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*Research Article*

# Comparative study on five commercial strains of *Saccharomyces cerevisiae* for wheat ethanol production

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## Abstract

The *Saccharomyces cerevisiae* yeast is one of the most widely used eukaryotes in a large variety of industrial processes such as ethanol production. Alcoholic fermentation is one of the critical points. There are salient differences between the yeast strains employed for neutral spirits and those used in whisky, rum, and brandy fermentation. The former white spirit processes aim to minimize the synthesis of volatile compounds. Five commercial strains of *Saccharomyces cerevisiae* were compared for ethanol production. The appearance and rehydration, bacterial contamination, reproductive ability, fermentation activity and metabolism of yeast were studied. A significant difference was established in the total, viable and dead cell counts: between $15.71\times10^9$ and $58.59\times10^9$ total cells.g$^{-1}$ dry product. The levels of bacterial cells were considerably below the permissible limits. Similar reproduction and fermentation activities were found but there were differences in the fermentation dynamics, which is very important for this production. The coefficients of sugar conversion into alcohol were between 0.574 and 0.595. The differences in the substrate utilization and synthesis of certain higher alcohols and acetic acid were established. Some of the commercial strains studied were recommended for wheat ethanol production on the basis of the results of the study.

## Keywords

active dry yeast, wheat ethanol production, reproductive ability, fermentation activity and dynamics, yeast metabolism

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## Introduction

The *Saccharomyces* cerevisiae yeast is undeniably the best studied and one of the most widely used eukaryotes in a large variety of industrial processes such as ethanol production (Parapouli et al 2016). Currently, the annual production of alcohol worldwide is over 100 billion liters, *S. cerevisiae* being the predominantly used industrial microorganism for ethanol production (Walker et al. 2018).

The importance of the *Saccharomyces* genus in the technological development of fermentation processes and as a model in scientific studies is unquestionable; however, little is known about its evolution. Each species is in a direct relationship with the natural environment since populations that coexist in the same habitat develop phenotypic convergence. These interactions define metabolic traits and survival strategies, and a determining factor is the different use and availability of resources in each environment (Liti et al. 2009; Spor et al. 2009; Fernandes et al. 2022).

Alcoholic fermentation is one of the critical points in the production of alcoholic beverages (Tsvetanov et al. 1990; Fugelsang et al. 2007). As a result of the metabolic processes in yeast cells, during its growth on a certain substrate yeast forms the main products of alcoholic fermentation, i.e. ethyl alcohol and carbon dioxide, along with a number of intermediate products (Mukhtar et al. 2010), higher alcohols accounting for about 50% of all volatile metabolites (Lakatošová 2016). This leads to the accumulation of a number of substances in the medium that affect both aroma and taste. Some of these substances have a positive effect, whereas the effect of others, even in low concentrations, is negative (Herraiz et al. 1993; Thomas et al. 1993; Bambalov 1998; Torrea et al. 2003; Barbosa et al. 2009)

The production of neutral distilled spirits is increasing worldwide due to the popularity of beverages such as vodka and gin. There are salient differences between the yeast strains employed for neutral spirits and those used in whisky, rum, and brandy fermentation. The former white spirit processes aim to minimize the synthesis of volatile compounds, whilst the opposite is valid for the latter (Black et al. 2023).

Although in spirit production the spirit composition can be influenced by the application of certain distillation and rectification modes, the fermentation process and yeast metabolites are essential for the quality of the spirit and the products obtained from it (Iarovenko et al. 2002).

Some small-scale distilleries have adopted spontaneous fermentation relying on wild yeast and bacteria; however, this applies to specific products since a lot of secondary metabolites accumulate after fermentation. This is not a good practice in the production of neutral spirits, and although other microorganisms may be present in the medium, the lead should be *Saccharomyces* cerevisiae (Pauley et al. 2017; Walker 2017).

The requirements concerning yeast used in the production of neutral spirits are as follows: fast utilization of the substrate in the medium, high alcohol yield at the end of the fermentation, thermotolerance and tolerance to osmotic stress, low production of congeners (Black et al. 2023). Yeast strain purity, viability, and vitality are key analytical parameters that can ensure consistent fermentation (Walker 2012).

The fermentation conditions (aeration intensity, sugar concentration, acidity, temperature) and yeast specificity should be aimed at maximum alcohologenic capabilities of the yeast and low concentrations of the secondary metabolites produced (Kosaric et al. 2001; Jacques et al. 2003). Higher alcohols, fatty aldehydes, volatile fatty acids with a high molecular mass, volatile sulfur compounds, as well as some simple and complex esters, have a negative impact on the aroma and taste of the spirit. As for glycerin, it is a metabolic product of yeast fermentation, the synthesis of which can lead to corresponding losses in ethanol yields (about 4%) (Nissen 2000). Aalst et al. (2022) described the procedure of reducing or eliminating this synthesis while increasing ethanol yield.

The presence of the above components in the spirit cannot be completely eliminated; however, the possibilities of reducing their concentrations in the fermented wort should be explored (Franceschin et al. 2008).

The aim of this study was to conduct a comparative investigation of commercial preparations of dry yeast strains of *Saccharomyces cerevisiae* for use in the production of alcohol from wheat.

## Materials and Methods

**Materials.** The study involved the commercial dry yeast preparations listed below. All of the yeast

strains were in compressed dry form.

1. Fali active dried alcohol yeast, *AB Mauri, United Kingdom*
2. Fermiol dry yeast *Saccharomyces cerevisiae DY 7221 for the production of distilled spirits, DSM Food Specialities B.V., France*
3. Ethanol Red dry ethanol yeast for the industrial ethanol industry, *Leaf by Lesaffre, France*
4. Conferm Rouge dry yeast for red wine alcoholic fermentation, *Begerow, Germany*
5. Saf Instant Red baker's dry yeast, *Lesaffre Yeast Corporation, France*

For comparison, a K-32 control strain from the collection of the Wine and Beer Technology Department was also included.
The other materials used were: industrial saccharified wheat grain mash with dry matter concentration of 27%, grape juice agar, MRS agar, nutrient medium with actidione, WLD and glucoamylase enzyme (1,4-alpha-D-glucan glucohydrolase, EC 3.2.1.3*)*, *Novozymes Switzerland AG*.

