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Research Article

Yeast selection for non-alcoholic and low-alcoholic beverages based on wort

Petar Nedyalkov[1]✉, Rositsa Denkova[3], Desisilava Teneva[4], Vesela Shopska[1], Bogdan Goranov[5], Zapryana Denkova[2], Georgi Kostov[1], Maria Kaneva[1]

[1]*Department of Wine and Beer Technology, Technological Faculty, University of Food Technologies, Plovdiv, Bulgaria*
[2] *Department of Microbiology, Technological Faculty, University of Food Technologies, Plovdiv, Bulgaria*
[3]*Department of Biochemistry and Molecular Biology, Technological Faculty, University of Food Technologies, Plovdiv, Bulgaria*
[4]*Institute of Organic Chemistry, Centre of Phytochemistry, Bulgarian Academy of Sciences*
[5]*LB Lact BAS Ltd.*

Abstract

A series of wort fermentations with eight yeast strains were carried out in laboratory conditions. The strains used were: *Saccharomyces cerevisiae* (2 strains), *Saccharomyces diastaticus* (3 strains), *Saccharomyces carlsbergensis* (1 strain), *Saccharomyces lactis* (1 strain), *Saccharomyces sake gekkeikan* (1 strain). Selection of yeast strains has been performed in order to study the possibilities for their application to obtain fermentable non-alcoholic and low-alcoholic beverages based on wort. Three yeast strains (two of *Saccharomyces cerevisiae* and one *Saccharomyces diastaticus*), were selected based on their good growth in the used medium and the pleasant organoleptic profile formed as a result of the fermentation carried out. The accumulated alcohol values varied between 0.05 and 0.22 % (w/w).

Keywords: yeast selection, non-alcoholic, low-alcoholic, wort-based beverages

✉*Corresponding author: Assis.Prof., Petar Nedyalkov, PhD; Department of Wine and Beer Technology, Technological Faculty, University of Food Technologies, 26 Maritza Blvd. BG-4002 Plovdiv, Bulgaria, tel.: ++359 32 603 642; mobile: ++359 878 742 475; E-mail: inj_petar_nedyalkov@abv.bg*

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Introduction

Wort alcoholic fermentation is a major process in beer production. Wort composition and parameters vary depending on the raw materials used (barley malt, wheat malt, unmalted grain raw materials) and mashing regime applied. Generally, wort is rich in fermentable sugars, dextrins, amino acids, peptides and polypeptides, minerals and vitamin B-complex. The presence of important nutrients for people makes wort suitable for the production of fermentable beverages other than beer. Traditional cereal fermentable beverages are rarely made from malt or wort, but there are data that the cereal nutritional value is increased during the malting process due to the synthesis of many functional bioactive components such as enzymes, antioxidants, vitamins (Rao and Muralikrishna 2006). The germinated grains have a higher content of certain vitamins compared to non-germinated ones (Hassani et al 2016). Germination also affects the nutritional fiber content. The amount of β–glucan decreases during germination, but the concentration of water–soluble arabinoxylans increases (Rao and Muralikrishna 2006; Hassani et al. 2013). Nowadays, the investigations related to fermented cereal foods and drinks are very intensive. Non-alcoholic fermented cereal beverages are seen as a new generation of fermented cereal beverages (Bogue and Huan Yu 2009). When wort is used for obtaining non-alcoholic fermented beverages, it gives the possibilities for enhancing the health benefits for the consumers. Traditional cereal-based fermented beverages are produced by spontaneous lactic acid, acetic acid and alcohol fermentations. The presence of yeast in the fermenting media leads to the accumulation of alcohol, which is an obstacle to the production of non-alcoholic beverages. On the other hand, if only bacteria are used, the obtained beverages would lose their natural carbonization and freshness, as well as the specific organoleptic profile resulting from the secondary yeast metabolites. Therefore, the selection of suitable yeast strains and fermentation conditions is of great practical importance for the production of non-alcoholic or low-alcoholic cereal-based fermented beverages.

