



Production and characterisation of non-alcoholic beer using special yeast

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Abstract

Non-*Saccharomyces* yeast strains *Saccharomyces ludwigii*, *Schizosaccharomyces pombe*, *Lachancea fermentati* and *Pichia angusta* together with a hybrid yeast strain cross-bred between genetically modified *Saccharomyces cerevisiae* W303-1A G418^R and *Saccharomyces eubayanus* as well as the parent yeasts of the hybrid were studied for potential use for non-alcoholic beer production. The hybrid yeast, its *Saccharomyces cerevisiae* W303-1A G418^R parent and *Saccharomyces ludwigii* were not able to metabolise maltose during Durham tube tests. *Schizosaccharomyces pombe*, *Lachancea fermentati* and *Pichia angusta* metabolised maltose, however, showed limited ethanol production. Parameters, volatile and non-volatile organic compounds of beers produced by the studied yeast were analysed and compared to a beer produced by bottom fermented brewer's yeast *Saccharomyces pastorianus*.

Keywords: low-alcoholic beer, non-alcoholic beer, non-*Saccharomyces* yeast, hybrid yeast

1 Introduction

Over the last years, the consumption of alcohol-free beer has been rising significantly due to the fact that it represents an alternative to standard soft drinks (Kyselová and Brányik, 2015). Consumer interest in health increases and no alcohol policies for drivers and pregnant women are advocated (De Francesco et al., 2018). However, most of the available non-alcoholic beers present a poor flavour profile that is not well accepted (Catarino and Mendes, 2011). The European Union has no law that refers to “low-alcohol” and “alcohol free” beers, but each country has its own legislation (Montanari et al., 2009). Beers with reduced alcohol content are often classified as “low-alcohol” beers containing 1.2% v/v of ethanol, and “non-alcohol” or “alcohol-free” beers containing 0.5% v/v of ethanol (Varela and Varela, 2019). Two main methods can be used to produce these beers. One of them, also called physical, is based on removing alcohol from the alcoholic beer produced by classical technology. This method requires considerable investments into the special equipment for alcohol removal, and the final beer is

characterised by poor sensory qualities caused by losing higher alcohols and esters, which must be refilled later (Brányik et al., 2012). The aim of the other method, often referred to as biological, is to reduce alcohol production during fermentation process (Ivanov et al., 2016). Biological methods include using wort with a lower content of simple sugars, early ending of fermentation, lower temperature of fermentation or lower metabolism activity caused by immobilization of the yeasts (Kochláňová et al., 2016b). Non-*Saccharomyces* yeasts are known for their important contribution to the flavour profile of fermented foods and beverages and have therefore been investigated for their targeted application in bioflavouring and, not least, non/low-alcoholic brewing (Bellut et al., 2019). Several non-*Saccharomyces* species have been tested for the production of low-alcohol and alcohol-free beers.

The rationale behind this study was to use yeast species that are unable to utilise maltose and/or maltotriose, the main sugars present in wort (Varela and Varela, 2019). It is not necessary to stop fermentation by cooling or yeast

separation, since fermentation will naturally come to a halt by depletion of fermentable sugars. *Saccharomyces ludwigii* can produce alcohol-free beers with rich flavour profiles owing to its aroma production and low performance in fermenting maltose and maltotriose from wort (Michel et al., 2016). In comparison to brewer's yeast some strains of *S. ludwigii* produce higher amounts of esters, higher alcohols and negligible concentrations of diacetyl and other undesired compounds (Kochláňová et al., 2016b). *Schizosaccharomyces pombe* is fission yeast with a very slow growth rate, a long lag phase and a high vitamin requirement. In addition to glucose, this yeast is able to use glycerol, sucrose, raffinose and maltose as carbon sources. It can also survive very low pH environments and wide range of temperatures (Loira et al., 2018). *Sch. pombe* is associated with a high fermentative power similar to *S. cerevisiae* with the production of ethanol up to 15% v/v (Benito et al., 2019). Together with lactic acid and acetic acid bacteria *Sch. pombe* is also part of microbiological consortium in Kombucha production (Villarreal-Soto et al., 2018).

