

## **Distillate composition of fermented media based on by-products of sugar beet processing**

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### **Abstract**

*Composition of fermented media's distillate is very significant because it dictates choice of downstream processes to obtain appropriate ethanol quality. The aim of this research paper is the analysis of the distillates composition of fermented media based on raw, thin and thick juices and molasses in terms of ethanol, methanol, higher alcohols and aldehydes content. All applied substrates, which represent intermediate and by-products of sugar beet processing, are fermented by different commercial starter cultures of *Saccharomyces cerevisiae* - dried distiller's yeast, dried wine yeast, dried wine yeast tolerant of high ethanol concentration, dried baker's yeast and fresh baker's yeast. If the fermentation is carried by commercial fresh baker's yeast there is statistically significant difference only in aldehydes content in distillate of fermented media based on molasses. However, if commercial starter cultures in dried forms are used as production strains, distillates of fermentation broths prepared from effluents of sugar industry are not statistically significant different regarding the content of the analyzed components.*

**Keywords:** sugar beet, *Saccharomyces cerevisiae*, lower alcohols, higher alcohols, aldehydes

### **1. Introduction**

Ethanol is widely used for the production of strong alcoholic drinks and to less degree for medical or pharmaceutical purposes (BARAS& al. [1]). Nowadays it also represents a modern type of energy and an important substitute for liquid fossil fuels or, in mixtures with gasses, a substitute for natural gas. The basic concern over ethanol production expansion is depletion of natural resources and demands for environmentally acceptable fuels – biofuels from renewable feedstocks, emitting less carbon dioxide into the atmosphere (ERICSSON and NILSSON[2]; FARRELL& al. [3]).

Ethanol can be produced from a wide range of feedstock, which includes sugar-based (sugar beet or sugarcane juice and molasses), starch-based (corn and wheat) and cellulosic (bagasse and wood) resources. In particular, sugar-based feedstock contains readily available fermentable sugars and can be an ideal substrate for ethanol production, since starch and cellulosic substances require an additional pre-treatment step for conversion into fermentable sugars (HATANO& al. [4]). Less expensive production of sugar from sugarcane indicates that the application of sugar beet for bioethanol production has great potential. Also, increased yield and sugar production efficiency have led to a reduction in sugar beet requirements. For these reasons, the majority of existing sugar plants in Europe started simultaneous production of ethanol in refineries built as their extensions (POPOV& al. [5]). Introducing the concept of sugar and ethanol co-production is an attractive option for sugar

factories, as it provides flexibility in terms of variation of produced quantities of sugar and ethanol, depending on the conditions prevailing on the market (GRAHOVAC & al. [6]). Molasses is commonly used feedstock for bioethanol production. In the sugar beet processing raw and thin juices are intermediate products with production costs considerably lower in comparison with molasses, obtained at the end of the process. The only disadvantage of these intermediate products is low storability and easy decomposition by the action of microorganisms. Thick juice is an intermediate product with significantly higher price mostly due to evaporation with storability comparable with molasses (HINKOVÁ and BUBNÍK [7]). Some domestic factories, that adopted co-production, use pure cultures of production microorganism for ethanol production. However, additional financial resources are needed for constructing part of the plant for inoculum preparation. To avoid these investments, most of existing factories apply commercial starter cultures.

*Saccharomyces cerevisiae* is the preferred fermenting microorganism for ethanol production because of its well documented industrial performances such as superior ethanol tolerance and high ethanol yield (PĂTRAȘCU & al. [8]). In addition to ethanol and CO<sub>2</sub>, various by-products are also produced during ethanol fermentation, for example, glycerol, acetaldehyde, organic acids and higher alcohols (BAI & al. [9]). The presence of pectin in feedstock may also result in methanol generation in the fermented medium (ANLI & al. [10]). Ethanol obtained by fermentation is on the world market classified in ten quality groups. Potential use of commercial ethanol depends on characteristic of these groups (BARAS & al. [1]).

The aim of this research paper is the determination of distillates composition of fermented media based on raw, thin and thick juices and molasses as intermediate and by-products of sugar beet processing. Distillates of fermented media were analysed in terms of ethanol, methanol, higher alcohols and aldehydes content. This research also includes comparison of distillates composition regarding the content of the analyzed components for media fermented by different strains of *Saccharomyces cerevisiae*, which are commercially available in the domestic market.