There are many studies related to the production of non- and low-alcohol beer (Evellin et al. 1999; Navártil et al. 2002; Nevogit et al. 2002; Selecký et al. 2008; Brányik et al. 2012). Different methods like ethanol removal by thermal or membrane processes and restricted ethanol formation are used. One way of restricted ethanol formation is the use of special yeast strains - genetically modified yeast or non-typical yeast strains (Brányik et al. 2012). However scarce information is still available on the selection of yeast suitable for fermented wort-based non-alcoholic or low-alcoholic beverages other than beer. The aim of the present study is to select yeast strains having a suitable metabolite profile for the preparation of non-alcoholic and low-alcoholic wort-based beverages.

Materials and Methods

Materials

Cereals. Pilsner malt from WEYERMANN Germany was used. The malt was ground on a Corona hand mill.

Yeast. The yeast strains used were from the collection of the Department of Microbiology at UFT – Plovdiv. The strains used were as follows: *Saccharomyces cerevisiae* (3-0635, 36G), *Saccharomyces diastaticus* (28B, 25G, 31-C1), *Saccharomyces carlsbergensis* (18G), *Saccharomyces lactis* (11-5964), *Saccharomyces sake gekkeikan* (6G).

Chemicals and reagents. All chemicals used were of analytical grade and were obtained from local producers.

Experimental design. The experimental work was carried out in two stages. In the first stage, the temperature and the duration of the fermentation, suitable for a slower fermentation process and less alcohol accumulation, were determined. In the second stage, the wort was inoculated with two different concentration of yeast cells. Three strains of yeast, suitable for non-alcoholic and low-alcoholic wort-based beverages, were selected.

The wort was obtained from barley malt by infusion method in laboratory conditions with Braumaister 20L from Speidel, Germany. For each stage of work, a fresh wort was prepared. After cooling and filtration, the wort was inoculated with the examined yeast strains. In the first stage, the fermentation was carried out at two temperatures (5 and 10°C) for 5 days. The quantity of the yeast suspension was 2.5% of the wort volume. The carbon dioxide amount released during the fermentation was monitored and the quantity of ethanol was calculated. In the second stage, two fermentation variants were performed at a temperature of 10°C for 4 days. The initial concentration of viable yeast cells was 10^8 CFU/cm^3 in the first variant and 10^4 CFU/cm^3 in the second variant. The changes in the concentration of viable yeast cells were monitored by taking samples on the 0th, 1st, 4th day of the fermentation. The ethanol content of the samples on the fourth day was determined by the distillation method. Sensory evaluation of the samples was carried out at the different stages of the experiment.

Analyses

Viable cell concentration. The viable cell concentration of yeast was determined by preparation of appropriate tenfold dilutions and spread plating on malt agar medium. The inoculated Petri dishes were incubated at 30°C for 24 hours until the appearance of countable single colonies. The number of single colonies was used to estimate the concentration of viable yeast cells (N, CFU/cm^3) according to the formula:

$$N = \frac{a}{V.d} \qquad (1)$$

where: a – average number of single colonies from a specific dilution; V – volume of the inoculum, cm^3; d – dilution.

Amount of released carbon dioxide. The amount of released carbon dioxide was determined according to Ivanov et al. (1979).

Alcohol content. Alcohol content was determined by distillation according to Method 9.2.1 (Analytica – EBC 2005) and by calculation depending on the amount released carbon dioxide, following the formula:

$$E = \frac{\Delta m.92}{88.V}.100,\% \text{ by volume} \qquad (2)$$

where: Δm – mass difference for a certain period of time, g; V – volume of the fermented liquid, cm^3; 92 – molar weight of 2 molecules of ethanol; 88 – molar weight of 2 molecules CO_2 (Kostov 2015).

Wort extract and pH. Wort extract was determined using a pyknometer according to Method 8.3 (Analytica – EBC 2005). The pH value was determined using pH meter Sartorius PB-11 according to Method 8.17 (Analytica – EBC 2005).

Sensory analysis. The sensory analysis of the beverages obtained in the second stage, variant 1 was carried out by a descriptive method and of those obtained in the second stage, variant 2 - by a ranking test according to Method 13.11 (Analytica – EBC 2005).