**Microscopic examination.** Microscopic examination of the appearance and rehydration of the dry yeast products was made according to the OIV regulations (OIV 2023);

**The total cells' concentration.** The total concentration of cells in the dry preparation was determined by diluting the rehydrated samples and counting in a Bürker chamber. The concentration of viable cells in the preparations was determined by inoculating the rehydrated preparations after they had been diluted into two complex nutrient media (grape juice-agar; MRS medium-agar) run in triplicate, and the dead cell count was obtained as the difference between the two values (Ivanov et al. 1979).

**The bacterial contamination.** The bacterial contamination in the dry preparations was determined by dilutions of the rehydrated samples and inoculation into selective nutrient media (grape juice agar + actidion 20 $mg.dm^{-3}$ and WLD medium), each in run in triplicate (Ivanov et al. 1979);

**The reproductive ability**. Sterile grape juice was used to determine reproductive ability. The dry preparations were rehydrated in advance under standard conditions, and the control strain was activated in sterile grape juice. The sterile grape juice was inoculated in 10 $cm^3$ test tubes with the respective volumes of inoculum from the rehydrated suspension and the broth culture in order to provide about $1\times10^6$ $cells.cm^{-3}$. The samples were cultured in a thermostat at 25℃, and the yeast cell concentration was determined daily using a Bürker counting chamber (Ivanov et al. 1979).

**The fermentation activity.** The fermentation activity of the studied yeasts was determined using the gravimetric method. The dry preparations were rehydrated under standard conditions, and the control strain was activated in grape juice. The samples were seeded with amounts providing about $2\times10^6$ $cells.cm^{-3}$. In order to approximate the real conditions of alcohol production, saccharified wheat grain mash with a dry matter concentration of 27%, sterilized in a Koch steam sterilizer for 20 minutes, was used as a substrate. For additional saccharification, after the sterilization the glucoamylase enzyme was divided into doses recommended by the manufacturer (0.35 $kg.t^{-1}$ starch) and kept for 1 hour at 60℃. In the experiment, two replicates of each variant were included, along with a sample where no enzyme had been dosed in order to determine the enzyme influence during fermentation. The concentration of fermentable sugars in the starting material was 185 $g.dm^{-3}$ in the samples with added enzyme and 160 $g.dm^{-3}$ in the sample without enzyme addition. In this part of the experimental work, the main metabolites in the fermented samples were determined. The sugar to ethanol conversion rates and the alcoholic fermentation efficiency (the percentages of metabolized sugars used for ethanol, and the by-products and native biomass, respectively) were calculated (Ivanov et al. 1979).

**The metabolism study.** For the study of the metabolism of the experimental yeast preparations, industrial saccharified wheat mash, sterilized and with glucoamylase added, was used again. The concentration of fermentable sugars in the saccharified mash was 168 $g.dm^{-3}$. Again, the inoculum was 2% of the liquid culture and of the 100-fold diluted rehydrated preparations. The experimental samples prepared in two replicates were placed in flasks in a 0.4 $dm^3$ volume, and the

fermentation was carried out at a temperature of 25-27°C.

The alcohol amount was determined by pycnometry; the volatile acids were separated by steam distillation and titrated using standard sodium hydroxide; the reducing sugar content was defined using the Schoorl method (Ivanov et al. 1979). The principal volatile substances (acetaldehyde, ethyl acetate, n-propanol, isobutyl alcohol, isoamyl alcohol, acetic acid) were determined by gas chromatography according to the OIV regulations (OIV 2023).

**Statistical analysis.** The results are the mean values of three determinations, and the coefficients of variation expressed as the percentage ratios between the standard deviations and the mean values were found to be <5 % in all cases. The means were calculated using Microsoft Excel™ at a 95 % confidence level (Donchev et al. 2007).

## Results and Discussion

**Appearance and rehydration of dry preparations.** The dry yeast preparations were similar in nature. There were differences in the size of the individual particles, their color and the adhesion between the individual structural elements. In the Fermiol preparation, the particles were very pale, while in the Fali, Saf instant Red and Ethanol red preparations they were creamy, and in the Conferm Rouge preparation they were beige to light cream. Most preparations consisted of medium shaped rods, only the Fermiol preparation particles were rounded. Stronger adhesion was found with the Fali and Conferm Rouge preparations. During the rehydration under standard conditions, the Saf instant Red yeast formed a higher foam volume with bubbles larger than the others, which formed a fine-grained, medium foam volume. Almost all preparations exhibited very easy rehydration; after homogenization, homogeneous yeast milk was formed, the cells were suspended very easily and remained stable in a dispersed state.

**Concentration of viable, dead cells and bacterial contamination in the dry preparations.** The results have been presented in Table 1. The total cell concentration in the preparations varied greatly and ranged between 15.71 and 58.59 billion cells per gram of dry product. The lowest concentration was found in the Fermiol preparation, and the highest concentration of cells in the Conferm Rouge preparation.

**Table 1.** Viable and dead yeast cell concentration in the active dry yeast products

| Active dry yeast products | Yeast cells concentration, $\times 10^9.g^{-1}$ product | | | Dead, | Viable, |
|---|---|---|---|---|---|
| | Total | Viable | Dead | % | % |
| Fali | 29.47 ± 0.88 | 20.10 ± 0.57 | 9.37 ± 0.28 | 31.80 | 62.20 |
| Fermiol | 15.71 ± 0.45 | 8.77 ± 0.25 | 6.94 ± 0.22 | 44.18 | 55.82 |
| Ethanol Red | 35.16 ± 0.89 | 26.10 ± 0.75 | 9.06 ± 0.24 | 25.77 | 74.23 |
| Conferm Rouge | 58.59 ± 1.47 | 35.52 ± 1.01 | 23.07 ± 0.65 | 39.38 | 60.62 |
| Saf instant Red | 19.75 ± 0.53 | 16.15 ± 0.49 | 3.60 ± 0.11 | 18.23 | 81.77 |

*The results have been expressed in cells, $\times 10^9.g^{-1}$ product ± SD of three replicates*

The differences between the individual preparations decreased in the viable cell concentrations. The Fermiol preparation was characterized by very low concentrations. It is worth noting that the available literature indicates quite high differences in the concentrations of viable cells in dry yeast preparations: between 5 and 30 billion cells per gram of dry product.