## **2. Materials and Methods**

### **2.1. Microorganisms and inoculum preparation**

Five different commercial types of *Saccharomyces cerevisiae* were used throughout this research: dried distiller's yeast – DD (Lallemand Inc., Rexdale, Ontario, Canada), dried wine yeast – DW<sub>1</sub> (Lallemand Inc., Rexdale, Ontario, Canada), dried wine yeast tolerant of high ethanol concentration – DW<sub>2</sub> (Lallemand Inc., Rexdale, Ontario, Canada), dried baker's yeast – DB (Alltech, Senta, Serbia) and fresh baker's yeast – FB (Alltech, Senta, Serbia). In order to rehydrate dried yeasts and metabolically acclimatize the cells prior to fermentation, yeasts were suspended in a small quantity of culture medium under aerobic conditions for 2 h (temperature of 30°C, agitation rate of 200 rpm) and then introduced to the rest of the culture medium. On the other hand, fresh baker's yeast was also suspended in a small quantity of culture media and immediately used for inoculation.

### **2.2. Substrates**

Raw, thin and thick juices and molasses obtained from a domestic sugar factory were used as fermentation media. Raw and thin juices were diluted with water to give total sugar

mass fractions of 5 and 10% and also used without dilution with a total sugar mass fraction of approx. 13%, obtained from sugar beet processing technology. Thick juice and molasses were diluted with water to give a total sugar concentration of 5, 10, 13, 15, 20 and 25% (w w<sup>-1</sup>). The substrates were adjusted to pH 5.0 with 10% sulphuric acid (v v<sup>-1</sup>).

### 2.3. Fermentation

Fermentations were carried out in a 2.0 l laboratory bioreactor with the fermentation media of 1.5 l. The laboratory bioreactor with the substrate was sterilized by autoclaving at 121°C and pressure of 2.1 bars for 20 min. The sterile medium was inoculated to give the initial yeast cell concentration of 10<sup>8</sup> cells ml<sup>-1</sup> (approx. 3 g of yeast dry solids per 1000 ml of media). The fermentations were carried out in batch mode under anaerobic conditions for 48 h at the temperature of 30°C and agitation rate of 150 rpm.

### 2.4. Analytical methods

Samples of the substrates were taken for analysis. The pH was measured directly in the media by the laboratory multiparameter analyzer Consort C863 (Consort, Turnhout, Belgium) with the glass electrode. Total dissolved salt (TDS) content and conductivity were determined using conductivity electrode. The amount of soluble ash was calculated based on the conductivity of the sample. Dry matter (dm) was determined by the standard drying method in an oven at 105°C to a constant mass (AOAC [11]).

The samples of the substrates were centrifuged at 4000 rpm for 15 min. Then sucrose and reducing sugar content (sum of glucose and fructose) of the supernatant were determined (Jasco, Inc, Easton, MD, USA, pump PU-980, detector RI-930, sampler AS-950, 20 ml injection loop, column sugar KS-801, eluent: water at flow rate of 0.6 ml min<sup>-1</sup> and elution time 30 min). Fermentable sugar content was expressed as the sum of sucrose and reducing sugars. Sucrose, glucose, and fructose standards were purchased from Supelco (Bellefonte, PA, USA). Free nitrogen content of the supernatant determined by Kjeldahl method (HERLICH[12]).

Ethanol content in distillate of fermented media was determined by gas chromatography, using a HP 5890 Series II GC (Agilent Technologies Inc, Santa Clara, CA, USA) equipped with a flame ionization detector, a Carbowax 20 M column at 85°C and the carrier gas was helium. Injector and detector temperature was maintained at 150°C.

The content of methanol, higher alcohols and aldehydes in the distillate of fermented media were determined by standard AOAC methods (AOAC [11]).

During this experiment three independent fermentations were carried out with raw, thin and thick juices and molasses from domestic sugar factories as fermentation media. The results were statistically tested by analysis of variance and the means were compared by Scheffé's test at a significance level of  $p=0.05$ , using the StatPro for Microsoft Excel 2003 software.

## 3. Results and Conclusions

The compositions of substrates (raw, thin and thick juices and molasses) before dilution and fermentation were presented in Table 1. Results from Table 1 show that compositions of raw, thin and thick juices and molasses were characteristic and usual for sugar beet processing in domestic factories. With their compositions, the given by-product, as

well as the intermediate products are to be considered as convenient raw materials for fermentation media preparation for bioethanol manufacturing process.