Members of *Lachancea* genus, *L. thermotolerans* and *L. fermentati* have been associated with grape must and wine fermentation processes in several wine producing countries (Porter et al., 2019). Domizio et al. (2016) proposed *L. thermotolerans* to be a good choice for the production of sour beers in a single fermentation without the need of lactic acid bacteria. There has been a very limited research into *L. fermentati* in connection with a production of volatile compounds. Analyses of the common compounds suggest a strain differentiation regarding the production of higher alcohols, isoamyl alcohol and propanol (Porter et al., 2019). Bellut et al. (2020) studied different strains of *L. fermentati* isolated from kombucha in a low-alcoholic beer production with the focus on the production of lactic acid at the expense of an ethanol. They prepared low-alcoholic beer with a balanced profile of sweetness and acidity, but due to a stopped fermentation, high diacetyl levels were present.

Hansenula polymorpha (syn. *Pichia angusta*) is able to ferment glucose, but not galactose, sucrose, maltose or raffinose and is recognized for its enzyme alcohol oxidase, which can oxidise not only methanol, but short chained alcohols as well (Negrută et al., 2010). Use of non-*Saccharomyces* yeasts other than *Saccharomyces ludwigii* for the production of alcohol-free beer has not been studied to a great extent. It is a challenge to find such yeasts that are able to produce aromatic compounds which can mask the wort-like off-flavours created by residual wort sugars and aldehydes (Bellut et al., 2018).

The objective of this study was to investigate the application of the selected non-conventional yeasts for the production of non-alcoholic beer. Further we compared profiles of produced beers and beer prepared by standard bottom fermenting brewer's yeast *S. pastorianus* W96.

2 Materials and methods

2.1 Microorganisms

Yeasts (Table 1) were gained from yeast collections and the control strain of *Saccharomyces pastorianus* W96 was obtained from a local brewery (its origin is in the Collection of Brewing Yeast of the Research Institute of Brewing and Malting, Prague, Czech Republic). The hybrid yeast was prepared at the Faculty of Natural Sciences of the Comenius University in Bratislava, Slovakia. One hybrid parent, an auxotroph haploid of *Saccharomyces cerevisiae* W303-1A G418^R, was constructed by a replacement of a wild type *AAC1* gene with *aac1::kanMX4* allele from the international systematic *S. cerevisiae* gene disruption project (Baker Brachmann et al., 1998). The other hybrid parent was a culture strain of *Saccharomyces eubayanus* PYCC121. The construction of the hybrid and its use in this work was motivated by the effort to imitate a natural hybridisation event and to reveal the influence of recombination on the inherited features of the offspring.

Table 1 List of yeast strains

| Yeasts | Descriptions | Abbreviations |
|---|--|---------------|
| Hybrid (SCP × SEP) | Hybrid strain mtDNA from <i>Saccharomyces cerevisiae</i> W303-1A G418 ^R | HYB |
| <i>Saccharomyces cerevisiae</i> W303-1A G418 ^R | <i>MATa</i> , <i>ade2-1</i> , <i>trp1-1</i> , <i>leu2-3,112</i> , <i>his3-11,15</i> , <i>ura3-1</i> , <i>can1-100</i> , <i>aac1::kanMX4</i> , <i>Gal+</i> , <i>psi+</i> , <i>ρ+</i> parent of the hybrid | SCP |
| <i>Saccharomyces eubayanus</i> PYCC121 | Wild strain, parent of the hybrid | SEP |
| <i>Saccharomyces ludwigii</i> CCY 34-1-2 | | SLU |
| <i>Schizosaccharomyces pombe</i> NRRL Y-12796 | | SPO |
| <i>Lachancea fermentati</i> CBS 4506 | | LFE |
| <i>Pichia angusta</i> CBS 7073 | | PAN |
| <i>Saccharomyces pastorianus</i> W96 | Control bottom-fermenting yeast | W96 |
| CCY = Culture Collection of Yeasts (Slovakia), CBS = Central Bureau of Fungal Cultures (The Netherlands), NRRL/ARS = Agricultural Culture Collection (USA), PYCC = Portuguese Yeast Culture Collection (Portugal) | | |

2.2 Carbohydrate fermentation tests

Tests were performed in accordance with Kurtzman et al. (2011) in glass tubes containing Durham tubes and 2% w/v sugar solution with bromothymol blue as an acid base indicator. Production of CO₂ in Durham tubes was evaluated after 7 days of static cultivation at 25 °C.