The rather high percentage of dead cells in almost all preparations was impressive: from 18 to 44%. On the one hand, this could be attributed to the harsh cultivation conditions, i.e. temperature, aeration, late generations, applied in order to obtain high cell

concentrations. A typical example was the Conferm Rouge preparation. Another reason could be an unsuitable culture medium and mode, little trehalose and glycogen amounts accumulated in the cells, the "hard" drying conditions, and others. In the Fermiol sample, despite the relatively low cell concentrations reached, the dead cells amounted to 44%. It should be pointed out that along with the concentration of viable cells, their fermentation activity is also of great importance.

The concentrations of infecting bacterial cells in the preparations studied have been presented in Table 2.

The concentration of infecting bacterial cells in four of the preparations were significantly below the permissible limit. According to the regulations, the tolerance for bacteria is $1\times10^6$ bacteria.$g^{-1}$ of dry product. Only in the Fali preparation, the amount of bacteria found was $4.65\times10^6$ bacteria.$g^{-1}$ of dry product. The bacteria were lactic acid, rod-shaped. At higher concentrations of infecting bacterial cells, the risk of initiation of their growth increases when the onset of alcoholic fermentation is delayed or when inhibitory factors are present.

**Table 2**. Bacterial cell concentration in the active dry yeast products

| **Active dry yeast products** | **Bacterial cell concentration, $\times10^3$.$g^{-1}$ product** |
|---|---|
| Fali | 4.65 ± 106.14 |
| Fermiol | 5.00 ± 0.16 |
| Ethanol Red | 20.00 ± 0.41 |
| Conferm Rouge | * |
| Saf instant Red | 5.00 ± 0.12 |

*The results have been expressed in cells, $\times10^3$.$g^{-1}$ product ± SD of three replicates.*

** Note: The bacterial cell concentration was less than $5\times10^3$ cells.$g^{-1}$ dry product.*

**Breeding ability of yeast products.** The results for the breeding ability of the commercial preparations and the control yeast strain have been presented in Table 3.

**Table 3.** Yeast strain breeding in the active dry yeast products

| **Active dry yeast products** | **Yeast cell concentration, $\times10^6$.$cm^{-3}$. $d^{-1}$** | | | | |
|---|---|---|---|---|---|
| | **Initially** | **24 h** | **48 h** | **72 h** | **96 h** |
| Fali | 1.01 ± 0.03 | 105.25 ± 3.06 | 135.00 ± 3.35 | 160.00 ± 4.41 | 152.50 ± 4.16 |
| Fermiol | 0.66 ± 0.01 | 72.50 ± 2.12 | 100.00 ± 2.94 | 110.00 ± 3.27 | 107.00 ± 3.27 |
| Ethanol Red | 1.43 ± 0.04 | 88.50 ± 2.37 | 130.00 ± 3.92 | 164.00 ± 4.41 | 159.72 ± 4.74 |
| Conferm Rouge | 1.09 ± 0.03 | 110.25 ± 3.14 | 147.50 ± 4.00 | 140.00 ± 4.08 | 144.10 ± 3.92 |
| Saf instant Red | 0.95 ± 0.03 | 64.50 ± 2.04 | 97.50 ± 2.94 | 126.50 ± 3.84 | 130.00 ± 3.76 |
| K-32 | 0.98 ± 0.02 | 49.25 ± 1.48 | 110.00 ± 3.27 | 122.50 ± 3.67 | 118.49 ± 3.52 |

*The results have been expressed in cells, $\times10^6$.$cm^{-3}$ ± SD of three replicates*

The initial cell concentrations differed from one another but generally followed the trends observed when determining the cell concentration in the preparations. The reproduction dynamics (Fig. 1) was similar for the individual preparations. The largest amount of cells accumulated between the 24$^{th}$ and the 48$^{th}$ h; the preparations with a lower total cell concentration (Saf instant Red and Fermiol) were still lagging behind the others by the 24$^{th}$ h but by the 48$^{th}$ h the differences decreased significantly. For all variants, the stationary phase of growth began after the 72$^{th}$ h. The Ethanol Red and Fali preparations accumulated the maximum amount of cells. The Fermiol sample remained with the lowest cell concentration, being about 30% lower than the first ones. The K-32 control strain

showed a slow start, lagging considerably behind most variants, but maintained exponential growth until the 72nd h and reached $123.10^6$ cells.cm$^{-3}$.

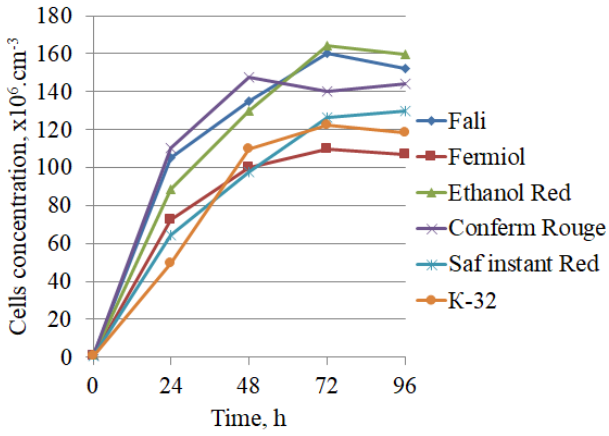



**Figure 1.** Breeding of yeast preparations expected

**Fermentation activity of the yeast preparations studied.** The fermentation curves from the experiment have been presented in Fig. 2. It indicates that the preparations studied and the control strain exhibited similar fermentation activities. There were minor differences in the initial stages: between the 24th and 72nd h and at the end of the 144-h period, the amount of $CO_2$ released was in the 12.0-12.5 g range. In the enzyme-free sample, a slightly lower fermentation rate was noticed and 3 g less $CO_2$ was released at the end of the process. This had been

