Investigation of yeast performances in the fermentation of beet and cane molasses to ethanol production

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Abstract In commercial ethanol production producers often use sugarcane or sugar beet molasses as raw material due to their abundance and low costs. The most employed microorganism used for fermentation is *Saccharomyces cerevisiae* yeast due to its ability to hydrolyze sucrose from cane or beet molasses into glucose and fructose, two easily assimilable hexoses. The aim of this study was to evaluate the influence of different five commercial dry yeasts of *Saccharomyces cerevisiae* on sugar beet and cane molasses fermentation by ethanol and secondary products yield quantification. When the different types of Saccharomyces cerevisiae ware used to ferment sugar beet and cane molasses an ethanol production of 7–9% v/v was obtained. Ethanol yield was calculated as milliliter of ethanol produced per 100 mL of fermentation broth. The most suitable yeast seems to be Safdistil C-70 when the ethanol yields obtained are compared.

Keywords: ethanol, *Saccharomyces cerevisiae*, molasses, fermentation

1. Introduction

Manufacture of alcoholic beverages is in fact as old as human civilization. Although a large amount of industrial alcohol produced goes into alcoholic beverages, further applications for ethanol were also exploited, as fuel, for lighting purposes and for various uses in the chemical industry [1, 12, 16].

Alcohol used for drinks is made primarily from potatoes, cereals and molasses [2].

Molasses, the noncrystallizable residue remaining after sucrose purification, has some advantages: it is relatively inexpensive raw material, readily available, it does not require starch hydrolysis and already used for ethanol production [10, 15, 16].

The molasses obtained after sugar beet processing contains about 60% sucrose and 40% other components. The nonsucrose substances include inorganic salts, raffinose, kestose, organic acids and nitrogen containing compounds. Molasses is used in the production of baker's yeast, in fermentation technology for ethanol, citric, lactic and gluconic acids production, as well as glycerol, butanol and acetone production, as an ingredient of mixed feeds or in the production of amino acids [2, 4].

The residual molasses after cane sugar processing contains about 4% inverted sugar, 30-40% sucrose, 10-25% reducing substances, a very low amount of raffinose and no betaine and about 5% of aconitic acid [2].

Molasses does not require saccharification. The saccharified mash is cooled to 30°C and then inoculated with a yeast starter which has been cultured on acid medium or directly with distiller's yeast. After 48 h of fermentation, the ethanol present at 6-10% v/v in mash is distilled off along with the other volatile constituents [2, 3].

Baker's yeast *Saccharomyces cerevisiae* is the preferred fermenting microorganism for ethanol production because of its superior and well-documented industrial performances [4].Although many researchers studied the ethanol fermentation with *Saccharomyces cerevisiae*, in some cases a lack of recognition of its metabolic pathway led to approaches that are unlikely to yield significant improvements. The main metabolic pathway involved in the ethanol fermentation is glycolysis (Embden–Meyerhof-Parnas or EMP pathway),

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through which one molecule of glucose is metabolized, and two molecules of pyruvate are produced [8, 13].

Other yeasts, referred to as nonconventional yeasts, were studied for ethanol production. Among these are those that endure much lower pH than *Saccharomyces species*, such as *Zygosaccharomyces sp.*, as well as those that perform ethanolic fermentation at temperatures above 40°C, such as *Hansenula polymorpha* [4]. *Zymomonas mobilis* has also been intensively studied over the past three decades and repeatedly claimed by some researchers to replace *Saccharomyces cerevisiae* in ethanol production, because this species possesses some "superior characteristics" compared to its counterpart *Saccharomyces cerevisiae* [5, 6, 7, 11, 13, 14].

But, *S. cerevisiae* is a better choice due to its superior ethanol tolerance and high ethanol yield [4, 14].

For sure the main challenge of the fermentation process is to reach the best yield (alcohol produced/sugar used) as well as the highest reaction rates [9].

The purpose of this study was to evaluate the influence of different five commercial dry yeasts of *Saccharomyces cerevisiae* on sugar beet and cane molasses fermentation by ethanol and secondary products yield quantification.

2. Experimental

For the alcohol production sugar cane and beet molasses were used. These molasses are obtained from sugar manufacturing plants. The characteristics of the molasses used are presented in table 1.

The yeasts used for fermentation process are various types of active dried yeasts *Saccharomyces cerevisiae*: Safdistil C-70 and Ethanol Red^{TM} from SC Enzymes & Derivates SA, Trockenhefe and Fali^{R} from SC Protect Consult SRL and Pakmaya from SC Pakmaya SA.