2.3 Pre-fermentation and fermentation

Yeasts were inoculated from Petri dishes into 100 ml Erlenmeyer flasks containing 20 ml of YPD (Yeast Extract-Peptone-Dextrose) pre-fermentation medium. They were cultivated on a rotary shaker for 24 h, at 25 °C and 180 rpm. Wort of the original gravity 10.42 °P prepared from Pilsen malt (100%) and Premiant Hops by infusion mashing in 20 l Speidel Braumeister microbrewery served as a fermentation medium. After 24 h of yeast cultivation, the volume of a pre-fermentation medium corresponding to the pitching rate of 1 × 10⁶ yeast cells/ml (cell count for each yeast strain was performed under a microscope with the Bürker chamber) was added into 500 ml flasks containing a fermentation medium to reach 400 ml. The primary fermentation was carried out in 500 ml flasks with fermentation seals at 12 °C for 7 days. After 7 days young beers were poured through filter into 300 ml fermentation flasks and sealed. Beers underwent maturing at 4 °C for 21 days. Matured beers were then analysed.

2.4 Analyses of produced beers

For analyses of beer parameters, 50 ml of beer samples were centrifuged for 10 min at 3000 rpm and degassed using sonification for 30 min). Ethanol concentration, pH, colour, both original and real extracts, and a real degree of fermentation were determined using a DMA 4500M density meter coupled with AlcoLyzer Beer ME, Haze QC ME Turbidity Measuring Module and pH ME Beverage Measuring Module (Anton Paar, GmbH, Graz, Austria). Samples for the analysis of organic compounds (OC) were prepared as follows: 6 ml of cool beer together with 0.5 g NaCl (Merck, Darmstadt, Germany) and 20 µl of ethanol solution of 1.60 mg/l benzophenone (internal standard) were placed into a 20 ml glass headspace vial and sealed with metallic hole cup and PTFE/Silicone septa. The analysis of OC in beer samples by HS-SPME-GC×GC-TOF-MS (headspace-solid phase microextraction comprehensive gas chromatography connected to high resolution time of flight mass spectrometry) was performed in accordance with methods used by Furdíková et al. (2020). Processing of the obtained data was performed using LECO Chroma TOF-HRT 1.90.60 Software and the US National Institute of Standards and Technology (NIST 2014) mass

spectra library. The identification of OC was confirmed by accurate mass measurements and comparison of the measured retention index (RI) with the RI obtained by an injection of authentic standards or with a reference value obtained from NIST WebBook (2020). Volatile organic compounds were considered as identified if the difference between the experimental and reference RI was less than 20 units. Relative concentrations of OC were calculated by the ratio of each individual peak area to the area of internal standard and converted to concentration equivalents based on the internal mass added. The following authentic standards obtained from Merck had the purity of ≥ 99.5% and were used to identify OC by GC×GC: ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, ethyl hexadecanoate, 2-phenylethyl acetate, 2-phenyl ethanol, amyl and isoamyl alcohol, 1-octanol, 1-decanol, guaiacol, 4-vinylguaiacol, 3-methylbutanoic, hexanoic, octanoic, decanoic, dodecanoic, tetradecanoic and hexadecanoic acid, 2,3-butanedione, benzeneacetaldehyde, benzaldehyde, furfural, 5-hydroxymethylfurfural and benzophenone.