and was logical in view of the fewer fermentable sugars due to the lack of additional enzymatic saccharification. Fig. 3 indicates that the largest amount of gas was released by the 72nd h, and about 95% of the total amount of $CO_2$ was released by the 96th h. The release of gas continued until the 312th h but the reported amounts were negligible. The different preparations exhibited differences in the dynamics of the alcoholic fermentation over the 96-hour period observed. The onset of the alcoholic fermentation was the fastest with Ethanol Red and Fali, which released 32-34% of the total amount of gas by the 24th h, and the other dry preparations released between 25 and 28% of $CO_2$. The K-32 control strain was lagging behind at this point, the gas released being 22% of the total amount.

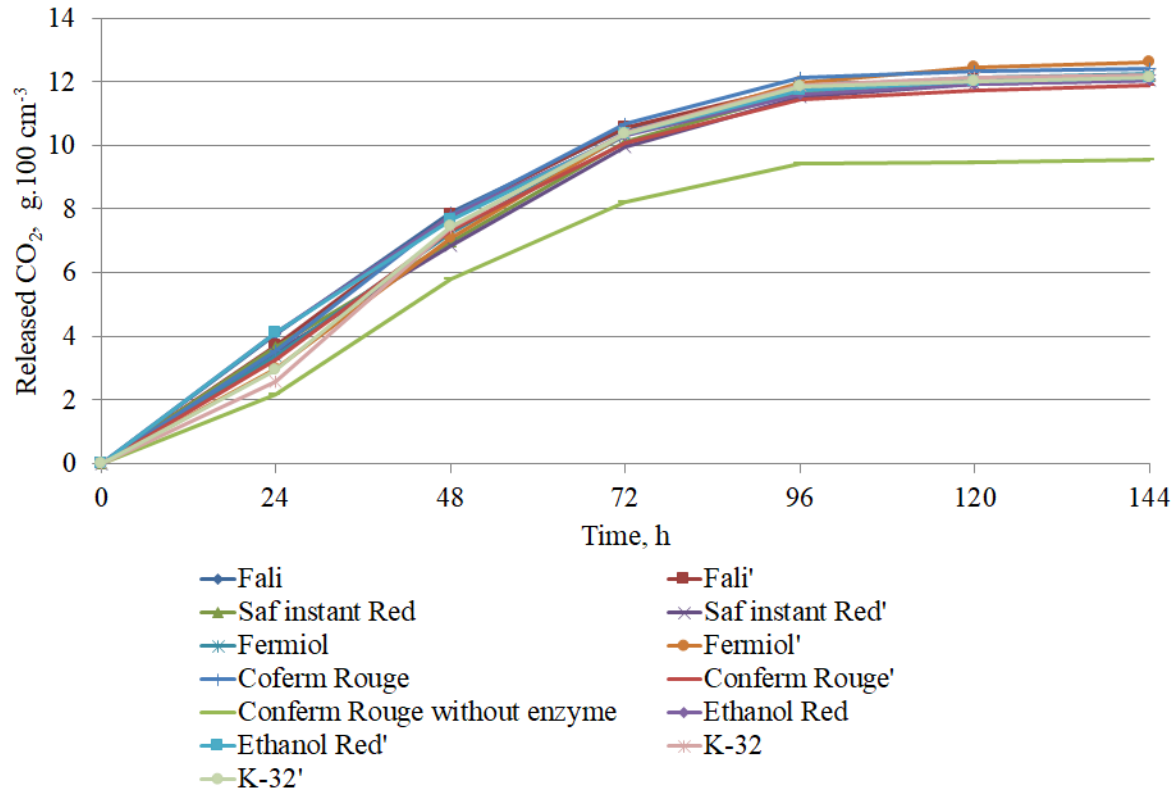



**Figure 2.** Fermentation curves of the experimental products

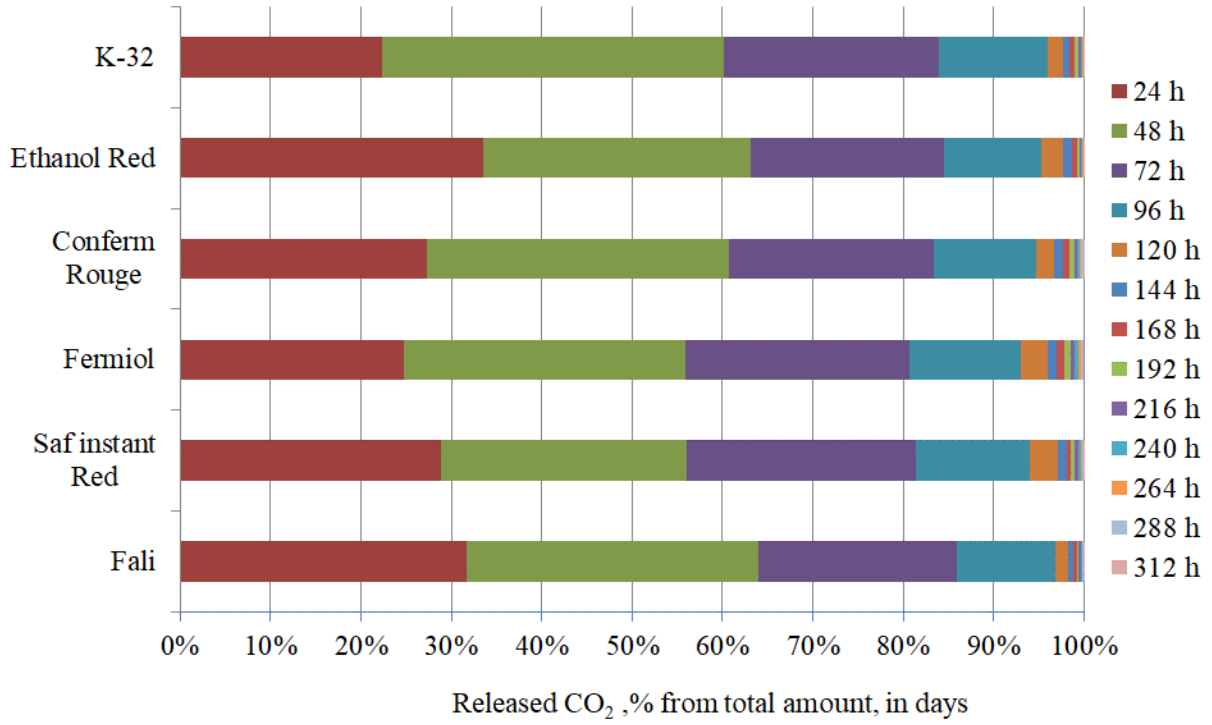



**Figure 3.** Dynamics of the $CO_2$ released by the experimental products

In the period until the 48$^{th}$ h, the Fali sample fermented most actively, the K-32 control sample caught up and the released gas was within 60-63% of the total; the Saf instant Red and Fermiol samples were slightly behind, releasing 55-57% of the total $CO_2$ amount. After 72 h, all samples had released more than 80% of the total amount of gas, the Fali preparation still having a slight advantage with 86%. At the 96$^{th}$ h, the experimental samples and the control sample were practically equal, the amount of $CO_2$ released being within the 95-97% range.

Both the Saf instant Red and the Fermiol samples were slower and released just under 95% of the total amount of gas.

The dynamics of the alcoholic fermentation and the rapid and steady substrate degradation in ethanol production are essential for creating a process flow that can ensure adequate distillation volumes and reduce the risk of growth of infectious microflora. This stimulates the search for yeasts that carry out faster alcoholic fermentation (Iarovenko et al. 2002).

Under the conditions of the experiment, this proved to be the Fali product, and the K-32 control strain also exhibited a very good result.