The dried yeasts have the following characteristics: dry matter 94-96%, nitrogen content 4-8% from dry matter, P_2O_5 1-4% from dry matter, living cells $6x10^8$ cells/g, NTG < 10^5/g, coliform bacteria < 10^2/g, lactic bacteria < 10^3/g.

The fermentation process was conducted at industrial scale at *S.C. Euroavipo S.A. Ploiesti, Romania*. The alcohol obtained was analyzed for ethanol concentration using the STAS 184/2-87 method (ethanol concentration determination using thermo-alcoholmeter). Also, the total acidity of the samples was determined according to STAS 184/2-87. The samples obtained were analyzed also using gas chromatography. The volatile compounds from spirits were separated and measured using a TRACE GC having the following characteristics: SPLIT injector, FID detector, fused silica capillary 30 m long, inner diameter 0,25 mm, stationary phase CARBOWAX 20M. The volatile compounds determined using gas chromatography were: ethanol, methanol, furfural, acetic acid, acetic aldehyde, ethyl acetate, isoamyl alcohol, n-propyl alcohol, isopropyl alcohol, n butyl alcohol, isobutyl alcohol, n-amyl alcohol, hexanol, propionic aldehyde, butyric aldehyde, ethyl formiate, isobutyl acetate, isoamyl acetate, ethyl lactate, propionic acid, butyric acid, pentanoic acid, hexanoic acid, acetone. The total content in volatile compounds was obtained as sum of each quantified component.

3. Results and discussions

The specific consumption (expressed as 50 type molasses, sucrose) was different for each different yeast strain used.

When sugar beet was used as fermentation medium, the highest practical yield (expressed as liters of ethanol obtained from 100 kg sucrose) was obtained when Safdistil C-70 dried yeast was used.

This practical yield represents about 95% from calculated theoretical yield. (Table 2)

Table 1. Physico-chemical composition of the sugar beet and cane molasses used for experiment

Molasses type	Water, %	Dry matter, %	Total sugar, %	Total nitrogen, %	Mineral substances, %	pH
Sugar beet	21.6	78.4	51.2	2.2	6.5	7.8
Sugar cane	18.2	81.8	54.6	0.5	6.2	7.6

Table 2. The specific consumptions and alcohol yield for all yeasts types used for sugar beet molasses fermentation

Yeast type	Molasses consumption mg/mL absolute alcohol	Sucrose consumption, mg/mL absolute alcohol	V (mL) absolute alcohol obtained from 100 mg sucrose	% from theoretical yield	Fabrication quality
Safdistil C-70	3.131±0.05[a]	1.565±0.07	63.80±0.15	94.18±0.15	excellent
Etanol Red™	3.206±0.07	1.603±0.04	62.38±0.09	92.08±0.09	very good
Fali®	3.385±0.01	1.692±0.05	59.08±0.15	87.22±0.15	good
Trokenhefe	3.217±0.08	1.608±0.01	62.16±0.16	91.77±0.16	very good
Pakmaya	3.410±0.06	1.705±0.06	58.65±0.10	86.58±0.10	good

[a] standard deviation

Table 3. The specific consumptions and alcohol yield for all yeasts types used for sugar cane molasses fermentation

Yeast type	Molasses consumption mg/mL absolute alcohol	Sucrose consumption. mg/mL absolute alcohol	mL absolute alcohol obtained from 100 mg sucrose	% from theoretical yield	Fabrication quality
Safdistil C-70	3.189±0.04[a]	1.594±0.06	62.71±0.10	92.57±0.15	excellent
Etanol Red™	3.202±0.03	1.601±0.04	62.46±0.08	92.20±0.09	very good
Fali®	3.202±0.06	1.601±0.03	62.46±0.09	92.20±0.15	good
Trokenhefe	3.207±0.03	1.603±0.03	62.38±0.12	92.08±0.16	very good
Pakmaya	3.238±0.04	1.619±0.05	61.76±0.15	91.10±0.10	good

[a] standard deviation

In Fig. 1 the fabrication yield obtained after alcoholic fermentation of sugar beet molasses with five different yeasts is presented. It can be observed that when Pakmaya yeast was used for sugar beet molasses fermentation, the lowest yield was obtained: 58.65 mL absolute alcohol/100 mg sucrose meaning 86.58% from calculated theoretical yield.