3 Results and discussion

3.1 Yeast characterisation

When characterising non-*Saccharomyces* yeasts for their suitability in alcohol-free beer production, several key attributes should be investigated. The first attribute is the ability to utilize wort saccharides, mainly maltose, which is the most abundant one (about 65%) in the wort (Briggs et al., 2004) and could be a significant feature of potential non/low-alcoholic brewing yeast candidate (Bellut et al., 2018). Over the years, various tests have been devised to detect the production of carbon dioxide from carbohydrates by fermenting yeast (van der Walt, 1970). An interesting approach was used by Michel et al. (2020) who determined the yeast fermentation ability through the correlation between sugar depletion and formed pressure. However, the Durham tubes test (Kurtzman et al., 2011) have been found to be the most useful for routine purposes. Therefore, this method was used in our study. The hybrid yeast (HYB) – a descendant of *Saccharomyces cerevisiae* W303-1A G418^R (SCP) and *Saccharomyces eubayanus* PYCC121 (SEP), as well as the parent yeast *Saccharomyces cerevisiae* W303-1A G418^R (SCP) were not able to ferment maltose. Similarly and as presumed, *Saccharomyces ludwigii* CCY 34-1-2 (SLU) did not utilise maltose (Table 2).

Table 2 Carbohydrate fermentation tests of tested yeasts

| Yeasts | Glucose | Maltose | Sucrose | Raffinose |
|---|---------|---------|---------|-----------|
| Hybrid (SCP × SEP) | +++ | - | +++ | +++ |
| <i>Saccharomyces eubayanus</i> PYCC121 | +++ | +++ | +++ | +++ |
| <i>Saccharomyces cerevisiae</i> W303-1A G418 ^R | +++ | - | +++ | - |
| <i>Schizosaccharomyces pombe</i> NRRL Y-12796 | +++ | + | +++ | +++ |
| <i>Lachancea fermentati</i> CBS 4506 | +++ | +++ | +++ | + |
| <i>Pichia angusta</i> CBS 7073 | +++ | +++ | +++ | + |
| <i>Saccharomycodes ludwigii</i> CCY 34-1-2 | +++ | - | +++ | - |
| <i>Saccharomyces pastorianus</i> W96 | +++ | +++ | +++ | +++ |

+++ = positive ability to ferment saccharide – Durham tube completely filled with CO₂, + = weak/delayed ability to ferment saccharide – Durham tube partially filled with CO₂, - = inability to ferment saccharide – no CO₂ in Durham tubes after 7 days of cultivation

3.2 Beer parameters

Beers prepared from the eight studied yeasts were tested for their ethanol content, which is the most important parameter of low/non-alcoholic beer. Further also pH value, colour, real degree of fermentation (RDF) and real extract (RE) – a portion of not fermented extract were examined (Table 3). The term RDF is the most accurate expression of fermentability (Huerta-Zurita et al., 2019) and it is closely related to the ethanol content in the beer. Ethanol level < 0.5% v/v was detected in beers produced by *Saccharomyces cerevisiae* W303-1A G418^R (SCP – 0.38%), *Schizosaccharomyces pombe* NRRL Y-12796 (SPO – 0.36%), *Pichia angusta* CBS 7073 (PAN – 0.14%) and *Saccharomycodes ludwigii* CCY 34-1-2 (SLU – 0.46%). Therefore, these beers can be labelled as non-alcoholic. *Saccharomycodes ludwigii* yeasts are well known as agents producing low/non-alcoholic beer producers. De Francesco et al. (2015) also used a strain of *S. ludwigii* and obtained beers with ethanol content < 1% v/v. Our beers prepared using the hybrid yeast (HYB – 1.02% of ethanol) and *Lachancea fermentati* CBS 4506 (LFE – 0.76%) can be classified as low-alcoholic ones. Control beer fermented by lager yeast *S. pastorianus* W96 contained 4.04% of ethanol. The highest RE value (PAN – 10.11% w/w) and the lowest RDF value (PAN – 2.04%) were detected in the beer prepared with *P. angusta* CBS 7073. As presumed, the lowest RE value (W96