It was in this part of the work that the main yeast metabolites in the fermented samples were determined. Some process efficiency coefficients were calculated as well. Table 4 presents the results on the reducing sugars, ethyl alcohol concentration and volatile acids in the experimental samples; Fig.4 demonstrates the sugar into ethanol conversion coefficient, and Fig. 5 shows the percentages of metabolized sugars used for ethanol and for secondary products and biomass, respectively.

Fig. 3 shows the amount of released $CO_2$ in days, as a percentage of the total released gas for the experimental samples, as mean values of the two replicates.

The alcoholic fermentation in all samples was complete, as evidenced by the amount of reducing sugars: below 3 g.dm$^{-3}$. In the sample without enzyme addition, more sugars were found, which could also be attributed to residual dextrins reducing Fehling's solution. The alcohol content in this sample, along with the sugars, corresponded to the lower amount of released $CO_2$. The values of the volatile acids were practically the same, except for the Conferm Rouge sample, where they were twice as high, but nevertheless remained within normal limits (Table 4).

**Table 4.** Physicochemical indicators of the experimental samples

| Active dry yeast products | Alcohol, % vol. | Reducing sugars, g.dm$^{-3}$ | Volatile acidity, g.dm$^{-3}$ |
|---|---|---|---|
| Fali | 10.55 ± 0.20 | 1.75 ± 0.08 | 0.21 ± 0.02 |
| Fermiol | 10.65 ± 0.24 | 2.17 ± 0.13 | 0.28 ± 0.04 |
| Ethanol Red | 10.50 ± 0.20 | 2.37 ± 0.10 | 0.22 ± 0.02 |
| Conferm Rouge | 10.75 ± 0.20 | 2.08 ± 0.10 | 0.44 ± 0.05 |
| Conferm Rouge – without enzyme addition | 8.80 ± 0.16 | 7.08 ± 0.26 | 0.42 ± 0.04 |
| Saf instant Red | 10.48 ± 0.22 | 2.47 ± 0.12 | 0.22 ± 0.03 |
| K-32 | 10.88 ± 0.22 | 2.31 ± 0.15 | 0.26 ± 0.04 |

*The results have been expressed in % vol and g.dm$^{-3}$ ± SD of three replicates*

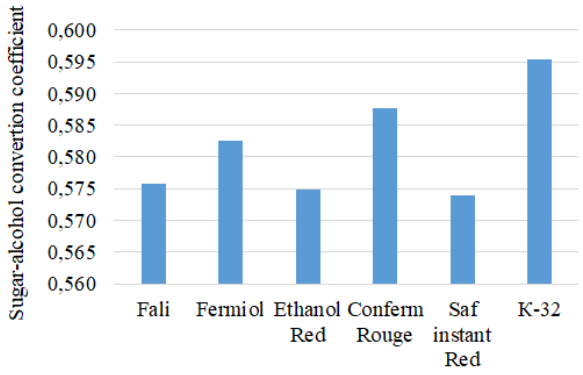



**Figure 4.** Sugar-alcohol conversion coefficients of yeast preparations

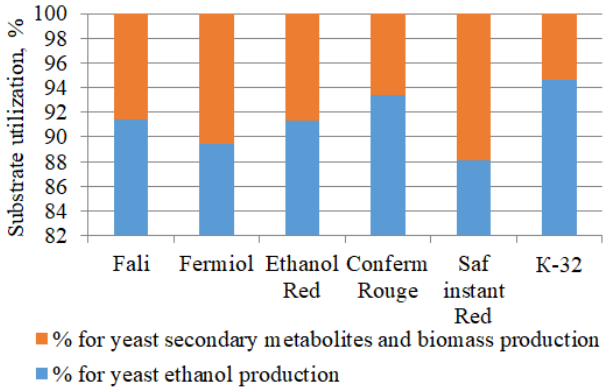



**Figure 5.** Yeast substrate utilization efficiency

The alcohol concentrations in the samples varied between 10.48-10.88 % vol. The K-32 and Conferm Rouge samples formed the most alcohol, about 3% more on average than Saf instant Red, the sample with the lowest alcohol.

