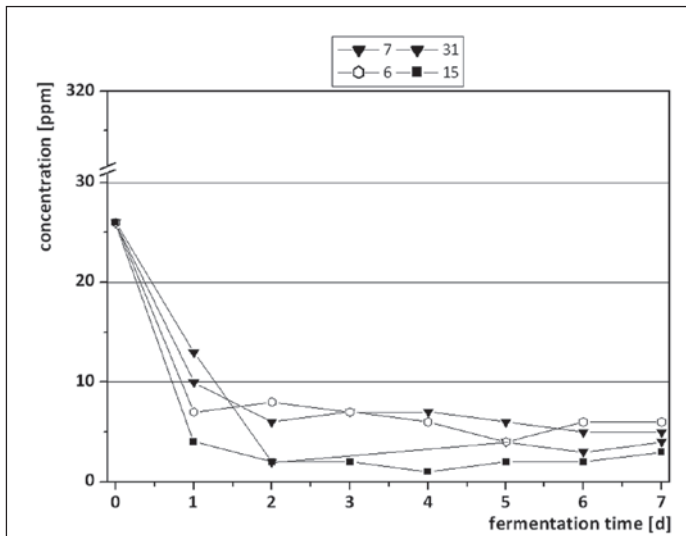


Fig. 11 Formic acid development during fermentation in EBC fermentation tubes with different yeast strains

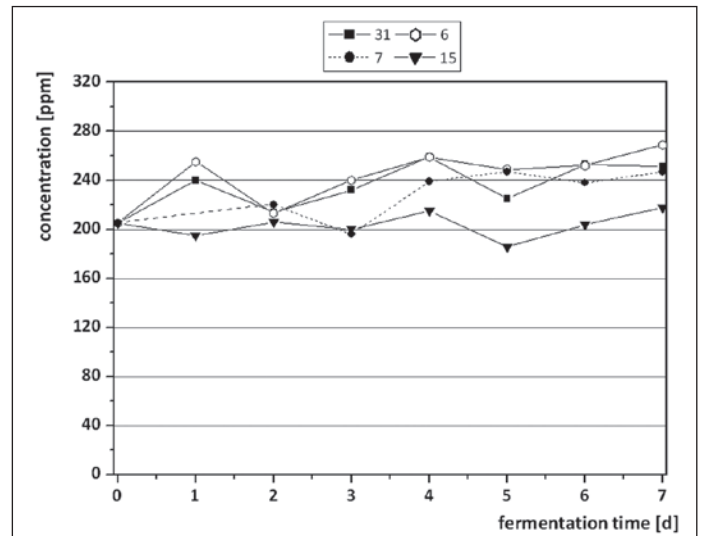


Fig. 12 Citric acid development during fermentation in EBC fermentation tubes with different yeast strains

Table 2 Concentration development of organic acids during beer fermentation with 4 different bottom fermenting yeast strains in 2 litre EBC tubes (Fig. 6 to Fig. 14)

Acid	Lower Krausen state	High Krausen state	Krausen collapsing	Difference between strains	Summary
Fumaric acid	0	0	0	no	Concentration stays below detection limit
Propionic acid	0	0	0	no	Concentration stays below detection limitw
Butyric acid	0	0	0	no	Concentration close to detection limit – slight decrease is observed
Pyruvic acid	--	0	0	slight	Strong concentration decrease at beginning of fermentation
Oxalic acid	--	0	0	no	Strong concentration decrease at beginning of fermentation
Formic acid	--	0	0	slight	Strong concentration decrease at beginning of fermentation
Acetic acid	++	o	-	slight	Increase at beginning and decrease towards end of fermentation: Overall a slight increase
Citric acid	0	0	0	no	Very slight increase over fermentation: Final concentration mostly determined by wort concentration
Malic acid	0	+	+	yes	Increase in concentration from high Krausen state on.
Lactic acid	0	+	+	yes	Increase in concentration from high Krausen state on.
Maleic acid	0	0	0; +	slight	Concentration close to detection limit, no changes over fermentation,; only one strain showed a slight increase towards end of fermentation
Succinic acid	+	+	0	yes	Increase at lower and high Krausen state.