Table 1. Compositions of substrates (raw, thin and thick juices and molasses)

Parameter	Raw juice	Thin juice	Thick juice	Molasses
Dry matter, % w w <sup>-1</sup>	14.56	14.37	60.31	81.32
Sucrose, % w w <sup>-1</sup>	12.94	13.07	54.12	50.14
Coefficient of purity, % w w <sup>-1</sup>	88.87	90.95	89.74	61.66
pH	6.41	9.09	8.03	7.05
Ash, % w w <sup>-1</sup>	0.281	0.338	1.97	9.92
Reducing substances, % w w <sup>-1</sup>	0.0692	0.0134	0.501	0.862
Total nitrogen, % w w <sup>-1</sup>	0.129	0.128	0.158	1.841

Figure 1A-D shows effect of the initial concentration of fermentable sugars from raw, thin and thick juices and molasses on ethanol, methanol, higher alcohols and aldehydes content in distillates of fermented media. During these experiments, fresh baker's yeast (FB) was used as a production microorganism.

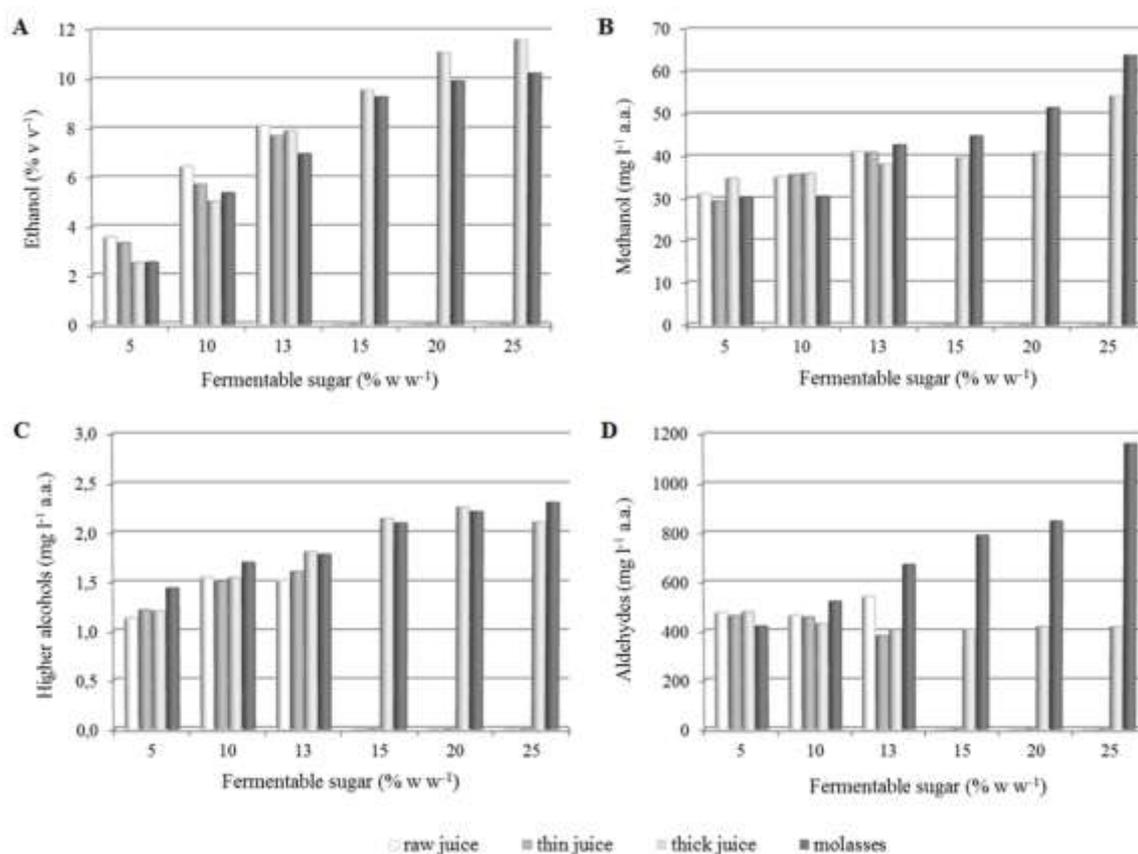


Figure 1. Effect of the initial concentrations of fermentable sugars from raw, thin and thick juices and molasses on ethanol (A), methanol (B), higher alcohols (C) and aldehydes (D) content in distillates of fermented media