The sugar to ethanol conversion coefficients in all samples were within normal limits of 0.574-0.595 (Fig. 4). The K-32 control strain exhibited the highest conversion coefficient: about 3.5% higher than the samples with the lowest conversion coefficient, i.e. Fali, Saf instant Red and Ethanol Red. In large fermentation volumes and numerous cycles, this difference is quite significant, both technologically and economically. In the fermentation of 1000 kg of carbohydrates, the K-32 preparation would yield about 23 dm$^3$ of 96% alcohol more.

The K-32 preparation used carbohydrates most efficiently: 95-96% of the substrate was used for ethanol production and only 4-5% for secondary metabolites and biomass. In addition to the higher yield, the rectification process itself was facilitated to some extent, in particular, the separation of the primary and tail fractions. Fali, Saf instant Red and Ethanol red used twice as much substrate for purposes other than ethanol synthesis. For spirit production purposes, where the target metabolite is ethanol, this is essential and economically significant. It is noteworthy that the control strain, which was a liquid culture and was supposed to have a better preserved reproductive ability, made very economical use of the substrate. We attribute this to the specifics of the strain, and it should be emphasized that this strain is typically alcoholic and has been used at the Katunitsa Distillery for years (Fig. 5).

**Investigation of the metabolism of experimental yeast preparations in close to real conditions.** Under the conditions of this stage of the study, which were much more similar to the real ones and more of an "open vessel" type, the ethanol concentrations in the samples were in much closer ranges than under anaerobic conditions. Table 5 presents the results of the determination of alcohol and reducing sugars and the total determination of volatile impurities using. The Conferm Rouge preparation formed the lowest amount, and Fali formed the highest. However, the difference was only about 2.4%. The evaporation losses under open fermentation vessel conditions also led to certain equalization of the ethanol concentrations.

**Table 5.** Physicochemical composition of the experimental samples

| Active dry yeast products | Alcohol, % vol. | Reducing sugars, $g.dm^{-3}$ | Total higher alcohols, $mg.dm^{-3}$ | Total esters, $mg.dm^{-3}$ | Total aldehydes, $mg.dm^{-3}$ |
|---|---|---|---|---|---|
| Fali | 9.69 ± 0.25 | 3.01 ± 0.12 | 235 ± 6 | 155 ± 5 | 84 ± 3 |
| Fermiol | 9.51 ± 0.23 | 2.94 ± 0.09 | 241 ± 9 | 266 ± 7 | 71 ± 3 |
| Ethanol Red | 9.51 ± 0.20 | 2.81 ± 0.09 | 203 ± 7 | 466 ± 10 | 74 ± 3 |
| Conferm Rouge | 9.46 ± 0.25 | 2.87 ± 0.15 | 215 ± 7 | 388 ± 9 | 96 ± 4 |
| Saf instant red | 9.63 ± 0.22 | 2.94 ± 0.15 | 271 ± 7 | 198 ± 4 | 96 ± 4 |
| K-32 | 9.56 ± 0.27 | 2.71 ± 0.14 | 129 ± 3 | 255 ± 7 | 38 ± 2 |

*The results have been expressed in % vol, $mg.dm^{-3}$ and $g.dm^{-3}$ ± SD of three replicates*

In all samples, the process was completely finished and the sugars were below 3 $g.dm^{-3}$. The values of the volatile components were within normal limits. The higher alcohols varied widely from 129 to 271 $mg.dm^{-3}$, the K-32 sample formed the lowest amount, while Saf instant Red, Fermiol and Fali formed twice as much as the control sample on average. The concentrations of aldehydes in the samples varied less and were in the range of 71-98 $mg.dm^{-3}$. The K-32 control strain formed between 1.8 and 2.5 times less aldehydes than the others. The concentration of esters was high and varied widely from 155 to 466 $mg.dm^{-3}$. The increase in esters could also be attributed to the fact that the methodology included distillation for their determination, wherein additional esterification occurred. Nevertheless, it was evident that the Ethanol Red preparation formed 1.2 to 3 times as many esters as the other strains. Conferm Rouge also formed a significant amount of esters. The lowest amounts of esters were found in the Fali and Saf instant Red samples.

Table 6 presents the results of the gas chromatographic determination of individual representatives of the volatile impurities.

The acetaldehyde concentrations in the samples varied between 57 and 111 $mg.dm^{-3}$ (49% differences), the ethyl acetate amounts in the samples were almost identical: between 10 and 16 $mg.dm^{-3}$. The i-amylol concentrations differed by about 50 % and ranged from 130 to 261 $mg.dm^{-3}$; those of i-butanol differed by about 52%, varying between 30 and 62 $mg.dm^{-3}$, and of propanol by about 45 %, between 11 and 20 $mg.dm^{-3}$.

The concentration of the individual volatile components in the individual yeast strains determined gas chromatographically were consistent with the total concentrations found. Once again, the acetaldehyde in the K-32 control sample was in the lowest concentration, the ethyl acetate amount was the highest in the Fermiol sample, and the higher alcohols were the highest in Saf instant Red.