Statistical analysis. The results of the ranking method were processed and analyzed statistically according to the requirements of the EBC (Analytica – EBC, Method 13.11 (2005), using MS Excel software.

Results and Discussion

First step – Determination of the fermentation temperature and duration

The experiment was carried out with 11.12% wort, with pH 6.28. As can be seen from the graphs (Fig. 1a), the quantity of released carbon dioxide during the fermentation at 5°C within 5 days was insignificant. The fermentation in the variant with *Saccharomyces diastaticus* 25G started on the third day. A weak fermentation process was observed on the fifth day in the variants with strains *Saccharomyces diastaticus* 31-C1 and *Saccharomyces carlsbergensis* 18G. The rest of the yeast strains did not carry out fermentation at 5°C

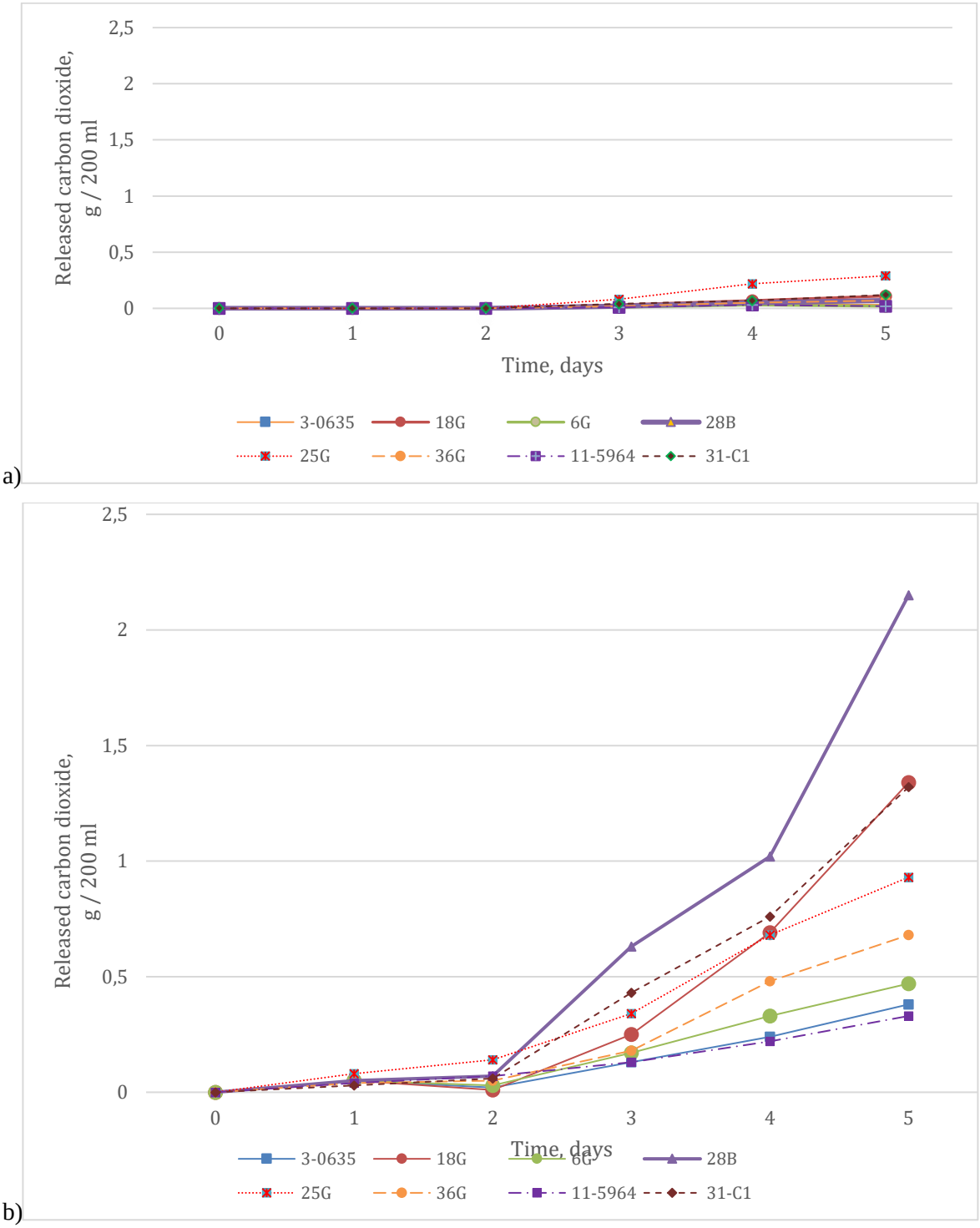


Figure 1. Dynamics of released carbon dioxide during wort fermentation with different yeast strains at 5°C (a) and at 10°C (b)

within 5 days. Based on the obtained results it can be assumed that the fermentation process was suppressed, because of the low temperature. The absence of alcohol or the low alcohol content was a desired effect, because the beverages would be non-alcoholic or low-alcoholic, but the lack of alcohol fermentation was accompanied by an absence of a natural freshness from the carbon dioxide accumulation as well as other yeast metabolites. The observed starting of the fermentation process from 3 to 5 days after inoculation is not acceptable from a technological point of view. The fermentation process was significantly more intensive in the variants fermented at 10°C (Fig.1b). *Saccharomyces diastaticus* 25G started the fermentation process first (on the second day). The fermentation process with the other strains started on the third day. During the fermentation process, the largest fermentation activity was observed at *Saccharomyces diastaticus* 28B. The fermentation activity of strains *Saccharomyces lactis* 11-5964 and *Saccharomyces cerevisiae* 3-0635 was the weakest. Depending on the amount of carbon dioxide released on the fifth day, the amount of ethanol in the samples was calculated according to formula (2) and converted in % by weight using a table. As can be seen (Table 1) *Saccharomyces lactis* 11-5964 accumulated the smallest amount of alcohol and *Saccharomyces diastaticus* 28B – the biggest alcohol amount. Based on the results of the first stage, the fermentation temperature was chosen to be 10°C and the fermentation duration - 4 days, because after the fourth day, there was greater fermentation activity of some strains, but the main goal of the present study was to obtain beverages with smaller alcohol content.