The main metabolic pathway involved in the ethanol fermentation is glycolysis (Embden–Meyerhof–Parnas or EMP pathway), through which one molecule of glucose is metabolized, and two molecules of pyruvate are produced. Under anaerobic conditions, the pyruvate is further reduced to ethanol with the release of CO<sub>2</sub>. Theoretically, the yield is 0,511 for ethanol and 0,489 for CO<sub>2</sub> on a mass basis of glucose metabolized (BAI& al.[9]). Results shown in Figure 1A indicate that with the increasing of fermentable sugars concentration from 5 to 20% (w w<sup>-1</sup>), ethanol content increased for all applied raw materials (raw, thin and thick juices and molasses). When initial sugar concentration of 20% (w w<sup>-1</sup>) was increased to 25% (w w<sup>-1</sup>), ethanol content in media based on thick juice and molasses were not significantly different. Low ethanol content at higher sugar concentrations were more expressed in fermentation medium prepared from molasses. This is a consequence of the higher dry matter content in molasses in comparison with thick juice. Higher dry matter content causes a higher osmotic pressure in the medium which adversely effects on yeast cell growth and ethanol production. In the industry, the ethanol yield that is calculated based on the total sugar feeding into the fermentation system without deduction of the residual sugar can be as high as 90–93% of its theoretical value of ethanol to glucose (BAI& al.[9]). The ethanol yields in applied experimental conditions were 0.61, 0.64, 0.61 and 0.54 (ml g<sup>-1</sup> fermentable sugars) for media based on raw, thin and thick juices and molasses, which is 93.85, 98.46, 93.85 and 83.08% of theoretical yield, respectively. In terms of the ethanol yield, the obtained results suggest that all of the substrates are suitable for the ethanol production.

Methanol is formed by pectinolytic enzymes that split the methoxyl group from the pectin present in raw material (ANLI& al.[10]). Sugar beet contains 1-2 g pectic substances per 100 g of beet. Only relatively small amounts of pectin (0.1-0.3% w w<sup>-1</sup>) are dissolved in colloidal form in the beet cell juice. Under normal conditions, a very small fraction of pectic substances (4-6%) exceeds in raw juice during extraction in sugar beet processing (van der POEL& al.[13]). Throughout further processing in the sugar industry content of pectic substances are reduced. However, a small amount of pectin is sufficient for the formation of methanol during fermentation. Also, with less dilution of applied raw material, substrate contains a higher content of pectic substances. This means that the content of methanol increases with increasing fermentable sugars content in all substrates (see Figure 1B). For fermented media based on thin and thick juices and molasses with sugar concentration of 13% (w w<sup>-1</sup>) in comparison with raw juice with the same sugar concentration *p*-values were 0.9474, 0.1515 and 0.6647, respectively. The obtained results suggest that there is no statistically significant difference in the content of methanol in the distillates of fermented media based on raw, thin and thick juices and molasses.

Higher alcohols are produced by yeasts during alcoholic fermentation through the conversion of the branched chain amino acids present in the medium: valine, leucine, isoleucine, threonine and phenylalanine. They are also produced *de novo* from a sugar substrate (LAMBRECHTSand PRETORIUS[14]). Sugar beet contains 0.2-0.3 g amino acids per 100 g of beet. Amino acids are found in free form dissolved in the beet cell juice. Roughly 92-97% of free amino acids in sugar beet pass into the raw juice. The total amount of amino acids decreases during processing. The concentration of free amino acids is markedly higher in raw juice than in thin juice. The total amount of amino acids falls during juice purification to about 70% of its level in raw juice, mainly because of the formation of amides. However, this does not apply to certain amino acids such as valine, leucine, isoleucine and threonine which are precursors of higher alcohols. Content of these amino

acids stays constant during sugar beet processing (van der POEL& al.[13]). This fact explains uniform content of higher alcohols in distillates of all fermented media with same concentration of fermentable sugars (see Figure 1C). For fermented media prepared from thin and thick juices and molasses with sugar concentration of 13% ( $w w^{-1}$ ) in comparison with raw juice with the same sugar concentration  $p$ -values were 0.6047, 0.1288 and 0.3104, respectively. These values suggest that there is no statistically significant difference in the content of higher alcohols in distillates of fermented media based on intermediate and by-product of sugar beet processing.