– 3.95% w/w) and the highest RDF value (W96 – 62.30%) were detected in the beer prepared with the control yeast *S. pastorianus* W96. Increased wort colour during brewing process is caused by the temperature-dependent, non-enzymatic colour reactions of the Maillard type (Phiarais et al., 2010). The colour of the wort used in this study contained 20.61 EBC units and decreased during fermentation and beer maturation. The colour of the prepared beers ranged from 10.09 EBC units (fermented with *S. pastorianus* W96) to 15.19 EBC units (*S. cerevisiae* W303-1A G418^R – SCP). Correlation between ethanol concentration and beer colour was observed: the higher the beer ethanol concentration, the lower the colour of the beer. Also, more acidic beer gives a lower colour value of the beer. This can be related to the acidic environment, which decreases the colour compounds. Other contributors to the colour of beer are oxidized polyphenols; they occur especially when traces of metals such as iron or copper are present (Briggs et al., 2004; řavel et al., 2008). The pH value of beer influences its foam as well as its colloidal and microbiological stability (Liguori et al., 2015). According to Phiarais et al. (2010) the wort pH value usually ranges between 5.6–5.9. In our study the wort pH was 6.00. The lowest pH was detected in the beer prepared with *S. cerevisiae* W303-1A G418^R (SCP – 4.64) and the highest pH value was measured in the beer prepared with hybrid yeast (HYB – 5.04).

Table 3 Parameters of wort and beers (Beer abbreviations correspond to yeast ones, see Table 1)

| Parameter/Beer | Wort | HYB | SEP | SCP | SPO | LFE | PAN | SLU | W96 |
|---------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Ethanol (% v/v) | - | 1.02 | 2.63 | 0.38 | 0.36 | 0.76 | 0.14 | 0.46 | 4.04 |
| Original extract (% w/w) | 10.42 | - | - | - | - | - | - | - | - |
| Real extract (% w/w) | - | 8.76 | 6.40 | 9.80 | 9.80 | 9.18 | 10.11 | 9.65 | 3.95 |
| Real degree of fermentation (%) | - | 15.45 | 39.60 | 5.74 | 5.52 | 11.55 | 2.04 | 6.90 | 62.30 |
| Colour (EBC) | 20.61 | 12.41 | 10.75 | 15.19 | 12.77 | 14.03 | 13.61 | 13.22 | 10.09 |
| pH | 6.00 | 5.04 | 4.69 | 4.64 | 4.97 | 4.96 | 4.91 | 4.82 | 4.86 |

- = not detected

3.3 Organic compounds

The presence of volatile and non-volatile organic compounds and their quantities were evaluated (Table 4). Esters are important sensory active substances produced by cultural yeast. Small differences in the concentration of esters can have a big impact on the final taste of beer (Kochláňová et al., 2016a).

Ethyl hexanoate responsible for apple/banana flavour (Viejo et al., 2019) was detected only in beers prepared with hybrid yeast (HYB – 0.082 mg/l), hybrid's parent *Saccharomyces eubayanus* PYCC121 (SEP – 0.160 mg/l) and *Lachancea fermentati* CBS 4506 (LFE – 0.004 mg/l). Significantly high concentration of ethyl octanoate with apple/banana flavour (Viejo et al., 2019) was detected in the beer prepared with hybrid yeast (HYB – 1.226 mg/l), which was higher than that in control beer prepared with *Saccharomyces pastorianus* W96 (W96 – 1.044 mg/l). The use of non-*Saccharomyces* yeast *Torulospira delbrueckii* by Canonico et al. (2016) for production of low/non-alcoholic beer resulted in alcoholic beer (2.66% v/v of ethanol) with levels of ethyl hexanoate (0.031 ± 0.013 mg/l) and ethyl octanoate (0.006 ± 0.003 mg/l). 2-Phenylethyl acetate with sweet/honey flavour (Kobayashi et al., 2008) usually ranges in beer from 0.05–2.0 mg/l (Buiatti, 2009) and was detected in all our beers except those prepared with *Pichia angusta* CBS 7073 (PAN). Low-alcoholic beer prepared with hybrid yeast (HYB) displayed the highest concentration of ethyl caprate (0.828 mg/l), ethyl laurate (0.756 mg/l), ethyl myristate (0.296 mg/l) and ethyl palmitate (0.230 mg/l).