Table 1. Alcohol content in wort beverages fermented by different yeast strains

Indicator	Yeast strain							
	11-5964	**3-0635**	**6G**	**36G**	**25G**	**31-C1**	**18G**	**28B**
Concentration of alcohol in % by weight	0,17	0,20	0,25	0,36	0,49	0,70	0,71	1,12

Second step – Yeast selection

All yeast strains were used for the preparation of wort-based fermented beverage in the first variant of wort fermentation. The concentration of viable cells after the inoculation was about 10^8 CFU/cm^3 and the wort was with an extract of 11.48 % and pH 6.19. The concentration of viable cells of all strains increased with 4 logN during the fermentation process and reached the maximum quantity (10^{12}CFU/cm^3) at the end of process (Fig.2a). It can be concluded that all yeast strains grew well under the experimental conditions applied. The alcohol content of the different samples, determined by the weight method, varied between 0.31% (w/w) and 2.17 % (w/w) (Fig.2b). As can be seen the different yeast strains accumulated different amounts of alcohol. The smallest amount of alcohol was accumulated by the strains *Saccharomyces lactis* 11-5964 and *Saccharomyces cerevisiae* 3-0635, and the biggest – by *Saccharomyces diastaticus* 25G and *Saccharomyces diastaticus* 28B. The terms non-alcoholic and low-alcoholic beverages are not unambiguously defined in different countries and for the different beverages. The limits for the alcohol content in low-alcohol beers vary in different parts of the world. In most of the EU countries beers with low alcohol are divided into alcohol free beers (containing alcohol ≤ 0.5% by volume) and low-alcohol beers (alcohol up to 1.2% by volume) (Brányik et al. 2012). In the EU regulation 1169/2011 it is written that the beer alcohol content is noted on the label if it is above 1.2% by volume. In the USA alcohol free beers means that there is no alcohol present, while the

upper limit of 0.5% by volume corresponds to so called non-alcoholic beer or "near beer" (Montanari et al. 2009). In countries that enforce religious prohibition, the alcohol content in beverages must not exceed 0.05% by volume (Brányik et al. 2012).