**Table 6.** Volatile components in the experimental samples

| Active dry yeast products | Acetaldehyde, mg.dm$^{-3}$ | Ethyl acetate, mg.dm$^{-3}$ | Propanol, mg.dm$^{-3}$ | Isobutanol, mg.dm$^{-3}$ | Isoamylol, mg.dm$^{-3}$ | Acetic acid, mg.dm$^{-3}$ |
|---|---|---|---|---|---|---|
| Fali | 103 ± 3 | 12 ± 0,4 | 14 ± 0,5 | 62 ± 2 | 238 ± 6 | 146 ± 4 |
| Fermiol | 84 ± 2 | 16 ± 0,3 | 20 ± 0,4 | 58 ± 2 | 227 ± 7 | 191 ± 6 |
| Ethanol Red | 77 ± 2 | 10 ± 0,3 | 11 ± 0,3 | 40 ± 1 | 185 ± 4 | 117 ± 2 |
| Conferm Rouge | 111 ± 3 | 14 ± 0,5 | 16 ± 0,3 | 53 ± 1 | 202 ± 6 | 230 ± 7 |
| Saf instant Red | 100 ± 3 | 11 ± 0,3 | 16 ± 0,3 | 49 ± 2 | 261 ± 7 | 128 ± 3 |
| K-32 | 57 ± 2 | 10 ± 0,3 | 19 ± 0,4 | 30 ± 1 | 130 ± 4 | 396 ± 10 |

*The results were expressed in mg.dm$^{-3}$ ± SD of three replicates*

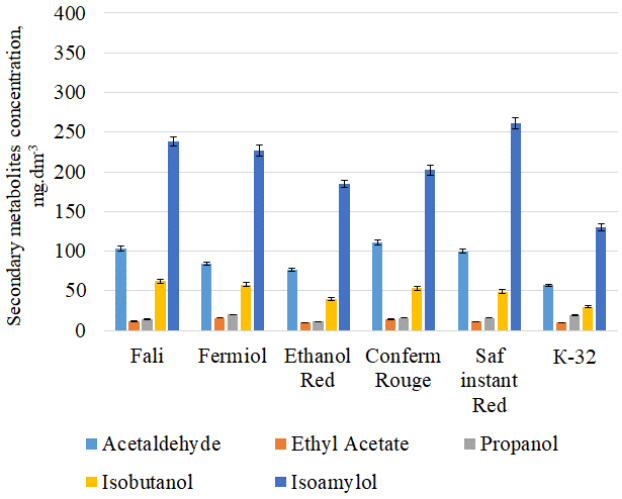



**Figure 6.** Secondary metabolites concentration in grain wort

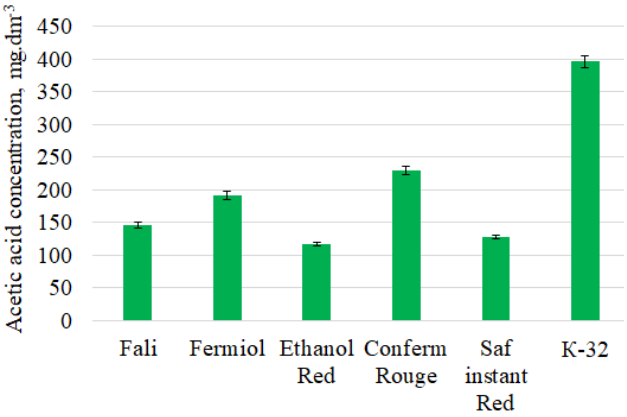



**Figure 7.** Acetic acid concentration in grain wort

In general, the lowest amounts of the secondary products formed were reached by the K-32 control strain, and they were the highest in the Fali and Saf instant Red preparations (Fig. 6). The concentration of acetic acid formed was an exception. Yeast from K-32 formed 2-3 times more of this metabolite, and although the absolute acetic acid values were within acceptable limits, the trend is highlighted (Fig. 7). A more salient synthesis of acetic acid was also observed in the Conferm Rouge sample.

## Conclusions

The results of the study of the dry yeast preparations provided can be summarized in the following directions. In general, the preparations were relatively close in terms of overall characteristics; the differences shown were related to individual indicators or to individual stages of the processes, and not to their overall duration. The preparations were similarly rehydrated and easily suspended in homogeneous yeast milk. This is important for ensuring optimal contact with the substrate and avoiding flocculation.

There was a substantial difference in the concentrations of total, viable and dead cells in the preparations. In general, preparations with a higher total number of cells also have more dead cells. The concentrations of total and viable cells in the preparations were the highest in Conferm Rouge. The presence of infectious bacterial microflora in the preparations remained within

normal limits, the most bacteria having been found in the Fali preparation.

The breeding ability of the preparations studied was similar, and within 96 h, Fali and Ethanol Red demonstrated the most intensive breeding activity, while Fermiol demonstrated the weakest.

The fermentation activity of the preparations was close; therefore, the more cells in the individual preparations did not lead to intensification of the course of the process. The Fali preparation was the most active one, the K-32 control strain also showed a very good result.

The K-32 preparation used the carbohydrates most efficiently, produced more ethanol, had a higher sugar to alcohol conversion coefficient, consumed less of the substrate for secondary metabolites and biomass. The Conferm Rouge metabolism was also effective. In all preparations, the secondary metabolites were within normal limits; there were more differences in the amounts of certain higher alcohols and acetic acid. In total, the secondary metabolite amounts were the lowest in K-32, and the highest in the Fali and Saf instant Red preparations.

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## References

Aalst A., Valk S., Gulik, Jansen M., M., Pronk J., Mans R. Pathway engineering strategies for improved product yield in yeast-based industrial ethanol production. *Synthetic and Systems Biotechnology*, 2022, 7(1): 554-566. https://doi.org/10.1016/j.synbio.2021.12.010

Bambalov G. Yeasts and their impact on the hydrogen sulfide aroma in wine (overview). Wine-Growing and Winemaking, 1998, 1: 47-49. [in Bulgarian]

Barbosa C., Falco V., Mendes-Faia A., Mendes-Ferreira A., Nitrogen addition influences formation of aroma compounds, volatile acidity and ethanol in nitrogen deficient media fermented by *S. cerevisiae* wine strains, *Journal de Bioscience and Bioengineering*, 2009, 108(2): 99-104. https://doi.org/10.1016/j.jbiosc.2009.02.017

Black K., Walker G. Yeast fermentation for production of neutral distilled spirits. *Applied Sciences*, 2023, 13(8): 4927. https://doi.org/10.3390/app13084927

Donchev D., Dilcheva M., Kinova V. *A Practical Guide to Statistics* (Second Edition). Auto Spectrum. 2007, 138 pages. Print ISBN: 978-954-8932-40-0. [in Bulgarian]