In table 3 the specific consumptions and ethanol yields are presented for sugar cane molasses fermentation using the same five yeast types.

It can be observed, comparing the data from tables 2 and 3, that the differences in the specific consumption and ethanol yield for the same yeast type between the cane and beet molasses used are low.

When cane molasses is used as raw material for alcohol obtaining, the ethanol yield is different depending of the yeast strain used for fermentation. It can be observed from table 3 that when Safdistil, Ethanol Red, Fali and Trokenhefe yeasts are used, the practical yield vary between 92.08 and 92.57% of the theoretical yield. Pakmaya yeast performances instead are lower as when sugar beet molasses was used as fermentation medium.

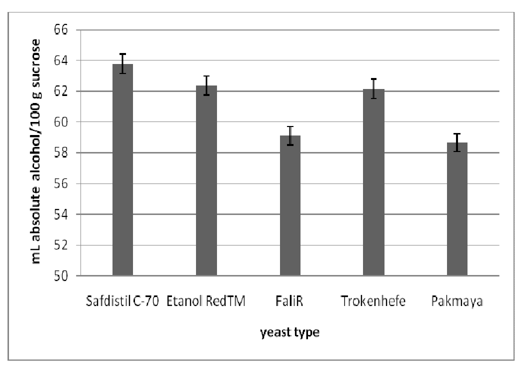

Fig. 1. The practical fabrication yield obtained after alcoholic fermentation of sugar beet molasses using different yeast types

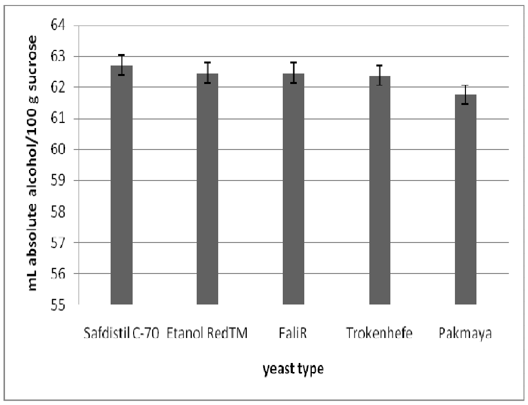

Fig. 2. The practical fabrication yield obtained after alcoholic fermentation of sugar cane molasses using different yeast types

The influence of the yeast strain used for fermentation on mash composition was also studied. The differences between the fermented mashes were insignificant, unaffected by molasses type, as it can be seen from the figures 3-8. The results represent the average composition of the fermented mash.

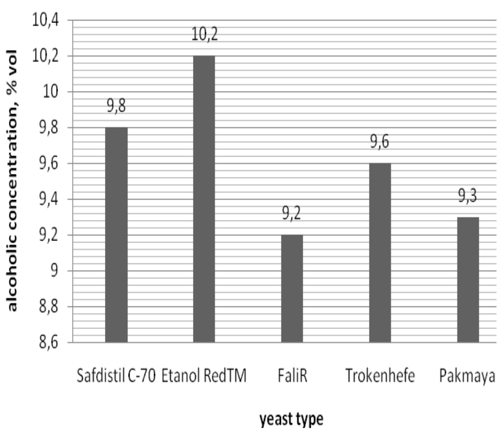

Fig.3. The alcoholic concentration of the fermented mashes in % volume

Although the yeast Ethanol Red is commercialized by the same company as Safdistil, the fermented mash by Ethanol Red yeast had the highest alcoholic concentration, as it can be observed from Fig. 3.

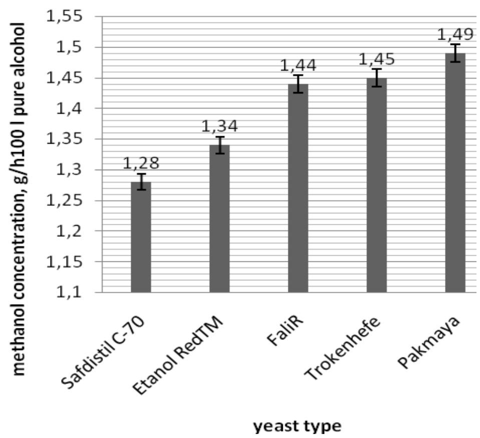

Fig. 4. The methanol concentration of the fermented mash

When the mash quality is analyzed, it can be seen that the methanol concentration is lowest when Safdistil yeast is used for mash fermentation (Fig. 4), 1.28 g/100 L pure ethanol. This mash had the lowest volatile substances too.