+increase, ++ strong increase, 0 no change, - decrease, -- strong decrease in concentration

by yeast it was seen, that organic acids can be put together into groups. This is summarised within Table 2.

As mentioned the main focus of these trials was set on development of succinic acid (Fig. 14). As shown it is possible to observe differences between strains during fermentation. Figure 15 shows succinic acid production in relation to extract decrease and cell count development. It can be seen, that strain 7 is able to ferment with comparable fermentation speed and yeast cell count development as other 3 strains without producing higher amounts of succinic

acid. Succinic acid production during fermentation (subtracting wort derived concentrations from final ones) is doubled or tripled with strains 6, 15 and 31 compared to strain 7. A higher acetic acid production as described in literature in case of low succinic acid production was not confirmed with this trial. For yeast strain 6 low succinic acid production was not confirmed by this larger scale fermentation.

Trials in EBC fermentation tubes were as well carried out with top-fermenting yeast strains 1, 3, 17 and 19. For these strains

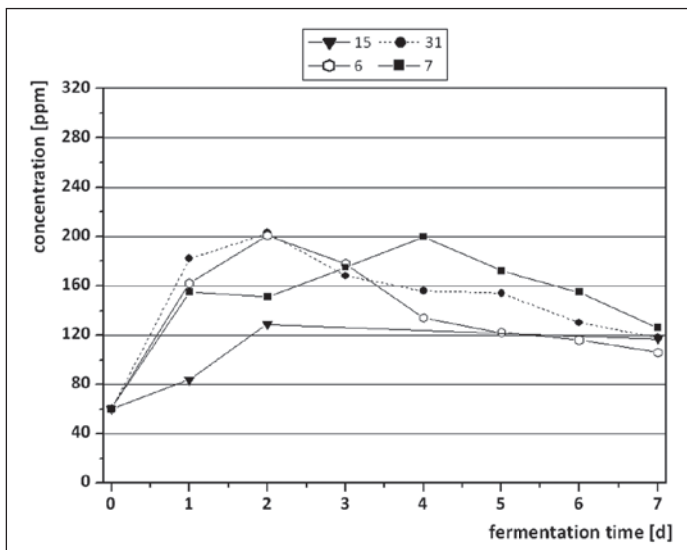


Fig. 13 Acetic acid development during fermentation in EBC fermentation tubes with different yeast strains

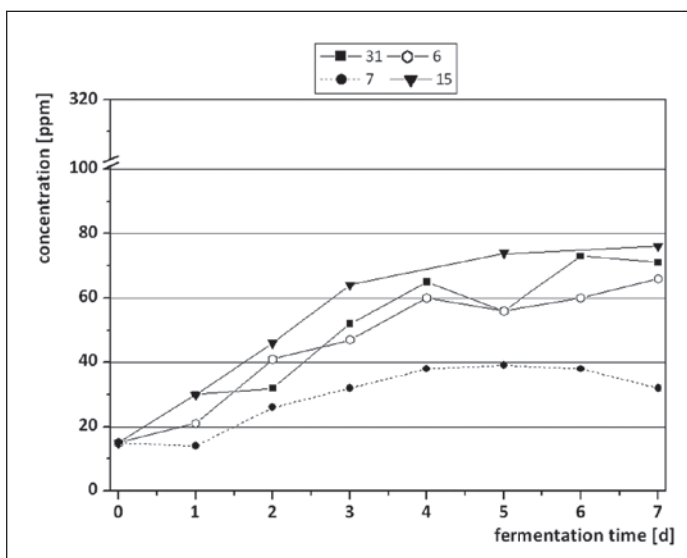


Fig. 14 Succinic acid development during fermentation in EBC fermentation tubes with different yeast strains