Franceschin G., Zamboni, A., Bezzo, F., A. Ethanol from corn: a technical and economical assessment based on different scenarios. *Chemical Engineering Research and Design*, 2008, 86(5): 488-498. https://doi.org/10.1016/j.cherd.2008.01.001

Fernandes F., Souza E., Carneiro L., Silva J., Souza J., Batista J. Current ethanol production requirements for the yeast *Saccharomyces cerevisiae*. *International Journal of Microbiology*, 2022, 2022(8): 7878830. https://doi.org/10.1155/2022/7878830

Fugelsang K., Edwards C. *Wine microbiology. Practical applications and procedures* (Second Editon). Springer. 2007, pages 394. eBook ISBN978-0-387-33349-6 Available at: https://link.springer.com/book/10.1007/978-0-387-33349-6#about-this-book

Herraiz T., Ough C. Formation of ethyl esters of amino acids by yeasts during the alcoholic fermentation of grape juice. *American Journal of Enology and Viticulture*, 1993, 44(1): 41-48. https://doi.org/10.5344/ajev.1993.44.1.41

Iarovenko V., Marinchenko V., Smirnov V., Ustinnikov B., Tsigankov P., Shvets V., Belov N. *Alcohol Technology*. Colos-Press. 2002, pages 464. Print ISBN: 5-901705-08-4 [in Russian]

International Organisation of Vine and Wine. *Compendium of International Methods of Wine and Must Analysis*. OIV. 2023, pages 1679. ISBN: 978-2-85038-068-6 Available at: https://www.oiv.int/sites/default/files/publication/2023-05/Compendium%20MA%20complet_EN.pdf

Ivanov T., Gerov S., Yankov A., Bambalov G., Tonchev T., Nachkov D., Marinov M. *Practicum in Wine Technology* (First Edition). Hristo G. Danov. 1979, pages 530. [in Bulgarian]

Jacques K., Lyons T., Kelsall D. *The Alcohol Textbook* (Forth Edition). Nottingham University Press. 2003, pages 448. Print ISBN 1-897676-13-1

Kosaric N., Pieper H., Vardar-Sukan F. *The Biotechnology of Ethanol. Classical and Future Applications* (First Edition). Wiley-VCH. 2001, pages 244. ISBN: 3-527-30199-2

Lakatošová J., Priesolová L., DokuPiLová I., Yoncheva T., Šmogrovičová D., Pátková J. Classical oenological parameters and concentration of selected higher alcohols and esters in traditional fermented Slovakian and Bulgarian wines. *Journal of Food and Nutrition Research*, 2016, 55(1): 1-10. Available at: https://www.vup.sk/en/index.php?mainID=2&navID=34&version=2&volume=55&article=1993

Liti G., Carter D., Moses A. et al. Population genomics of domestic and wild yeasts. *Nature*, 2009, 458(7236): 337-341. https://doi.org/10.1038%2Fnature07743

Mukhtar K., Asgher M., Afghan S., Hussain K., Zia-ul-Hussnain S. Comparative study on two commercial strains of *Saccharomyces cerevisiae* for optimum ethanol production on industrial scale ethanol production on industrial scale. *BioMed Research International*, 2010, 2010(4): 419586. https://doi.org/10.1155/2010/419586

Nissen T., Hamann C., Kielland-Brandt M., Nielsen J., Villadsen J. Anaerobic and aerobic batch cultivations of *Saccharomyces cerevisiae* mutants impaired in glycerol synthesis. *Yeast*, 2000, 16(5): 463-474. https://doi.org/10.1002/(SICI)1097-0061(20000330)16:5%3C463::AID-YEA535%3E3.0.CO;2-3

Parapouli M., Vasileiadis A., Afendra A., Hatziloukas E. *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiology*, 2020, 6(1): 1-31. https://doi.org/10.3934/microbiol.2020001

Pauley M., Maskell D. Mini-review: The role of Saccharomyces cerevisiae in the production of gin and vodka. *Beverages*, 2017, 1(3): 0013. https://doi.org/10.3390/beverages3010013

Spor A., Nidelet T., Simon J., Bourgais A., Vienne D., Sicard D. Niche-driven evolution of metabolic and life-history strategies in natural and domesticated populations of *Saccharomyces cerevisiae*. *BMC Evolutionary Biology*, 2009, 9(12): 296-312. https://doi.org/10.1186/1471-2148-9-296

Thomas C., Boulton R., Silacci M., Gubler W. The effect of elemental sulfur, yeast strain, and fermentation medium on hydrogen sulfide production during fermentation. *American Journal of Enology and Viticulture*, 1993, 44(2): 211-216. https://doi.org/10.5344/ajev.1993.44.2.211

Torrea D., Fraile P, Garde T., Ancin C. Production of volatile compounds in the fermentation of chardonnay musts inoculated with two strains of *Saccharomyces cerevisiae* with different nitrogen demands. *Food Control*, 2003, 14(8): 556-571. https://doi.org/10.1016/S0956-7135(02)00146-9

Tsvetanov O., Luchev S, Angelova Zh. Enological characteris significance of some wine yeast strains. Wine-Growing and Winemaking, 1990, (1): 10-11; 15-16. [in Bulgarian]

Walker G. Yeast Vitality and Stress Responses: Novel Investigative Approaches. In: *Yeast Flocculation, Vitality and Viability* (A. Speers Ed). The American Society of Brewing Chemists. 2012, pp. 85-96. ISBN 978-0-9787726-4-2 Available at: https://rke.abertay.ac.uk/en/publications/yeast-vitality-and-stress-responses-novel-investigative-approache

Walker G. Physiology of ethanol-producing yeasts. In: *The Alcohol Textbook* (Sixth Edition). Lallemand Biofuels & Distilled Spirits. 2017, pp. 257-271. ISBN 9780692930885 Available at: https://rke.abertay.ac.uk/en/publications/physiology-of-ethanol-producing-yeasts

Walker G., Walker R. Enhancing yeast alcoholic fermentations (Chapter Three). *Advances in Applied Microbiology*, 2018, 105(1): 87-129. https://doi.org/10.1016/bs.aambs.2018.05.003