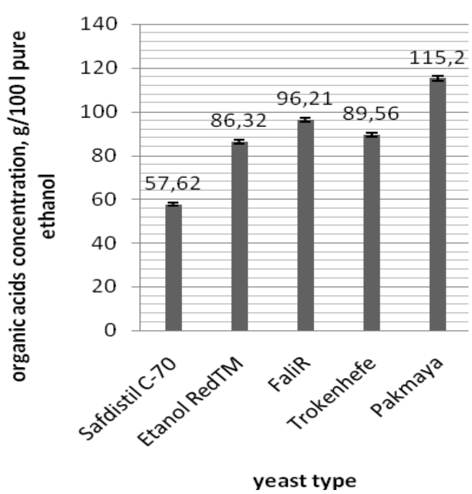

Fig. 5. The mash composition in organic acids

As it can be observed in Fig. 6, the ester composition of the mash differs with yeast type; the highest concentration in ester was obtained by using Fali and Trokenhefe yeasts.

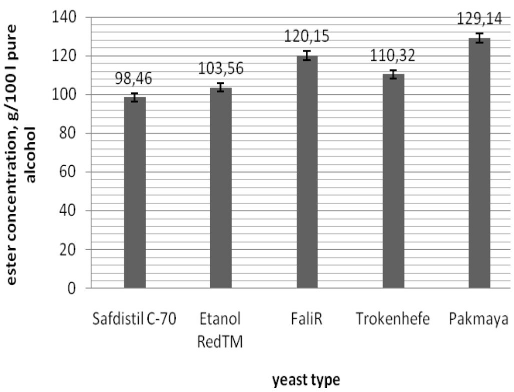

Fig. 6. The ester concentration in fermented mash

In Fig. 7 and 8 the higher alcohols concentration of the fermented mash and the aldehyde content of the fermented mash respectively are presented.

The total volatile substances content is lowest for the mash fermented with Safdistil yeast, 358.64 g/100L pure alcohol.

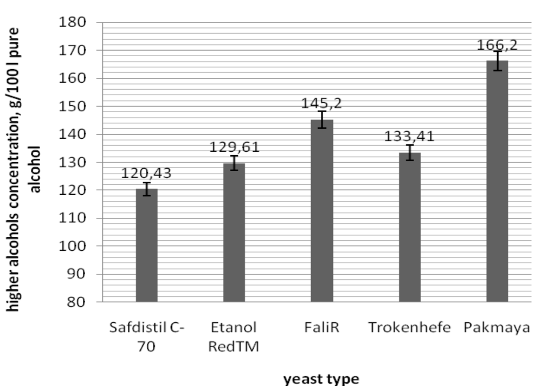

Fig. 7. The higher alcohols composition of the mashes after fermentation

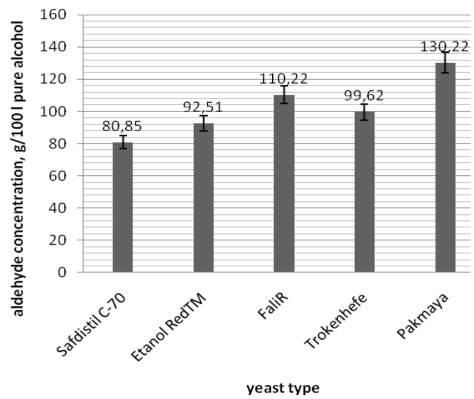

Fig. 8. The aldehyde composition of the fermented mashes

4. Conclusions

The yeast type influence on ethanol production yield was studied. It can be observed that when sugar beet molasses is used, the highest yield was obtained by using Safdistil dried yeast, commercialized by SC Enzymes & Derivates SA. The production yield represents about 95% of the theoretical yield. The lowest fabrication yield was obtained when Pakmaya yeast was used for mash fermentation. This yield means 86,58% from the theoretical calculated yield.

When cane molasses was used as fermentation medium, the same Pakmaya yeast lead to the lowest practical yield.

The differences obtained between the ethanol production yields are low, independent of the raw material origin.

The highest alcoholic concentration was obtained in the mash fermented with Ethanol Red yeast from SC Enzymes & Derivates SA.

Concerning mash's quality, it can be observed that when Safdistil yeast was used for mash fermentation, lower methanol was present in the mash and lower total volatiles amount was obtained.

Yeast type used for mash fermentation for ethanol production is an important parameter, because it influences ethanol yield and ethanol quality also.

5. Acknowledgments

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