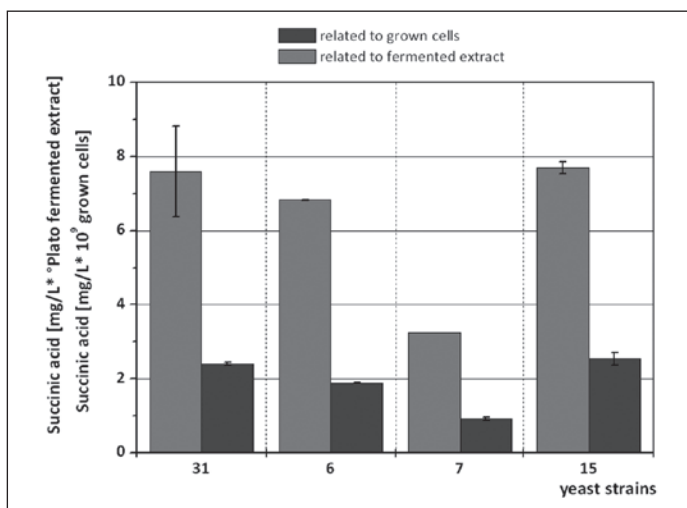


Fig. 15 Extract and yeast multiplication related succinic acid production of different yeast

low succinic acid levels were not confirmed with 2 litre fermentation scale (data not shown). This behavior was similar to bottom fermenting strain 6. As mentioned earlier the different conditions of screening and EBC tube scale must be seen as responsible for this observation.

Wort and beer of strains 7 and 31 had been compared additionally regarding their amino acid profile (data not shown). It was seen, that especially aspartatic acid, glutamic acid and to a certain point alanin had been taken up by strain 7 to a much higher degree as with strain 31. The different amino acid metabolism of these strains might therefore the reason for the difference in succinic acid production.

3.2.1 Additional observations regarding low malic acid producing yeast

It was found that as well malic acid production during brewers wort fermentation is very much strain depended. Even though trial set-up was not designed with the objective to find a yeast strain low in malic and succinic acid production data of the screening revealed difficulties to find a strain low in production of both acids.

3.3 Comparison of results of yeast screening with fermentation trials in EBC tubes and comercial beers

In order to make succinic acid production of trials comparable with beers of industrial production, analyses of succinic acid concentration of 60 commercial beers were carried out. An average value with the determined standard deviation is given with figure 16. It can be seen, that found concentrations are in the same range as the beers from EBC tube fermentations (40–90 ppm). Additionally one commercial beer fermented in industrial scale with strain 7 was analyzed. It was found to be on the lowest level of succinic acid concentration of commercial beers analyzed. The concentration of succinic acid was comparable to the concentration of beer of EBC tube fermentation with this yeast.

4 Discussion and conclusion

4.1 Scale-up

Regarding succinic acid development of fermentation within 200 ml Erlenmeyer flasks and 2 litre EBC tubes differences were observed and not all analyzed organic acids behaved the same. At the same time succinic acid concentration of commercial beer and of beer from EBC tube were in the same ranges. From this the assumption can be made, that laboratory scale screening as carried out within these trials is not as suitable as thought in the beginning. Nevertheless it was possible to find one strain able to produce low amount of succinic acid. This was confirmed by analyzing an industrial produced beer with this strain. The usefulness of dimensions of EBC tubes was confirmed by this trial and encouraged authors to further develop this practical fermentation scale as intermediate between laboratory and pilot plant [31, 32].

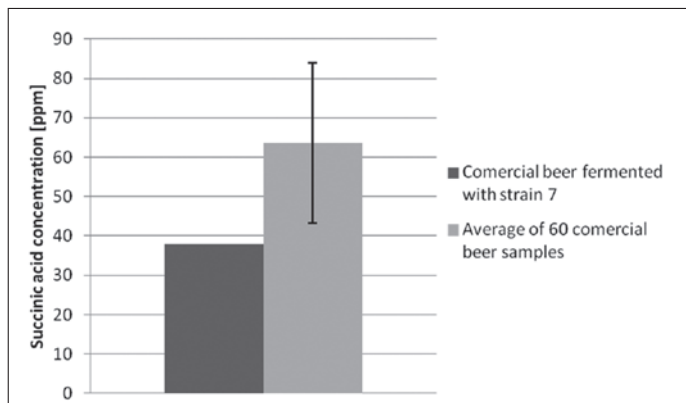


Fig. 16 Comparison of succinic acid concentration of commercial beers (all malt and with portion of adjuncts, mainly bottom fermenting) with one commercial beer fermented with yeast strain 7. In three commercial beers fermented with strain 31 succinic acid concentration were found in the range of 50 to 80 ppm

4.2 General observation of concentration development of organic acids and comparison with literature

Cited literature regarding organic acid development during fermentation was basically confirmed for succinic, fumaric, citric and formic acid. Additionally to this so far not monitored organic acids had been described the first time in an isothermal fermentation with 100 % malt based wort as substrate.