Higher alcohols act as the precursors of the more flavour-active esters (Anderson et al., 2019). 2-Phenylethanol with roses/sweetish flavour (Kobayashi et al., 2008) is formed during fermentation with phenyl-alanine catabolism (Andrés-Iglesias et al., 2016), however, it was detected in our wort (0.114 mg/l), as well. Its concentration ranged from 0.211 mg/l in the non-alcoholic beer prepared with *Schizosaccharomyces pombe* NRRL Y-12796 (SPO) to 0.706 mg/l in the low-alcoholic beer prepared with hybrid yeast (HYB). Amyl alcohol with fusel/fruity flavour (The Goodscents Company, 2020) was detected only in beers prepared with hybrid yeast (HYB – 0.008 mg/l) and hybrid's parent *S. eubayanus* PYCC121 (SEP – 0.007 mg/l). Isoamyl alcohol with banana/sweetish flavour (Kobayashi et al., 2008) was detected only in the control beer prepared with *S. pastorianus* W96 (W96 – 0.066 mg/l). Senkarcinova et al. (2019) reported that enhanced formation of higher alcohols is caused by increased fermentation temperatures, and also by shorter maturation times which was observed by Landaud et al. (2001). Hence, the primary fermentation temperature (12 °C) and maturation time (21 days) could have a critical

impact on the formation of amyl alcohol and isoamyl alcohol in prepared beers. 1-Octanol (citrus/orange flavour) ranges from 10 to 40 µg/l in ales and 1-decanol (waxy/fatty flavour) from 5 to 20 µg/l (Phiarais et al., 2010). An increased concentration of 1-octanol was detected in the low-alcoholic beer prepared with hybrid yeast (HYB – 0.51 mg/l) and 1-decanol in the non-alcoholic beer prepared with *Saccharomyces ludwigii* CCY 34-1-2 (SLU – 0.54 mg/l). Fatty acids are beneficial for yeast growth at the beginning of fermentation, but when exposed to oxidation, they can cause an unpleasant off-flavour of beer. Infusion mashing can lead to a higher contents of middle-chain fatty acids (Olšovská et al., 2014).

Octanoic and decanoic acids are important contributors to the flavour of many lager beers (Clapper-ton and Brown, 1978). The concentration of hexanoic (0.188 mg/l), octanoic (0.286 mg/l) and decanoic acid (0.197 mg/l) in the control beer prepared with *S. pastorianus* W96 (W96) were comparable to those in the low-alcoholic beer prepared with hybrid yeast (HYB): hexanoic (0.119 mg/l), octanoic (250 mg/l) and decanoic acid (0.185 mg/l). The concentration of isovaleric acid (0.33 mg/l) in the non-alcoholic beer prepared with *S. ludwigii* in the work of Jiang et al. (2017) was compared to that in a commercial non-alcoholic beer (0.34 mg/l) and a Pilsner type beer (0.12 mg/l). The concentration of isovaleric acid in the non-alcoholic beer prepared with *S. ludwigii* CCY 34-1-2 (SLU) was 0.013 mg/l.

A significant concentration of dodecanoic acid, which plays an important role in the disruption of the beer lacing (Briggs et al., 2004), was detected in the low-alcoholic beer prepared with hybrid yeast (HYB – 0.150 mg/l). In the beer prepared with control *S. pastorianus* W96 (W96) it was 0.102 mg/l. Concentrations of myristic (0.019 mg/l) and palmitic acid (0.015 mg/l) were the highest in the low-alcoholic beer prepared with hybrid yeast (HYB). Diacetyl (2,3-butanedione) and 2,3-pentadione (vicinal diketones) which results from the chemical oxidative decarboxylation of excess α -acetolactate leaked from the valine/leucine biosynthetic pathway to the extracellular environment (De Francesco et al., 2015). The flavour threshold of diacetyl, which imparts beer with a butter or toffee-like flavour commonly ranges between 0.1–0.2 mg/l in lager and 0.1–0.4 mg/l in ales (Krogerus and Gibson, 2013). Diacetyl was detected only in our beer prepared with the control bottom fermenting brewer's yeast of *S. pastorianus* W96 and its level was above the minimum of the flavour threshold range (W96 – 0.118 mg/l). A formation of aldehydes in beer involves different substrates and mechanism and occurs mainly during wort mashing and boiling (Gernat et al., 2019), but it is also affected by the storage temperature and dissolved