Aldehydes are usual products of alcoholic fermentation. The precursors of aldehydes, the 2-keto acids, are formed as intermediates in both the anabolic and catabolic formation of amino acids or higher alcohols. Conditions which favor higher alcohols production also favor formation of small quantities of aldehydes. These may be secreted but can be reabsorbed and reduced by yeast to the corresponding alcohol during the later stages of the fermentation. Acetaldehyde is the major component. It is an intermediary product formed from pyruvate. Oxidation of alcohols, degradation of amino acids and autooxidation of fatty acids may also produce aldehydes (LAMBRECHTS and PRETORIUS[14]). Results shown in Figure 1D indicate that fermentable sugars concentrations in fermented media based on raw, thin and thick juices no affected on change of aldehydes content in their distillates. Also, can be seen increase of aldehydes content with the increasing of fermentable sugars concentrations in fermented media prepared from molasses. This can be explained by the high concentration of non-sugar compounds in molasses from which arise aldehydes during distillation. There is no statistically significant difference in the content of aldehydes in distillates of fermented media based on thin and thick juices with sugars concentration of 13% ( $w w^{-1}$ ) compared to the raw juice with the same sugar concentration ( $p$ -values were 0.0703 and 0.0544, respectively). However, there is a statistically significant difference in aldehyde content in distillate if used medium based on molasses in comparison with medium based on raw juice ( $p=0.0269$ ).

Fermentation media, prepared from raw, thin and thick juices and molasses with fermentable sugars concentration of 13% ( $w w^{-1}$ ), were also inoculated with different types of yeast in the dried forms in order to evaluate their fermentation productivity. Examined types of yeast are commercially available and extensively used as starter cultures in various branches of the fermentation industry. Dried distiller's yeast (DD) is intended for use in fuel ethanol and beverage alcohol production. Yeasts  $DW_1$  and  $DW_2$  are in use for wine production and are selected because of very short lag phase and fast fermentation. Strain  $DW_2$  is also tolerant on high ethanol concentrations. Strain of commercially produced fresh baker's yeast (FB) is also applied in form as dried yeast (DB).

Figure 2A-D shows the dependence of the ethanol, methanol, higher alcohols and aldehydes content on the applied media and yeast strains.

The effects of applied media and yeast strains on ethanol content are presented in Figure 2A. Average values of the ethanol content in distillates of fermented media based on raw, thin and thick juices and molasses were almost uniform. For ethanol content in media fermented by types of yeast in dried forms, DB, DD,  $DW_1$  and  $DW_2$ , in comparison with fresh baker's yeast (FB),  $p$ -values were 0.9636, 0.3838, 0.8034 and 0.4912, respectively. The obtained results suggest that there is no statistically significant difference in ethanol production when commercial starter cultures are used as production strains.

Figure 2B illustrates the dependence of the methanol content on the applied media and yeast strains. For methanol content in the distillates of media fermented by strains DB, DD,  $DW_1$  and  $DW_2$ , in comparison with fresh baker's yeast (FB),  $p$ -values were 0.3628, 0.7152, 0.0414 and 0.0417, respectively. These results indicate that there is no statistically

significant difference in methanol content in distillates of media fermented by the applied yeast strains.