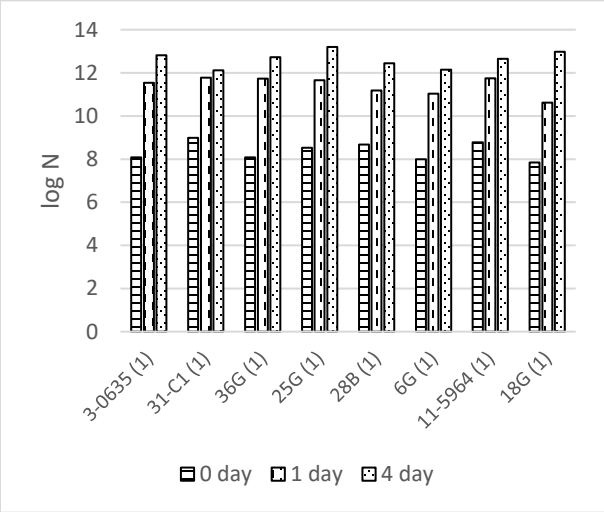


Figure 2a). Changes in the concentration of viable yeast cells during wort fermentation (variant 1)

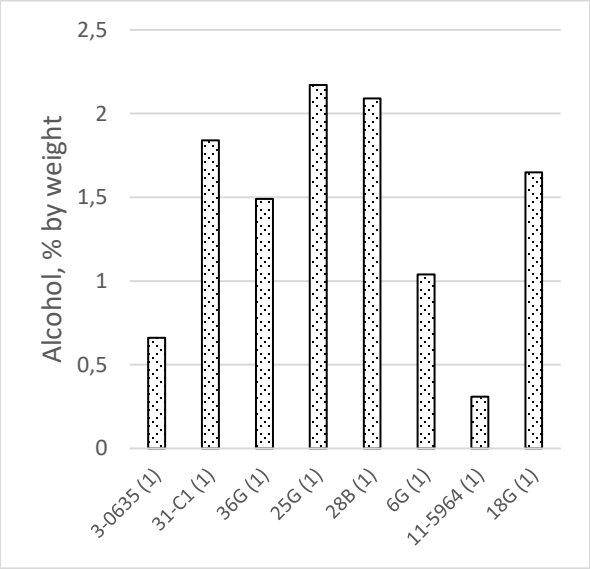


Figure 2b). Alcohol content in wort beverages fermented by different yeast strains (variant 1)

The alcohol content in juices of different fruits is allowed to be up to 3 g/dm^3 (AIJN Code of Practice 2018), and for the non-alcoholic water based beverages, the alcohol content should not exceed 0.5% (Kabzev et al. 1973). In the present article, it is assumed, that the beverages with alcohol up to 0.5% by volume (0.4% by weight, respectively) are non-alcoholic and those with alcohol content between 0.5 and 1.2% by volume (0.4 - 0.96% by weight, respectively) are low alcoholic beverages. According to this and based on the results (Fig.2b) it can be concluded that only the sample fermented with *Saccharomyces lactis* 11-5964 met the requirement for a non-alcoholic drink. The alcohol content of the variant fermented with strain *Saccharomyces cerevisiae* 3-0635 met the requirements of low-alcoholic drink. In the other samples, the concentration of alcohol exceeded the limit for non- and low-alcoholic beverages. It was observed by the sensory analysis that the samples fermented with strains *Saccharomyces sake gekkeikan* 6G, *Saccharomyces carlsbergensis* 18G, *Saccharomyces diastaticus* 25G, *Saccharomyces diastaticus* 28B, *Saccharomyces diastaticus* 31-C1 and *Saccharomyces cerevisiae* 36G had a distinct carbonation taste with different intensity. The samples with *Saccharomyces diastaticus* 31-C1 and *Saccharomyces cerevisiae* 36G stood out with a very pleasant and balanced flavour. The boiled cereal flavour was detected in the sample fermented with *Saccharomyces lactis* 11-5964 and impure notes in the flavour were observed in the sample fermented with strain *Saccharomyces carlsbergensis* 18G. Therefore, these two strains (*Saccharomyces lactis* 11-5964 and *Saccharomyces carlsbergensis* 18G) were eliminated in the next stage of work. In the second fermentation variant, the yeast inoculum quantity was reduced so that the count of viable cells after inoculation was 10^4CFU/cm^3 in order for less alcohol to be produced by the yeast strains. The wort had extract of 11.36% and pH 6.28.