oxygen presence (Soares da Costa et al., 2004). In this study, the concentration of benzeneacetaldehyde (green/honey odour) ranged from 0.017 mg/l in the low-alcoholic beer fermented by hybrid yeast (HYB) to 0.058 mg/l in the non-alcoholic beer prepared with *Sch. pombe* NRRL Y-12796 (SPO). A significant concentration of benzaldehyde, a Strecker aldehyde of almond/cherry odour, which is formed during the ageing of beer (Riu-Aumatell et al., 2014), was detected in our non-alcoholic beer prepared with *Sch. pombe* NRRL Y-12796 (SPO – 0.130 mg/l). Furfural with threshold ≥ 0.015 mg/l and 5-hydroxymethylfurfural with threshold ≥ 0.0358 mg/l (Saison et al., 2009), the most important aldehydes formed during Maillard reactions, which serve as markers for the heat load impact on the mash, wort and beer (Contreras-Calderón et al., 2008; Germat et al., 2019), were not detected in any of our beers.

During boiling or under the influence of some bacteria and wild yeasts, some ferulic acid is decarboxylated to yield 4-vinyl guaiacol with smoky/bacon odour (The Goodscent Company, 2020), which confers an undesirable flavour in most beers (Briggs et al., 2004). Its formation from ferulic acid during yeast metabolism depends on temperature and yeast strain, but also on lagering duration and pasteurization of the beer (McMurrough et al., 1996). The flavour threshold of 4-vinyl guaiacol in beers (lagers, ales and stouts) is 0.2–0.3 mg/l (Becker and Gerhäuser, 2008) and in this study its concentration did not exceed the maximum for the flavour threshold level. The highest concentration of guaiacol with woody/phenolic flavour (The Goodscent Company, 2020) was detected in our non-alcoholic beer prepared with *S. ludwigii* CCY 34-1-2 (SLU – 0.029 mg/l).

4 Conclusions

Non-*Saccharomyces* yeasts were regarded to be responsible for microbial-related problems during beer production, which resulted in an almost global use of pure yeast cultures. Numerous previous reports described the potential of the so-called non-conventional yeasts which introduce desirable aromas and flavours to beer during fermentation. Thanks to these reports the role of the non-conventional yeast in the production of beers was revised. Our results confirm that some of the non-conventional yeasts which we tested are suitable for production of non/low-alcoholic beer. It was found that even yeast able to ferment maltose can produce low or non-alcoholic beers, because their ethanol production was limited. The submitted paper was primarily intended as a screening study, in which the yeast strains were exam-

ined only on a laboratory scale. It is necessary to perform pilot plant scale-ups of the most interesting strains and to assess their sensory and organoleptic quality by a panel of trained testers.

5 Acknowledgements

This article was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences VEGA 1/0063/18, by the Slovak Research and Development Agency APVV-15-0333 and with the support of the Ministry of Education, Science, Research and Sport of the Slovak Republic within the Research and Development Operational Programme for the project 'University Science Park of STU Bratislava', ITMS 26240220084.

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Table 4 Organic compounds in wort and produced beers (Beer abbreviations correspond to yeast ones, see [Table 1](#))