4.3 Objective to find low succinic acid yeast

The objective of this yeast screening was to find a yeast with very low succinic acid production during normal brewery fermentation. One strain revealed characteristics to produce much lower amounts as most brewery yeast strains. Control of a commercial beer fermented with this strain confirmed this characteristic even for industrial scale.

4.4 Brewing process parameters

Besides knowing the impact of the yeast strain on succinic acid production it is of importance to know as well impact of process parameters like temperature profile, yeast multiplication, substrate concentration. Trials concerning these parameters have been done as well. Results will be shown and discussed in a different paper.

Acknowledgement

Authors like to thank VLB Berlin and especially the teams of department of Brewing and Beverage Science and Applications and of the Central Laboratory for the support received within this research project.

This research project realized by Versuchs- und Lehranstalt für Brauerei in Berlin e.V. was supported by the German ministry of economy and technology within the program framework of industrial collaborative investigation (IGF) as an enactment of the German Bundestag.

5 Literature

- Singer, M. V.; Eysselein, V. E. and Goebell H.: Beer and wine but not whisky and pure ethanol do stimulate release of gastrin in humans, *Digestion* 1983; **26**, pp. 73-79.
- Singer, M. V.; Leffmann, C.; Eysselein, V. E.; Calden, H. and Goebel H.: Action of ethanol and some alcoholic beverages on gastric acid secretion and release of gastrin in humans, *Gastroenterology* 1987, **93**, pp. 1247-1254.
- Singer, M. V.; Teyssen, S. and Eysselein, V. E.: Action of beer and its ingredients on gastric acid secretion and the release of gastrin in humans, *Gastroenterology* 1991, **101**, pp. 935-942.
- Teyssen, S.; Lenzing, T.; Gonzalez-Calero, G.; Korn, A.; Riepl, R. L. and Singer, M. V.: Alcoholic beverages produced by fermentation but not by distillation are powerful stimulants of gastric acid secretion in humans, *Gut* 1997, **40**, pp. 49-56.
- Liszt, K.; Walker, J. and Somoza, V.: Identification of organic acids in wine that stimulate mechanism of gastric acid secretion, *J. Agric. Food Chem.* 2012, **60**, pp. 7022-7030.
- Walker, J.; Hell, J.; Liszt, K.; Dresel, M.; Pignitter, M.; Hofmann, T. and Somoza, V.: Identification of beer bitter acids regulating mechanisms of gastric acid secretion, *J. Agric. Food Chem.* 2012, **60**, 1405-1412.
- Mattos, R. and Moretti, R.: Beer drinkability – A review, *MBAA TQ*, 2005, **42** (1), pp. 13-15.
- Conner, M. T.: An individualized psychological approach to measuring influences on consumer preferences, *Measurement of Food Preferences* 1994, pp. 167-201.
- Boulton, C. and Quain D.: *Brewing yeast and fermentation*, Blackwell science Ltd., 2001.
- Briggs, D. E.: *Brewing science and practice*, Woodhead publishing limited, Cambridge England, 2004.
- Oura, E.: Reaction products of yeast fermentation, *Process biochemistry*, 1977, pp. 19-35.
- Coote, N. and Kirsop, B. H.: The content of some organic acids in beer and other fermented media, *J. Inst. Brew.*, 1974, **80**, pp. 474-482.
- Klopper, W. J.; Angelino, S. A. G. F.; Tunning, B. and Vermeire, H. A.: Organic acids and glycerol in beer, *J. Inst. Brew.* 1986, **92**, pp. 225-228.
- Püschner, C.; Zenz, H. and Schwarz, H.: Änderung des Carbonsäureprofils während des Brauprozesses, *Mitteilungen Österreichisches Getränke Institut*, 1995, **5/6**, pp. 52-59.
- Sroka, P. and Tuszyński T.: Changes in organic acid contents during mead wort fermentation, *Food Chemistry* 2007, **104**, pp. 1250-1257.
- Whiting, G. C.: Organic acid metabolism of yeasts during fermentation of alcoholic beverages – a review, *J. Inst. Brew.*, 1976, **82**, pp. 84-92.
- Yang X. and Yin L.: Factors influencing the organic acids content in Final Malt, *ASBC Journal* 2006, **64** (4), pp. 222-227.
- Enebo, L.; Blomgren, G. and Johnsson, E.: Low molecular non-volatile organic acids in wort and beer, *J. Inst. Brew.* 1955, **61**, pp. 408-411.
- Shindo, S.; Sahara, H. and Koshino, S.: Relationship of production of succinic acid and methyl citric acid pathway during alcohol fermentation with immobilized yeast, *Biotechnology letters*, 1993, **15** (1), pp. 51-56.
- Engan, S.: Organoleptic threshold values of some organic acids in beer, *J. Inst. Brew.* 1974, **80**, pp. 162-163.
- Vanderhaegen, B.; Neven, H.; Coghe, St.; Verstepen, K. J.; Verachtert, H. and Derdelinckx, G.: Evolution of Chemical and Sensory Properties during Aging of Top Fermented Beer, *J. Agric. Food Chem.* 2003, **51**, pp. 6782-6790.