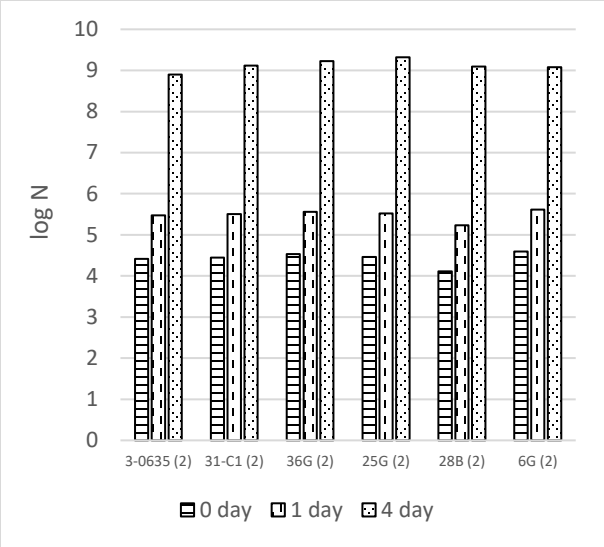


Figure 3a). Changes in the concentration of viable yeast cells during wort fermentation (variant 2)

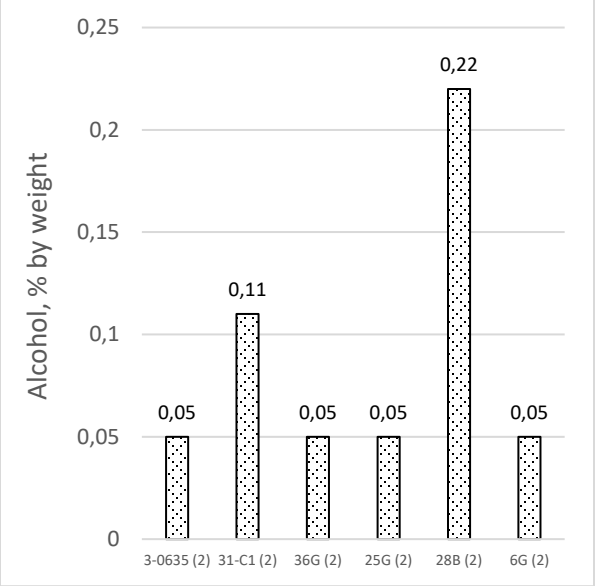
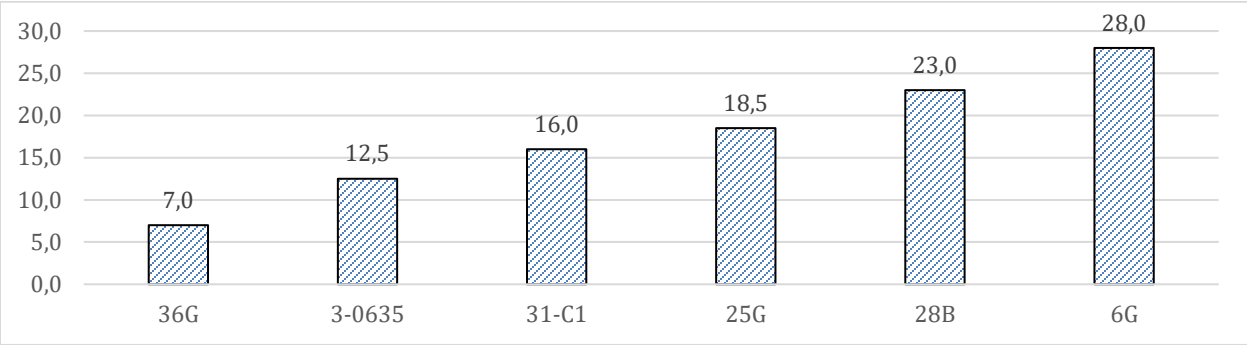