| Compounds (mg/l) | Wort | HYB | SEP | SCP | SPO | LFE | PAN | SLU | W96 |
|---|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| <u>Esters</u> | | | | | | | | | |
| Ethyl hexanoate (caproate) | - | 0.082 | 0.160 | - | - | 0.004 | - | - | - |
| Ethyl octanoate (caprylate) | - | 1.226 | 1.126 | 0.278 | - | 0.043 | 0.069 | 0.006 | 1.044 |
| Ethyl decanoate (caprate) | 0.031 | 0.828 | 1.245 | 0.127 | 0.005 | 0.022 | 0.215 | 0.038 | 0.792 |
| Ethyl dodecanoate (laurate) | 0.026 | 0.756 | 1.735 | 0.081 | 0.013 | 0.026 | 0.188 | 0.053 | 0.614 |
| Ethyl tetradecanoate (myristate) | 0.010 | 0.296 | 0.401 | 0.014 | - | 0.019 | - | - | 0.156 |
| Ethyl hexadecanoate (palmitate) | - | 0.230 | 0.346 | - | 0.023 | - | - | 0.033 | 0.330 |
| 2-Phenylethyl acetate | - | 0.778 | 0.829 | 0.135 | 0.008 | 0.887 | - | 0.213 | 0.992 |
| <u>Alcohols</u> | | | | | | | | | |
| 2-Phenyl ethanol | 0.114 | 0.706 | 0.833 | 0.332 | 0.211 | 0.693 | 0.326 | 0.509 | 0.746 |
| Amyl alcohol | - | 0.008 | 0.007 | - | - | - | - | - | - |
| Isoamyl alcohol | - | - | - | - | - | - | - | - | 0.066 |
| 1-Octanol | 0.037 | 0.051 | 0.015 | 0.040 | 0.010 | 0.037 | 0.028 | 0.030 | 0.027 |
| 1-Decanol | 0.024 | 0.034 | 0.018 | 0.015 | - | 0.008 | 0.005 | 0.054 | 0.038 |
| <u>Phenols</u> | | | | | | | | | |
| Guaiacol (2-methoxyphenol) | 0.001 | 0.001 | - | 0.009 | - | 0.001 | - | 0.029 | 0.001 |
| 4-Vinylguaiacol (2-methoxy-4-vinylphenol) | 0.202 | 0.247 | 0.292 | - | 0.055 | 0.024 | 0.070 | 0.172 | 0.063 |
| <u>Organic acids</u> | | | | | | | | | |
| 3-Methylbutanoic acid (isovaleric acid) | 0.009 | 0.003 | - | 0.005 | 0.020 | - | 0.005 | 0.013 | 0.003 |
| Hexanoic acid (caproic acid) | 0.077 | 0.119 | 0.071 | 0.131 | 0.065 | 0.068 | 0.082 | 0.108 | 0.188 |
| Octanoic acid (caprylic acid) | 0.098 | 0.250 | 0.195 | 0.227 | 0.147 | 0.119 | 0.097 | 0.201 | 0.286 |
| Decanoic acid (capric acid) | 0.062 | 0.185 | 0.239 | 0.108 | 0.051 | 0.051 | 0.047 | 0.104 | 0.197 |
| Dodecanoic acid (lauric acid) | 0.029 | 0.150 | 0.222 | 0.054 | 0.042 | 0.027 | 0.016 | 0.054 | 0.102 |
| Tetradecanoic acid (myristic acid) | - | 0.019 | 0.024 | - | - | - | 0.003 | - | 0.007 |
| Hexadecanoic acid (palmitic acid) | - | 0.015 | 0.017 | 0.007 | 0.015 | 0.004 | 0.004 | 0.002 | 0.009 |
| <u>Vicinal diketones</u> | | | | | | | | | |
| 2,3-Butanedione (diacetyl) | - | - | - | - | - | - | - | - | 0.118 |
| <u>Aldehydes</u> | | | | | | | | | |
| Benzeneacetaldehyde | 0.014 | 0.017 | 0.016 | 0.024 | 0.058 | 0.032 | 0.034 | 0.046 | 0.019 |
| Benzaldehyde | - | 0.005 | 0.003 | 0.012 | 0.130 | 0.014 | - | - | 0.003 |
| Furfural (FF) | - | - | - | - | - | - | - | - | 0.006 |
| 5-Hydroxymethylfurfural (5-HMF) | 0.007 | 0.013 | 0.001 | 0.003 | 0.003 | 0.008 | 0.022 | 0.001 | 0.016 |

- = not detected

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