22. Vanderhaegen, B.; Delvaux, F.; Daenen, L.; Verachtert, H. and Delvaux, F. R.: Aging characteristics of different beer types, *J. Agric.* 2007, **103**, pp. 404-412.
23. Saison, D.; de Schutter, D. P.; Uyttenhove, B.; Delvaux, F. and F. R.: Contribution of staling compounds to the aged flavour of lager beer by studying their flavour thresholds, *Food Chemistry* 2009, **114**, pp. 1206-1215.
24. Montanari, L.; Perretti, G.; Natella, F.; Guidi, A. and Fantozzis, P.: Organic and phenolic acids in beer, *Lebensm.-Wiss. u.-Technol.* 1999, **32**, pp. 535-539.
25. Castellari, L.; Magrini, A.; Passarelli, P. and Zambonli, C.: Effect of must fermentation temperature on minor products by cryo and non-cryotolerant *Saccharomyces cerevisiae* strains, *Italian Journal of Food Science* 1995, **7** (2), pp. 125-132.
26. Shimazo, Y. and Watanabe, M.: Effects of yeast strains and environmental conditions on formation of organic acids an must during fermentation, *J. Ferment. Technol.* 1981, **59** (1), pp. 27-32.
27. Heerde, E. and Radler, F.: Metabolism of the anaerobic formation of succinic acid by *Saccharomyces cerevisiae*, *Arch. Microbiol.* 1978, **117**, pp. 269-276.
28. Arikawa, Y.; Kuroyahagi, T.; Shiosaka, M.; Muratsubaki, H.; Enomoto, K.; Kodaira, R. and Okazaki, M.: Effect of genedisruptions of the TCA Cycle on production of succinic acid in *Saccharomyces cerevisiae*, *Journal of Bioscience and Bioengineering* 1999, **88** (1), pp. 28-36.
29. Kubo, Y.; Takagi, H. and Nakamori, S.: Effect of gene disruption of succinate dehydrogenase on succinate production in a sake yeast strain, *Journal of Bioscience and Bioengineering* 2000, **90** (6), pp. 619-624.
30. Yano, S.; Asano, T.; Kurose, N.; Hiramtsu, J.; Shimoi, H. and Ito, K.: Characterization of an α -keto glutarate resistant sake yeast mutant with high organic acid productivity, *Journal of Bioscience and Bioengineering* 2003, **96** (4), pp. 332-336.
31. Tyrell, T.; Exner, R. and Folz, R.: Further development of EBC fermentation test tubes, EBC 2011 Glasgow, Great Britain, Poster 103,
32. Tyrell, T.; Eglin, S.; Exner, R.; Biering, J.; Gigla, K.; Marshall, K.; Uhde, C. and Folz, F.: Experiences with new fermentation test tubes, WBC 2012, Portland, USA, Poster 239.

Received 6 May 2013, accepted 30 June 2013