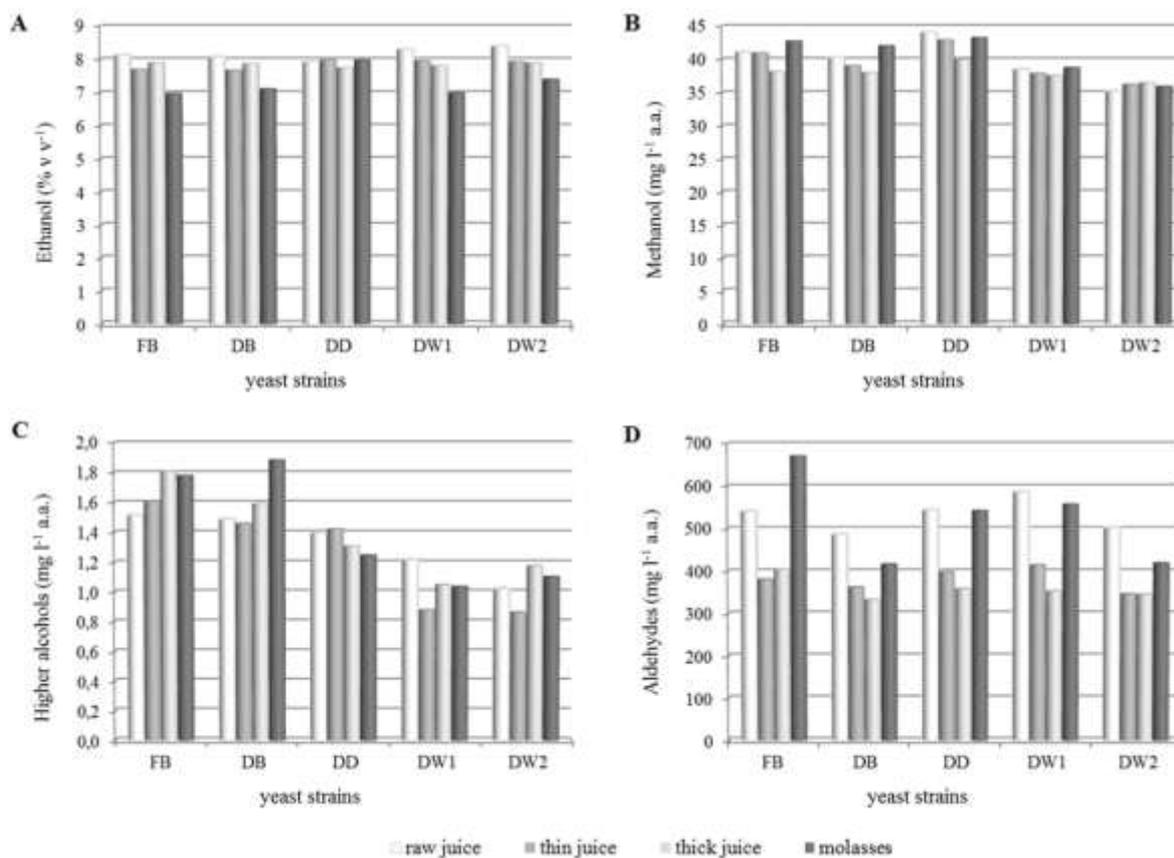


Figure 2. Dependence of the ethanol (A), methanol (B), higher alcohols (C) and aldehydes (D) content on the applied media and yeast strains

Experimental results indicate that yeast strains used for wine production give lower higher alcohols content in comparison with the other three yeast strains (see Figure 2C). For higher alcohols content obtained by fermentation with strains DB, DD, DW<sub>1</sub> and DW<sub>2</sub>, in comparison with fresh baker's yeast (FB), *p*-values were 0.4278, 0.2228, 0.0979 and 0.0619, respectively. These results suggest that there is no statistically significant difference in higher alcohols content in distillates of media fermented by the applied yeast strains.

Dependence of the aldehydes content on the applied media and yeast strains are shown in Figure 2D. From this figure it is evident that aldehydes content in distillates of fermented media prepared from thin and thick juices is lower in comparison with raw juice and molasses for all applied yeast strains. For aldehydes content in the distillates of media fermented by strains DB, DD DW<sub>1</sub> and DW<sub>2</sub>, in comparison with fresh baker's yeast (FB), *p*-values were 0.2531, 0.7021, 0.8607 and 0.2790, respectively. The obtained results suggest that there is no statistically significant difference in terms of aldehydes production during fermentation.

## 4. Conclusions

The obtained results suggest that there is no statistically significant difference in the content of ethanol, methanol and higher alcohols in distillates of fermented media based on raw, thin and thick juices and molasses if the fermentation is carried by commercial fresh baker's yeast. However, there is a statistically significant difference in terms of aldehyde content in distillate if used media based on molasses, in comparison with media based on raw, thin and thick juices. If commercial starter cultures are used as production strains - dried distiller's yeast, dried wine yeast, dried wine yeast tolerant of high ethanol concentration, dried baker's yeast and fresh baker's yeast, distillates of fermentation broths are not statistically different in terms of ethanol, methanol, higher alcohols and aldehydes content, regardless effluent of sugar industry used as base of fermentation media.

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