Figure 3b). Alcohol content in wort beverages fermented by different yeast strains (variant 2)

The results (Fig.3b) show that even at a lower initial concentration, the yeast strains grew up well under the specific fermentation conditions. They accumulated a significant amount of viable cells till the end of the process. An increase by 5 logN on the fourth day of fermentation was observed. The decreased initial concentration of viable yeast cells in the second variant of fermentation led to restricted ethanol formation. The alcohol concentration in the different samples was between 0.05 and 0.22% (w/v). *Saccharomyces diastaticus* 28B produced the biggest amount of alcohol, while the strains *Saccharomyces cerevisiae* 3-0635, *Saccharomyces sake gekkeikan* 6G, *Saccharomyces diastaticus* 25G and *Saccharomyces cerevisiae* 36G – the smallest. Even though the low alcohol concentration, fermentation tones were detected in the samples aroma and taste. Sensory evaluation of the samples on the fourth day of fermentation by the ranking test was performed. The results were processed statistically and using Friedman method (Analytica – EBC 2005) it was found that the ranking marks of the first 3 samples (fermented with strains *Saccharomyces cerevisiae* 36G, *Saccharomyces cerevisiae* 3-0635 and *Saccharomyces diastaticus* 31-C1) were not statistically different (Fig.4).

The same was found for the ranking marks of the other 3 samples fermented by the other three yeast strains. The ranking mark of the sample fermented with *Saccharomyces cerevisiae* 36G was statistically different from the marks of the last three samples (fermented with *Saccharomyces diastaticus* 25G, *Saccharomyces diastaticus* 28B and *Saccharomyces sake gekkeikan* 6G). The samples with *Saccharomyces diastaticus* 31-C1 and *Saccharomyces cerevisiae* 36G were with a pleasant and balanced flavour. The organoleptic profile of the samples with *Saccharomyces diastaticus* 25G and *Saccharomyces sake gekkeikan* 6G was unbalanced. Unpleasant flavours and aromas appeared in the sample with strain *Saccharomyces diastaticus* 28B. Compared to the other strains, *Saccharomyces diastaticus* 28B produced the greatest amount of alcohol at the experimental conditions applied. Based on the results from the sensory analysis and the alcohol concentration in the samples, the strains *Saccharomyces cerevisiae* 3-0635, *Saccharomyces diastaticus* 31-C1 and *Saccharomyces cerevisiae* 36G were selected as suitable strains for the preparation of non-alcoholic and low alcoholic wort-based beverages.



36G	3-0635	31-C1	25G	28B	6G
a	a,b	a,b	b,c	b,c	c

Figure 4. Ranking of the wort beverages fermented by different yeast strains (variant 2)

Further research is needed to evaluate the adaptation and activity of these three yeast strains during the fermentation of wort together with lactic acid bacteria, as well as their separate and combined influence on the sensory characteristics of the obtained fermented beverages.

Conclusions

The aim of the present study was to select yeast strains having a suitable metabolite profile for the preparation of non-alcoholic and low alcoholic wort-based beverages, but the optimum temperature and duration of fermentation for obtaining such beverages had to be determined first. Temperature of 10°C and duration of 4 days suitable for a slower fermentation process and less alcohol accumulation, were determined. In the next stage of work, three yeast strains (*Saccharomyces cerevisiae* 3-0635, *Saccharomyces diastaticus* 31-C1 and *Saccharomyces cerevisiae* 36G) were selected for wort fermentation to obtain beverages with low alcohol content and pleasant flavour. The ability these yeast strains to be used in combination with lactic acid bacteria for preparation of low- and non-alcoholic wort-based beverages will be investigated in the next step of the research.

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